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Microbiología

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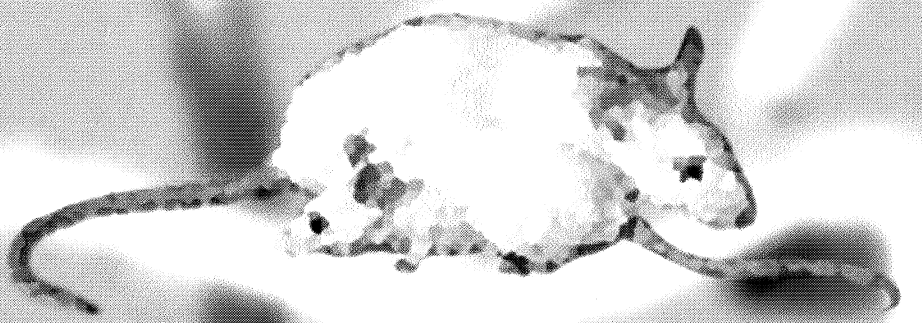

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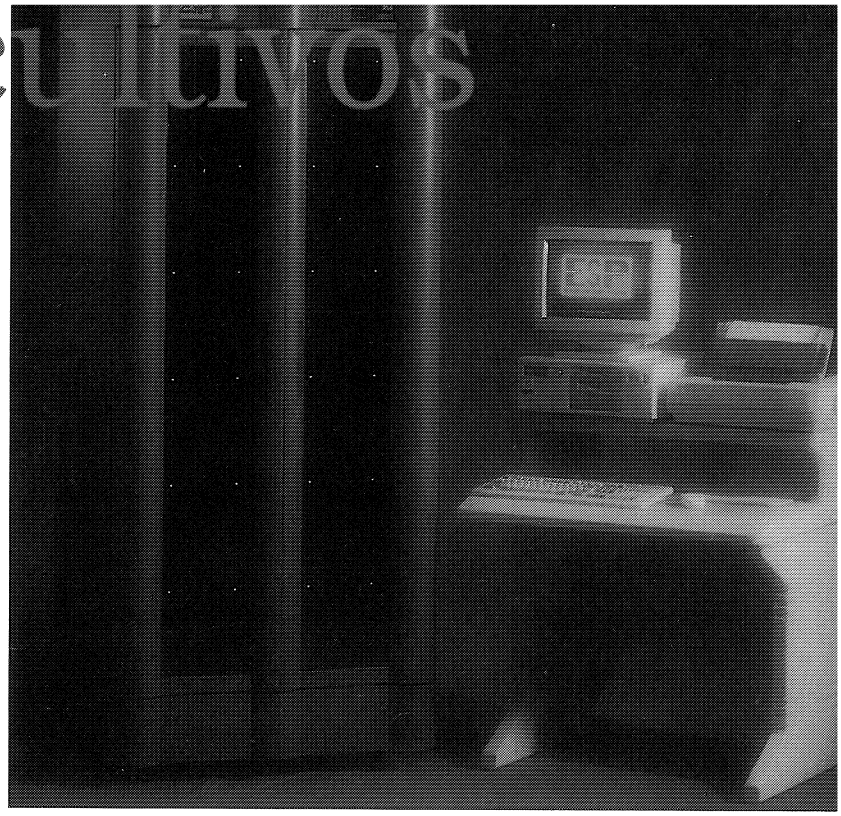

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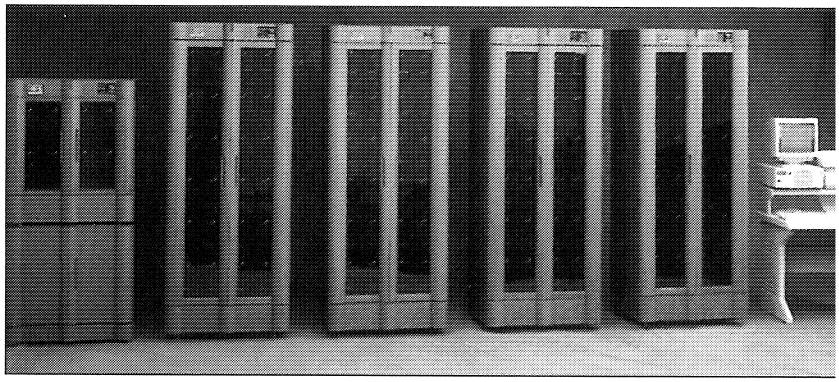

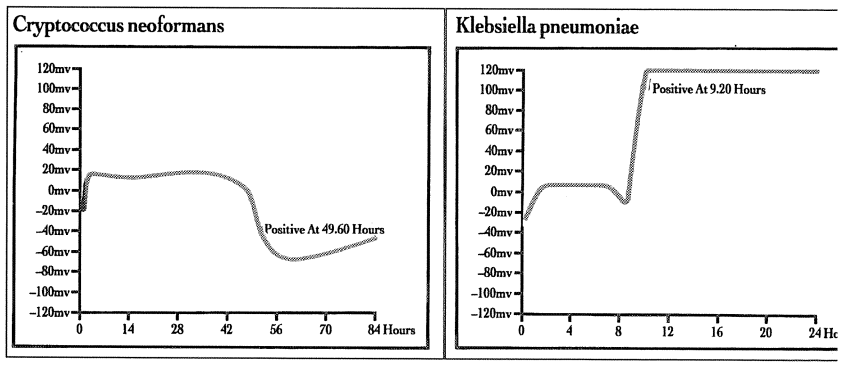

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[MICROBIOLOGÍA SEM 13(1): 1–120 (1997)]

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Continued on p. 115

CONTENTS

ISSN 02 13-4101

ÍNDICE

ISSN 02 13-4101

MICROBIOLOGÍA SEM **13** (1997), 7–10

Editorial*

Los retos de la ciencia en América Latina

Si bien es cierto que en los últimos años *Nature*, *Science*, *Scientific American* y algunas otras publicaciones internacionales han mostrado interés por reflejar la situación de la ciencia en los países del llamado Tercer Mundo, la actividad científica no es precisamente lo primero que viene a la mente cuando se piensa en América Latina. Para el lector no informado, somos la región del mundo donde parecen campear a sus anchas la inflación, el desempleo, los colapsos financieros y toda clase de desastres económicos, combinados con políticas inestables, tráfico de drogas, revueltas sociales y corrupción generalizada. La historia de gran parte del Nuevo Mundo parecería modelada por la combinación de brotes recurrentes de anarquía social y el poder devastador de huracanes, terremotos, erupciones volcánicas y otras fuerzas telúricas igualmente incontrolables.

Cualquier persona medianamente educada sabe que lo anterior no es más que una cara de la moneda. Es bien sabido que en los últimos años recesiones y devaluaciones han debilitado nuestra vida económica, reduciendo en forma dramática los servicios públicos, como salud y educación, y empujado a millones de personas hacia el desempleo y la pobreza. Pero es igualmente cierto que toda descripción del mundo latinoamericano tiene que incluir la extraordinaria vitalidad de nuestra cultura, hoy por hoy dotada de una diversidad y de una energía antes nunca vistas. Sin embargo, el gran ausente sigue siendo el componente científico. La vida intelectual en nuestras sociedades se está desplazando con enorme rapidez hacia un enorme abanico de posibilidades, pero la cultura científica sigue ocupando un lugar marginal en estos movimientos.

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* Tras la colaboración de científicos de Estados Unidos, que han escrito durante el año 1996 sobre la situación de la ciencia en los países de América Latina, *Microbiología SEM* ha invitado, para 1997, a prestigiosos investigadores latinoamericanos a que expresen su punto de vista sobre el tema, considerando que se puede obtener así una perspectiva general que incorpore diferentes opiniones y conocimientos, útiles para hacer un análisis lo más objetivo posible del estado de la cuestión.

Esta situación tiene raíces añejas. Somos, en parte, los hijos de la Contrarreforma y de la actitud taciturna de los Habsburgo (siglos XVI y XVII), que no pudieron comprender las transformaciones que se estaban dando en Europa y terminaron por darle la espalda a los cambios tecnológicos y científicos que acompañaron a la Revolución Industrial. El despotismo ilustrado de Carlos III, un Borbón (a mediados del siglo XVIII), promovió expediciones científicas y permitió la difusión de las ideas de Newton, Linneo y Lavoisier en los Virreinatos de Perú y la Nueva España (México), pero le asestó un golpe sin igual a la vida cultural de las colonias con la expulsión de los jesuitas. En el siglo XIX, mientras seguíamos luchando por nuestra independencia, debíamos enfrentarnos a las invasiones extranjeras. Para colmo de males, en esas épocas, mientras nos debatíamos en discusiones acerca de nuestra identidad histórica, surgieron y se multiplicaron caudillos de vocación tiránica, como el General Juan Manuel Rosas, un terrateniente que dominó la vida política y económica de Argentina durante más de veinte años, y a quien Charles Darwin describió como un déspota caprichoso y seductor.

Si Darwin hubiera visitado México (y sabemos por su *Diario* que este viaje representaba para él una buena alternativa a la vida matrimonial), se hubiera topado con el Generalísimo Don Antonio López de Santa Anna, un personaje de opereta que fue el equivalente norteño de Rosas. Como resumió Carlos Fuentes en *El Espejo Enterrado*, las oligarquías latinoamericanas del siglo pasado, sumergidas en una vida de ocio y elegancia, admiraban a figuras como Rosas y Santa Anna, pero ni unas ni otros hicieron mucho para favorecer un crecimiento económico racional. Ríos de productos agrícolas, minerales y de petróleo partieron de nuestras tierras, pero este carácter de naciones exportadoras hizo poco por el desarrollo de una tradición científica y tecnológica. Es cierto que existieron excepciones notables, encarnadas en los sabios locales, sobre todo médicos, farmacéuticos e ingenieros, que intentaban promover visiones seculares y científicas, pero a menudo con un elevado costo personal y sin que hubiera continuidad en sus esfuerzos. El siglo XIX nos dejó una herencia pesada: la investigación científica no ha formado parte de nuestras fuerzas productivas, y sigue siendo un componente marginal tanto de nuestro desarrollo económico como de nuestra vida cultural.

América Latina es actualmente un continente heterogéneo en donde los parches de modernidad y bienestar limitado coexisten en elevado contraste con situaciones de extrema pobreza, algo que ha aumentado en forma espectacular con la aplicación estricta de las teorías neoliberales. El rápido incremento de nuestra deuda externa ha traído consigo la contracción del sistema educativo, que resulta incapaz de responder a las necesidades culturales de nuestro tiempo y al crecimiento demográfico y se encuentra lastrado, en muchos países, por un desdén social por la labor de los maestros. Hace poco Sir John Maddox (ex-director de *Nature*) afirmó que en pocos años la quinta parte de la población del Reino Unido tendrá un título universitario en alguna carrera científica; en México, en cambio, el número de científicos activos apenas si llega a los 6000. Los males que aquejan nuestro desarrollo científico son perfectamente visibles, e incluyen (a) comunidades académicas reducidas que, fieles a una vieja tradición centralista, se concentran en unas pocas ciudades; (b) ausencia de científicos jóvenes y de continuidad generacional; (c) imposibilidad de implementación

de planes a largo plazo, sacudidos siempre por convulsiones económicas y desajustes políticos; (d) porcentajes insuficientes del producto nacional bruto dedicado a promover la investigación básica y aplicada; y (e) escasa subvención privada a las actividades científicas, resultado de un capitalismo dependiente cuya industria descansa sobre todo en patentes extranjeras.

En estas condiciones, no resulta extraño que, salvo contadas excepciones, los científicos latinoamericanos estén alejados del panorama internacional. Es evidente que esta ausencia no se puede achacar a la falta de creatividad; la historia ha demostrado una y otra vez que somos naciones de creadores de gran fuerza y originalidad prodigiosa. Sin embargo, pocos investigadores latinoamericanos han logrado el mismo reconocimiento que nuestros novelistas y pintores. No es difícil adivinar las razones de esta asimetría: los logros intelectuales no son producto de la casualidad; para que cuajen, se requiere siempre de un clima apropiado, que permita traducir en hechos concretos el potencial creador de un ser humano. Como escribió Claude Bernard, "el arte es el *yo*: la ciencia es el *nosotros*". Pero la implantación de una tradición científica requiere de continuidad de esfuerzos, y en México, y en otros países de América Latina, ello se ha visto interrumpido una y otra vez por catástrofes monetarias y desastres políticos. Seguimos pagando el precio de esa situación: una cultura científica no puede desarrollarse ni echar raíces sobre una base de inestabilidad económica y ausencia de apoyo social.

Pero hay otras razones que permiten explicar esta situación, y de ello ofrece un buen ejemplo el artículo que W. Wayt Gibbs publicó en el número de agosto de 1995 de *Scientific American* (diciembre en la edición en español). Según Gibbs, la ausencia de científicos de países en desarrollo y de las publicaciones que se editan en estas naciones es el resultado de una situación compleja, en donde se mezclan por igual situaciones externas e internas: falta de apoyo económico y social, diferencias en las normas académicas, barreras lingüísticas y los prejuicios políticos y sociales de los sistemas científicos de los países industrializados.

¿Con qué criterios se debe evaluar nuestro trabajo científico? La respuesta es simple: con los mismos que se utilizan para los investigadores del resto del mundo, sean países ricos o pobres. Los latinoamericanos no estamos dispuestos ni a jugar el papel del pariente pobre del pueblo, ni el del espécimen tercermundista al que se le cede generosamente un espacio en los foros internacionales. Pero para ser interlocutores es preciso disponer de los recursos económicos, humanos y sociales apropiados, incluyendo la implantación de políticas científicas coherentes y realistas. Ello no depende sólo de los gobiernos, sino también de la voluntad colectiva. Isaiah Berlin escribió alguna vez que la soledad no es sólo ausencia de los demás, sino también el estar entre quienes no saben de lo que hablamos. La alienación histórica de los científicos latinoamericanos sólo acabará cuando se haga de esta actividad un componente activo de nuestra identidad cultural y de nuestra vida económica. Para lograr este objetivo, la comunidad científica latinoamericana debe enfrentar una serie de desafíos, uno de los cuales es volver a la ciencia asequible a un público amplio. Ésta es una tarea prioritaria, que no puede quedar reducida a los aspectos meramente pedagógicos, ni estar limitada tan sólo a los aspectos utilitarios de la investigación.

No existe una ruta única para arribar al conocimiento científico, y debemos ser eclécticos en el

uso de todos los medios disponibles: prensa, radio, televisión, museos. La red Internet es un indicador del tremendo potencial de las nuevas formas de comunicación para romper el aislamiento científico de las naciones en desarrollo, pero, como ha señalado el antes citado Gibbs, en las bibliotecas de algunos países ni siquiera están disponibles los libros básicos, las revistas de investigación y las bases de datos. El control de la información es una de las fuentes del poder, y el ensanchamiento cada vez mayor de la brecha informativa puede aislar aún más a las naciones pobres y en desarrollo. La letra escrita no está condenada, ni siquiera en estas épocas del hipertexto y las autopistas de la información. La industria del libro es poderosa, pero las políticas editoriales locales e internacionales tienen que desarrollarse para hacer frente a un peligro creciente: la transformación del libro y las revistas científicas en artículos de lujo inaccesibles no sólo al gran público sino incluso a nuestros investigadores e instituciones.

Según un estudio demográfico reciente, en Italia hay tres adultos por cada niño. Nuestra tendencia es diferente: la mitad de la población de América Latina tiene quince años o menos. Cada uno de los científicos que podemos ganar o perder a la vuelta del año 2000 está ya cursando la escuela elemental. Que estos niños crezcan en un clima en donde la ciencia se considere como un componente de la cultura moderna es una responsabilidad internacional. El potencial intelectual que desaparece cuando un estudiante del Tercer Mundo abandona una carrera científica es una pérdida no solamente para su país, sino para la comunidad científica del mundo entero. Éste no es un argumento meramente retórico. La buena ciencia es ciencia buena, pero no es una actividad pura, descarnada y sin raíces. Los científicos están insertos en los marcos culturales, económicos y políticos de las sociedades a las que pertenecen, y esta variedad genera una gama amplia de perspectivas y orientaciones, cada una de ellas con su propio lenguaje, sus formas de aprendizaje, y sus tradiciones intelectuales. Esta diversidad no sólo nutre a la ciencia misma, sino que contribuye a hacer de ella un patrimonio de todos los hombres y mujeres del mundo. A los latinoamericanos nos toca enriquecer estas tradiciones, adoptando y adaptando a la ciencia como parte de nuestra cultura. De no hacerlo, nos estaremos condenando a cien años de soledad intelectual.

Antonio Lazcano
Universidad Nacional Autónoma de México

Antonio Lazcano, es profesor de la Universidad Nacional Autónoma de México. Especialista en temas del origen de la vida y clasificación filogenética, es autor de numerosos artículos sobre ambos temas y otras materias de biología y culturales. Ha impartido conferencias en diversas universidades europeas y estadounidenses. Durante su período de formación y en su carrera profesional ha trabajado con científicos de reconocido prestigio como Stanley L. Miller, Joan Oró, Lynn Margulis, entre otros. Ha sido un difusor de la obra de Alexander I. Oparin, con quien trabajó personalmente.

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MICROBIOLOGÍA SEM **13** (1997), 11–22 **MINIREVIEW**

Midwife to the greens: the electron capture detector*

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Summary

James E. Lovelock makes an account of the path he has followed since he started his scientific research at the National Institute for Medical Research (NIMR) in London in the 1940s, emphasizing the aspects related to environmental sciences. Lovelock explains the origins of the electron capture detector (ECD). So far, the ECD is the most sensitive, easily portable and inexpensive analytical apparatus capable of detecting substances present in the atmosphere at concentrations as low as parts per trillion (10^{-12}). It has been the first device specifically sensitive to pollutants, and its use has provided the grounds for the development of environmental sciences, and green politics. The data gathered by the ECD about the persistence of pesticides in the environment led American biologist Rachel Carson to write her seminal book *Silent Spring*; data regarding the global presence of chlorofluorocarbons (CFC) in the atmosphere led Sherwood Rowland and Mario Molina to develop their theory of ozone depletion. The results of his research over the years led Lovelock himself to the development of the Gaia theory.

Key words: electron capture detector, chlorofluorocarbons (CFC), Rachel Carson, ozone depletion, environmental sciences

Resumen

James E. Lovelock narra la trayectoria seguida desde que inició su carrera científica en el Instituto Nacional de Investigación Médica (NIMR) de Londres, en la década de los cuarenta, destacando los

* Article based on the lecture given by Prof. James E. Lovelock (Fig. 1) in Brussels on October 9, 1996, on the occasion of having been awarded the 1996 Volvo Environment Prize.

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aspectos relacionados con las ciencias ambientales. Lovelock explica los orígenes del detector de captura de electrones (ECD), hasta ahora el instrumento analítico más barato, fácil de transportar y sensible que es capaz de detectar substancias presentes en la atmósfera a concentraciones tan bajas como partes por billón (10^{-12}). Es el primer dispositivo específicamente sensible a contaminantes y su uso ha servido de base para el desarrollo de las ciencias ambientales y la política de protección del ambiente. La bióloga estadounidense Rachel Carson se basó en los datos aportados por el ECD sobre la persistencia de plaguicidas en el ambiente para escribir su obra seminal *Silent Spring*. La información proporcionada por esos datos en relación a la presencia global en la atmósfera de clorofluorocarbonos (CFC) sirvió de base a Sherwood Rowland y Mario Molina para desarrollar su teoría sobre la disminución de la capa de ozono. La investigación llevada a cabo por el propio Lovelock a lo largo de los años le sirvió de base para el desarrollo de la teoría de Gaia.

First steps

The electron capture detector (ECD) is now 40 years old, yet it is still widely used. It was born at a different time from now, a time when science was still a way of life—a vocation—not just a means of employment. My story will be about its origins. How the electron capture detector was the first device to be specifically sensitive to pollutants and how its use led to the growth of environmentalism and green politics.

Let me begin by telling you something about the place where it was discovered. This was the National Institute for Medical Research (NIMR) in London. Here, gas chromatography itself was reduced to practice by Archer Martin and Tony James, and here I stumbled on the ECD. In the 1940's and 1950's NIMR was one of the leading laboratories of the world. It employed only about 100 scientists and, of these, seven were or were to become Nobel laureates, and more than twenty five, Fellows of the Royal Society. Its strength came from the character of the man who directed it, Sir Charles Harington. He was a brilliant biochemist and the discoverer of thyroxine, and he was uncompromising in his pursuit of excellence. At the National Institute there were very few tenured posts, three year contracts were the rule. The director would tell worried scientists who sought security. "If you are good, you don't need tenure; if not, you should not be here." He would then grin and say: "Don't worry; such is our reputation, that you will find no difficulty in getting a job elsewhere." We were among the best paid scientists in the country, but there was very little money available for equipment. Instead, we had either to make our own or have it made in the Institute workshops. There was no chain of command, good young scientists could follow their instincts without the need to seek the permission of a divisional head or professor. But we were all accountable to the director. Because of this structure, disciplinary barriers were minimal and all scientists collaborated freely. Physicists, chemists, biologists and medical scientists talked and planned together in the coffee room or the cafeteria. It was for a while a fertile island of creativity in a sea of mediocrity.

In a way I first became involved with the ECD in 1948 when I was working on the problem of the common cold. In those days, precious little was known about the science of this subject but, as often is the way in states of scientific ignorance, people in the street knew all that there was to know about the common cold. To them it was quite simple: you caught colds in the winter by getting cold, hence the name. Then the Medical Research Council was a government institu-

tion and therefore not entirely unaffected by public opinion and political pressure. We thought it might be wise, therefore, to consider the possibility that colds were caught through getting cold. My job was to determine the extent of chilling objectively, then compare it with clinical data on the frequency of colds in different indoor climates. The three factors important in chilling are temperature, humidity, and air movement. The first two are easy to measure, but the air movements in a closed room—draughts, as the English call them—were so slight as to be undetectable by the simple anemometers then available.

In those days it was customary to build, not buy, instruments. Indeed, scientists were expected to invent and, in such circumstances, I soon found myself with two novel anemometers. The first was an ultrasonic device that exploited the change in wavelength of sound due to air motion. It worked well, but was still too insensitive to detect 5 mm/s air motion we needed to measure. The second method I tried was an ion-drift anemometer. Positive ions move in air at a speed of 10 mm/s in a field of 1 V/cm. Draughts easily perturbed the drift of these slowly moving ions. It was great fun to make such an anemometer and to find that it worked even better than expected.

When I say make, I mean it literally. Everything from the electronic equipment to the sensor itself was made by hand. Remember also in those days we used vacuum tubes, not solid state electronics. I even made the radioactive source needed to ionize the air by scraping the dial paint from gauges taken from the flight deck of old aircraft. These gauges provided a rich harvest of radium or mesothorium. I made the sources by ashing this paint, then resuspending the ash in lacquer. I then painted this radioactive lacquer onto the anemometer ion source.

It worked well and I was able to take it on an Arctic expedition in the winter of 1949. The only

FIG. 1. James E. Lovelock. (© Sandy Lovelock, 1995)

snag was that cigarette smoke perturbed its response; it was as sensitive to this as is a reformed smoker. To discover the source of this drawback, I exposed it to a number of different gases and smokes and found that, in addition to smoke, chlorofluorocarbons (CFCs) disturbed its function. Looking back, I realize the key to invention is need. We did not at that time need a device to detect low levels of halocarbons, and so the electron capture detector was in a sense prematurely discovered.

In 1951 having by then found out little more about common cold—other than that, chilling was not the cause—I was moved back to our parent institute in London. My new task was to work on the preservation of life in the frozen state. My colleagues were the biologists Alan Parkes, Audrey Smith, and Christopher Polge.

It was easy to preserve suspensions of cells in the frozen state for years. The challenge was to freeze, preserve and then bring back to life a whole animal. The difficult part was the rewarm-

ing, and if a cold or frozen animal was placed in a warm bath it would die. Quite simply because the skin and subcutaneous tissue would warm and start consuming oxygen before the heart did. When the heart warmed enough to beat, it would draw in anoxic blood from the surface tissues and fail. So my colleagues heated the heart first, keeping the skin cool so it did not use oxygen. They did this by pressing a hot metal spoon against the animal's chest just above the heart. A few animals were revived this way but only at the expense of severe chest burns. My biologist colleagues had that stoicism about the feelings of animals that one sees in working zoologists and farmers. They were not cruel or sadistic in what they did, nevertheless it was more than I could stomach. There was a painless way to heat the animals internally while keeping the skin cool. This was diathermy, radio frequency heating.

My apparatus budget was too small to allow me to buy a diathermy equipment so I made one. With it Audrey Smith reanimated her animals, almost without harming them. Later I borrowed a 500 watt continuous wave magnetron from the navy, and used it to reanimate small animals. I also used it to bake potatoes for my lunch. This was 1955. Maybe I had prematurely invented the microwave oven, but not being an entrepreneur I did not realise its great commercial potential.

As I continued these freezing experiments, I became aware that the sensitivity to freezing damage was connected with the fatty acid composition of the cell membrane lipids. Archer Martin and Tony James were in a lab one floor above me and I knew of their newly invented gas chromatograph (4, 5). I asked them what chance there was of analyzing the fatty acid composition of my cell lipids. At first they were enthusiastic. When they saw how small my samples were, just a few hundred micrograms, they advised me to extract larger samples. As an afterthought Martin added, "or maybe you can invent a more sensitive detector than my gas density balance." Larger scale experiments would have required about two months work. To go back to inventing seemed much more fun.

I remembered a sensitive ionization anemometer I had made in 1949 and how easily it was disturbed by the presence of CFCs. I wondered if this disadvantage of the anemometer might be turned to advantage, and made the basis for an ionization detector.

At the National Institute it was the tradition of those days never to read the literature, especially text books. Senior scientists warned that our job was to make the literature, not to read it. It was a recipe that worked well for me. Had I read the literature of ionization phenomena in gases before doing my experiments, I would have been hopelessly discouraged and confused. Instead, I just experimented. Fortunately we were not hampered, like now, by the excesses of the health and safety bureaucracy. Scientists who used dangerous chemicals or radioactive materials were expected to be personally responsible. There was some risk but I doubt if under the stifling restrictions of today I would have had the persistence to carry on with so uncertain a project as the infant ECD.

The first detector I made was modeled on the Dutch scientist Boer's design of the ionization cross-section detector. It was in effect a gas thickness gauge. The denser the gas, the greater the numbers of ions and consequently the larger the flow of current. I tried a detector made from a simple cylindrical ion chamber, about 2 ml and with a 20 millicurie Sr-90 beta source in it.

I remember bending the stiff foil of this radioactive source behind a sheet of thick glass until it fitted the detector cavity. The case of the chamber was connected to a source of direct current and the central electrode to a home made electrometer. It used a pair of vacuum tubes in a

balanced cathode follower circuit and it was made on our kitchen table. I purchased the electronic components from surplus equipment vendors in downtown London. The chromatograph itself consisted of a 1.2 m long straight glass column filled with a powder coated with a non volatile hydrocarbon called apiezon. The column was mounted vertically within a solid rod of aluminum, 2.5 cm in diameter and electrically heated. It ran at a temperature of about 100°C for most of the time.

This ionization cross section detector works best with light carrier gases such as helium or hydrogen; it is similar to the thermal conductivity detector in its performance. Helium was then expensive in Europe and hydrogen was unacceptable for use in a high-temperature apparatus expected to run overnight unattended. I was obliged therefore to use nitrogen as the carrier gas—as did Martin and James with their gas density balance. It was easy enough to confirm Boer's performance figures, but these were miserably insensitive when compared with those of Martin's gas density balance. The first ionization detector did not seem to be promising.

Deciding what next to do

Sometimes, when one is confronted with a failed experiment or an unsatisfactory device, it is better to cut one's losses and move on to something else. In this instance, however, I remembered the success of the ion anemometer and how its sensitivity was very dependent upon the applied potential. I thought it at least worth trying a few experiments to see if different ranges of applied potential would improve the performance of the ionization cross-section detector.

It was easier to try low potentials first. I soon found that if the detector was polarized with less than 30 V, the ion current in pure nitrogen became a little less, but that when other substances were present, it became much less. Tony James had supplied me with a test mixture of fatty acid methyl esters, from methyl propionate to methyl caproate. With the detector which was operating at 100–300 V, 1 mg of this mixture gave four small peaks. When I tried it with only 10 V and reversed the recorder connection in order to reveal negative peaks positively, the 1 mg sample gave what seemed to be a never-ceasing range of off-scale peaks. I thought that the search was over and we now had a truly sensitive detector. I asked James and Martin to come try it, which they did, bringing with them an allegedly pure sample of methyl caproate. I shall never forget the look of amazement on Tony Jame's face as peak after peak was drawn from a small sample of this substance. Worse, none of them had the retention time of methyl caproate or of any other fatty acid ester. We now know that what was seen were traces of electron absorbing impurities in the sample, but at that time it seemed to be a useless and wholly anomalous device.

In spite of this disappointment, I continued to play with it whenever there was time, and by trying compounds chosen at random from the lab shelves I discovered a certain sense in its behaviour. It seemed to respond to polar compounds like ketones and alcohols but not to hydrocarbons and ethers. When I tried a mixture of compounds made up in the nonpolar solvent carbon tetrachloride, the ion current fell to zero and remained there, resisting all attempts to restore normal operation. I later realized how unwise it was to connect the column to the detector using a silicone rubber seal, which became an almost permanent source of vapour of that intensely electron absorbing substance, carbon tetrachloride.

For the ordinary gas chromatograph, we clearly needed something more sensitive than the original ionization detector, but less tem-

peramental than the electron capture detector. I wondered if other ionization processes might be exploited to detect vapours of organic compounds in a nitrogen carrier gas.

This line of thought led by a happy accident to the discovery of the "argon" detector. This device exploited the ionizing collisions between rare gas metastable atoms and vapour molecules for detection. For a brief few years this was the principal sensitive detector used in gas chromatography (5, 7). There was a vast need for a sensitive detector to exploit fully the enormous possibilities of Archer Martin's invention. There was at that time little interest in the ECD, but immense interest in the argon detector. Until the more reliable flame ionization detector became the detector of choice in about 1959, everyone doing gas chromatography seemed to want an argon detector. For me this meant that life for a while was entirely dominated by detectors and the people who wanted to use them. Because the argon detector had the disadvantage of needing a radioactive source and had other drawbacks, I invented the photoionization detector. This never was however a serious competitor for the flame ionization detector. I continued to experiment with the ECD until by the end of 1959 it was developed to the point where it was very little different from those now used (Fig. 2). It was then, and still is, the most sensitive, easily portable and inexpensive analytical device in existence. The ECD is exquisitely sensitive. If a few litres of a rare perfluorocarbon were evaporated somewhere in Japan we could with little effort detect it in a 100 cubic foot sample of the air in Brussels a few weeks later. It would take between one and two weeks to reach there. Within two years it would be detectable anywhere in the world.

The early ECD was prone to all manner of anomalies and was an extraordinarily difficult device to use. Most of these problems I found were due to space charges and contact potentials that developed inside the detector when it was operated at low DC potentials. The key to the cure of the electron capture detector's bad habits came from an encounter with Ken McAffee of Bell Telephone Laboratories. He had developed a pulse method for observing electron attachment in a drift tube. After I considered his idea, it occurred to me that most of the difficulties with the ECD were due to its operation in weak electrical fields. These difficulties could be resolved if the electrons were collected by brief pulses of high potential. The high-potential pulses overcame the all too-frequent contact potentials and space charges that unpredictably either enhanced or opposed electron collection. Later the pulse method was further developed. I found that by observing the frequency of pulse needed to sustain a constant electron population the detector became even more stable and reliable. This method is now the one almost universally used. Its only drawback is a non linear response to strongly electron attaching compounds.

As experience in the analysis of different molecular species by electron capture grew, an odd and interesting association between electron capture and biological activity grew even more apparent.The great bulk of electron-absorbing substances fell into two categories: those that were important components of the biological system of energy transport and those that were highly toxic, especially to this system, or carcinogenic.

It was tempting to speculate that the free electron might be a fundamental particle of biology as well as of chemistry and physics. It was a challenging coincidence that each alternate acid of the Krebs' cycle (the major pathway for the oxidation of lipids and carbohydrates) was one of the few organic compounds that reacted vigorously with free electrons. These include pyruvate, oxaloacetate, fumarate, ketoglutarate, and

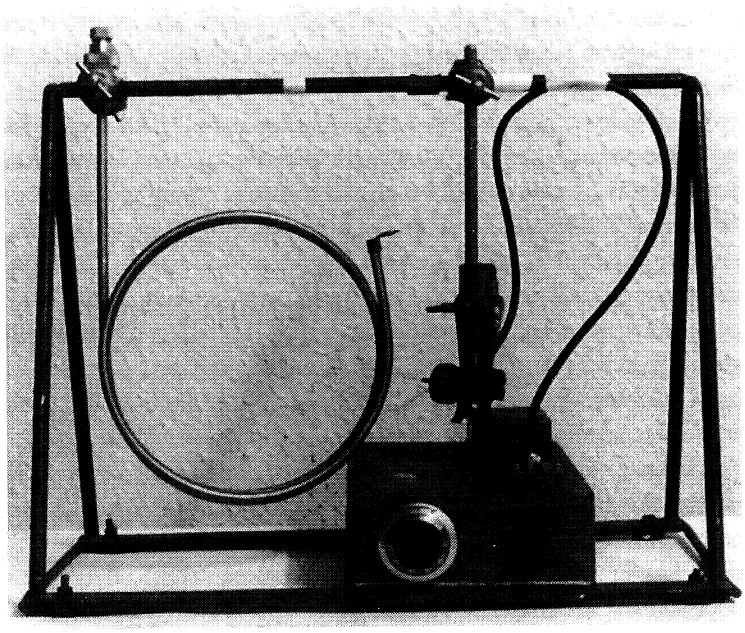

FIG. 2. The home-made electron capture detector.

cis-aconitate. The coenzymes ubiquinone and NADH are also electron-attaching, as well as compounds such as the thyroid hormones, which are able to mediate in this system. Other electron-attaching compounds are the iodo- and nitrophenols, which are toxic and act by uncoupling oxidative phosphorilation. It is still unclear whether this association is real or coincidental, but there is no doubt that a remarkably high proportion of electron absorbers are biologically active, which makes the ECD so important a device in environmental science.

Nowadays, whenever I come across a chemical substance that is strongly electron-absorbing, I tend to regard it cautiously. I well recall it being argued that the apparent association between carcinogenesis and electron capture was illusory, since so many of the halocarbons were not carcinogenic. Vinyl chloride, chloroform and trichloroethylene were quoted as examples of substance safe enough for use as anaesthetics in surgery. Now, we know them to be carcinogenic. I sometimes wonder about the phthalate esters. These ubiquitous plasticisers have long been a nuisance as electron-absorbing contaminants. Do they also have a more sinister role?

The emerging of environmental sciences

While I was just experimenting, serious scientists were applying the detector to the practical analysis of pesticide residues in foodstuffs. In the USA, Watts and Klein of the Food and Drug Administration (FDA), and in the UK Goulden and his colleagues at Shell, together established the base data that started the environmental movement. Soon it was realized that pesticides like DDT and dieldrin were distributed throughout the global environment. They could be found in the fat of Antarctic penguins and in the milk of nursing mothers in Finland. This was the data that led Rachel Carson to write her seminal book, *Silent Spring* (1). A book that warned the world of the ultimate consequences if these chemicals continued to be used by farm-

ers against all forms of life that are not livestock or crops (6). It was a book that was bound to affect the course of politics and in many parts of the world her gloomy forecast of a silent spring has come true. Not, as she predicted, by pesticide poisoning but simply by habitat destruction.

When I first heard that the ECD was being used this way I was delighted. I shared with Rachel Carson a concern over damage to wild life and to natural ecosystems. Some parts of the chemical industry reacted in a shameful and foolish way by trying to discredit her as a person. It did not work. Quite the reverse, it made Rachel Carson the first saint and martyr for the infant and innocent Green movement.

As environmentalism evolved, Rachel Carson's vision and the data itself became corrupted. I do not mean that the data gathered was false but so sensitive is the ECD that it can detect utterly trivial quantities of pesticides and other chemicals. Before ECD was used it would have been quite easy and reasonable to set zero as the lower permissible limit of pesticide residues in foodstuff. As you know, zero really means the least that can be detected. After the ECD appeared, zero as a limit became so low that to apply it in full would cause the rejection of nearly everythig that was edible. Even natural vegetation contains measurable levels of pesticides so sensitive is the device.

What was needed was common sense and the acceptance of the wisdom of the physician Paracelsus who said long ago "the poison is the dose." Even water is poisonous if too much is taken. Even the deadly nerve gases are harmless at the level of a picogram, easily detectable by an electron capture detector.

The next intervention of measurements with the ECD into Green politics was in the relatively clear cut problem of ozone depletion by halocarbons. So let me tell you something of my personal experiences in what has sometimes been called the ozone war during the past two decades.

In 1966 we purchased a holiday cottage in far western Ireland on the shores of Bantry Bay. It was sited on the slopes of a mountain called Hungry Hill. It was a small mountain of warm sandstone slabs that looked out over the broad Atlantic. Most of the time the air was clear and islands 50 miles away could be seen from the mountain top. Occasionally a dense haze filled the air and objects less than a mile away became invisible.

The haze looked and smelt to me just like the photochemical smog of Los Angeles, but how could it have reached this remote rural region?

Next summer much to annoyance of my wife and family, I brought a portable gas chromatograph on holiday with me. My objective was to measure the atmospheric concentration of trichlorofluoromethane (F11) before, during and after one of the outbreaks of hazy air. I chose to look for F11 rather than a typical smog chemical such as ozone or peroxyacetylnitrate (PAN) because the fluorocarbons alone are unequivocally human made. The other substances I knew would be regarded by my doubting colleagues as possible emissions from natural sources (4).

My idea was that if the haze was pollution, it would come from an urban industrial area, and in it there would be more of these CFCs than in clean Atlantic air. On the first few days of our holiday the air was sparkingly clear, and I was surprised to find a small but easily measurable quantity, 50 parts per trillion (ppt, 10^{-12}) of F11, in the air. A few days later, the wind shifted and an easterly drift of air blew from Europe. With it came the haze and the confirmation of my idea about the origin of the smog. In the hazy air there was 150 ppt of F11, three times as much as in the clear air. So the haze was man made. Later investigations showed it to be photochemical smog. It was rich in ozone and seemed to have come from southern France and Italy, having

drifted in the wind nearly 1000 miles carrying the exhaust fumes of the millions of cars of European holiday-makers.

There, this small investigation might have ended. I was curious though about the 50 ppt of CFC in the clean Atlantic air. Had it drifted across the Atlantic from America or more excitingly were CFCs accumulating in the Earth's atmosphere without any means for their removal? To find out, the only thing to do would be to travel by ship to the southern hemisphere and back and measure the CFCs as the ship travelled across the world.

The journey of the *Shackleton*

In 1971 I was a Visiting Professor at Reading University and with the help of Peter Fellgett, then Professor of Cybernetics, we applied for support from the Natural Environment Research Council (NERC) and Scientific and Engineering Research Council (SERC). We asked for a small grant to make measurements of dimethyl sulphide, methyl iodide and the CFCs aboard the *Shackleton*, which was due to make its voyage to Antarctica and back later the same year (4). The civil servants of both councils thought the research worthwhile, but the academic peer reviewers condemned the proposals as pointless. They even suggested that they were bogus on the grounds that there was no way to measure CFCs at the ppt concentrations. An advantage of doing research independently is that the approval of "apparatchiks" is not needed. The apparatus I used was so simple I was able to make it in a few days. It ran without failure throughout the six month voyage. The total cost of the research, including the apparatus, was a few hundred pounds. But the discoveries of the voyage were published in three *Nature* papers (2, 3, 8).

The *Shackleton* was a very small ship, so small that when I went to board it in November 1971, I had great difficulty in finding it at the docks at Barry, in south Wales. The mast and superstructure of the ship were below the edge of the dock and it was necesary to look over the side to see this small vessel on which I was to travel all the way to the southern hemisphere. Smallness is no disadvantage for ocean research. On a small ship one is closer to the sea and small ships are far less contaminated than today's floating palaces of ocean research. The least source of contamination on a ship makes measurement at the ppt concentrations difficult or impossible.

This journey of research revealed the global presence of the CFCs. In addition, I discovered the unexpected presence of other gases. These were methyl iodide, dimethyl sulphide and carbon disulphide. These it now seems may be as significant a discovery as was that of the CFCs. The major point is that this was small science, the kind of science that went on back in the 1950's. Big science would not have made or supported such an investigation. It was too speculative to justify the cost of a global scale expedition.

The CFC measurements made daily aboard the *Shackleton* provided the base data from which Sherwood Rowland and Mario Molina developed their theory of ozone depletion and warned that the accumulation of these gases in the atmosphere represented a hazard to future generations (4, 6, 9). In the early days of this research emotions were high and there was a sort of ozone war over the Rowland and Molina hypothesis. As is usual in war, truth was the first casualty. An example is in the 1976 report of the US National Academy of Sciencies on ozone depletion. Here it is stated that the F11 measurements of the *Shackleton* voyage were inaccurate. This conclusion did not come from an enquiry into the method of analysis by the panel. It came from the fact that the observations did not fit the predic-

tions of their theoretical models. I am glad to have this opportunity to state that, although made with simple apparatus, I now know that the *Shackleton* data were accurate to about 5%. It was the early and incomplete versions of the model that were wrong.

I was fortunate to have another sea voyage in the offing, this time aboard the German research ship *Meteor*, which sailed from Hamburg to Santo Domingo in the Caribbean and whose route took us through the Bermuda Triangle and across the Sargasso Sea. My stated intention on this expedition was to gather further information about the global distribution of the halocarbons. This I did, but much more interesting was the discovery that the abundance of the air pollutant peroxyacetylnitrate (PAN) increased steadily as we sailed into the remote regions of the Atlantic. I should mention that PAN is another important air pollutant to which the ECD is sensitive. There was no doubt that it was a natural product, since its abundance closely followed that of the sunshine, rising by day and vanishing by night. It was necessary to make measurements a long way from the ship, for it was a veritable floating city and consequently a prime source of atmospheric pollution. I did sometimes wonder if my complaints about the excessive use of aerosol dispensers by the sailors would give them cause to leave me behind.

As the years went by, so did the need increase for a comprehensive global monitoring of the CFCs. Our holiday cottage at Adrigole became the site of the first station of what was to become the Global Atmospheric Gas Experiment (GAGE) a global monitoring network. We showed that a conventional laboratory gas chromatograph equipped with an ECD could be used to monitor CFCs automatically. I used a Hewlett Packard model which ran without problems for a year. The instrument was looked after by our neighbour Mr. O'Sullivan. The success of this trial run in 1978 led to the establishment of a network of five monitoring stations in Barbados, Oregon, Samoa, Tasmania and Adrigole. These have successfully monitored the atmosphere ever since. From the results, the probable atmospheric lifetimes of the CFCs have been calculated (Table 1). Sadly, in the mid 1980s the Adrigole station was closed and measurements in Ireland transferred to the Irish Government Station in Galway.

What makes the ECD work?

This is not the place to discuss in detail the theory of the electron capture detector, but it is an opportunity to mention a few interesting theoretical aspects of the device which otherwise are rarely revealed. I find it helpful to think of the detector as a small reaction vessel holding a dilute suspension of the reagent chemical gaseous free electrons. It is interesting that the free electron, in thermal equilibrium with a gas ambient temperature, behaves as if it were a very large particle, larger even than most of the molecules it encounters. The apparent obesity of the electron is reflected by the de Broglie wavelength it possesses (7 nm at room temperature). This large cross section is a way of expressing the great rapidity of electron reactions and accounts for the sensitivity of the detector. Finally, the chemical reaction between electrons and molecules is second-order, and many of the problems of analysis arise from this fact. If the electron capture detector were insensitive and the number of molecules present was vastly greater than the number of electrons, the device would be splendidly linear and predictable in its response. Unfortunately, in the compounds it detects sensitively (those the analyst seeks to measure), the numbers of molecules in the detector are comparable with the number of electrons. In

TABLE 1. Greenhouse gases*

Gas	Pre-industrial concentrations ppm	1991 concentrations ppm	Warming contrib. %	Warming effectiveness	Human sources
Carbon dioxide	280	350	49	1	Combustion of fossil fuels coal, oil, and gas Deforestation and changing land use Biomass burning
Methane	0.7	1.7	18	25	Wetland agriculture. Enteric fermentation in cattle and termites. Leakages from gas and oil exploitation Biomass burning
CFCs	–	CFC–11: 0.0002	14	CFC–12: 10,000	Used in refrigeration, air conditioning, plastic foam, and as propellant, solvent sterilant
	–	CFC–12: 0.0004			
Nitrous oxide	0.28	0.31	6	150	Nitrogen-based fertilizers Fossil fuel combustion Biomass burning

* Recent research by Hansen and cols. at Goddard in the USA and by Pyle at Cambridge, UK, suggests that the CFCs cause a net greenhouse reduction. Also more UV in the troposphere means less methane.

such circumstances, as textbook physical chemistry would tell you, the response of the detector to varying sample size is unlikely to be either linear or easily predictable.

The saying "There are no bad instruments, only bad analysts" implies that however unusual the relationship between samples size and signal, careful calibration can always get results. But calibration with a gas at a concentration of a few parts per trillion is easier said than done. Inter calibration exercises by the US Bureau of Standards show that even among professional laboratories, the scatter of results reported for the stable and easily analysed fluorocarbons can be as great as 400%.

My personal solution to this problem was two fold. First I calculated from first principles the number of electrons that had reacted with fluorocarbon in the detector. This provided an absolute analysis and calibration was not needed. It required operating the detector in an unusual way. Something impossible with commercial gas chromatographs. I was fairly sure that this method would not be in error by more than 20%. As it turned out later, it was only 5% in error. At the time of the *Shackleton* measurements other scientists were skeptical of my coulometric analyses. So my second step was to move my home and laboratory to a remote country region close to the Atlantic Ocean. Here I converted a barn into a 50 m^3 exponencial dilution chamber. For the fluorocarbons, at least the analysis by electron capture detector is now tamed. Both calibration in the chamber and coulometry agree and with absolute accuracies of 5% and at a precision of 0.5%.

Lack of sensitivity did not use to be a complaint levelled at the electron capture detector. Once the possibilities of using electron-attaching compounds as tags or tracers were realized, that ultimate of molecular detection was the new destination.

From molecules to Gaia

We are still far from taking a grab sample of air or water and finding in it one molecule of tracer. The best we can now do is to detect between 10^5 and 10^6 molecules of tracer in a cubic centimeter of air. The use of certain fluorinated hydrocarbons as tracers is already becoming established as a method of following air masses across regions covering hundreds or even thousands of miles. It is obviously applicable to other tracing needs such as the movement of water masses in the oceans. It is now possible to detect directly one part in 10^{14}, an improvement made possible by signal processing using gas-switching techniques. Every year of this odyssey I have expected to find this simple device that anyone could make superseded by some impressive flight of high technology. Instead, its use seems to be expanding into new territories.

I have tried to show how the ECD influenced the development of the environmental movement and how this simple detector has taken me literally around the world in search of new information. Similar journeys in the mind, especially those using the opportunities provided by the NASA space program, enabled us for the first time to see the world from outside. This view led me to the idea, which the novelist William Golding suggested I should call Gaia, the goddess of the Earth, which has been my most useful contribution. The Gaia theory, which refers to the geophysiology science, explains the origin and evolution of a system in dynamic balance, a system made up of the surface planet Earth and the living beings. Until recently science was triumphantly reductionist and had succeeded in displacing the great religions as the source of knowledge about life and the cosmos. What reductionist science has not done is to offer moral guidance. The President of the Czech Republic, Vaclav Havel gave a speech when he received the Freedom Medal of the United States in 1994. He said that Gaia was part of a new science that did offer moral guidance. It gives us something to which we are accountable, the Earth itself. Or to use Havel's own words "We are not here for ourselves alone."

References

1. Carson, R. (1962). Silent Spring. Houghton Mifflin Co., Boston, 368 pp.
2. Charlson, R. J., Lovelock, J. E., Andreae, M. O., Warre, S. G. (1987). Ocean phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature **326**, 655–661.
3. Lovelock, J. E. (1965). A physical basis for life detection experiments. Nature **207**, 568–569.
4. Lovelock, J. E. (1979). The independent practice of science. New Scientist, Sept., pp. 714–717.
5. Lovelock, J. E. (1982). From gas chromatography to Gaia. Chromatographia **16**, 26–31.
6. Lovelock, J. E. (1988). The Ages of Gaia: A Biography of Our Living Earth. W. W. Norton & Co. New York, 252 pp.
7. Lovelock, J. E., Hitchcock, D. R. (1967). Detecting planetary life from Earth. Science Journal, Abril, pp. 2–4.
8. Lovelock, J. E., Maggs, R. J., Rasmussen, R. A. (1972). Atmospheric dimethyl sulphide and the natural sulphur cycle. Nature **237**, 452–453.
9. Rowland, S., Molina, M. (1974). Stratospheric sink for chlorofluoromethanes: chlorine atom-catalysed destruction of ozone. Nature **249**, 810–812.

MICROBIOLOGÍA SEM **13** (1997), 23–36 **MINIREVIEW**

Chemical signals in Gram-positive bacteria: the sex-pheromone system in *Enterococcus faecalis*

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Summary

This review summarizes relevant aspects of the sex-pheromone system of *Enterococcus faecalis,* a novel form of bacterial conjugation that plays a major role in the horizontal dissemination of genes. The process is initiated by a chemical signal, the sex pheromones, and includes several stages of interaction between the donor cell and the recipient cell. Most work in this area has focused on three plasmids, the haemolysin-bacteriocin plasmid pAD1, the bacteriocin plasmid pPD1 and the Tec^r (Tn*125*) plasmid pCF10. These plasmids share many molecular and genetic features but exhibit some interesting differences at the regulatory level. Preliminary studies suggest that many of the major components of this system may also play a role in host-parasite interactions involving enterococci.

Key words: *Enterococcus faecalis*, conjugative plasmids, aggregation and exclusion substances, plasmid and DNA transfer, sex pheromones

Resumen

En esta revisión se describen los aspectos más destacados del sistema de feromonas sexuales en *Enterococcus faecalis*, una nueva forma de conjugación bacteriana que desempeña un importante

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papel en la diseminación horizontal de genes. El proceso, que incluye diferentes estadíos de interacción entre las células donadoras y receptoras, se inicia por la presencia de las feromonas sexuales que actúan a modo de señal química. La elucidación de los mecanismos moleculares y genéticos ha demostrado la existencia de grandes homologías en los tres sistemas plasmídicos mejor estudiados: los plásmidos pAD1 (bacteriocina/hemolisina), pPD1 (productor de bacteriocina) y pCF10 (que porta un gen de resistencia a la tetraciclina en el transposón Tn*125*); sin embargo, esos plásmidos presentan interesantes diferencias en cuanto a la regulación. Estudios preliminares sugieren que algún componente de este mecanismo de regulación puede ser también importante en las interacciones de los enterococos con el hospedador.

Introduction

Until the 1970s, it was generally accepted that the conjugative transfer of DNA was an exclusive capability of Gram-negative bacteria and Actinomycetes. This trait, nonetheless, has been found also in Gram-positive bacteria. The first evidence of a conjugative system found in *Enterococcus faecalis* was further confirmed in other bacterial genera such as *Bacillus, Streptococcus, Lactococcus* and *Staphylococcus.*

Although functionally equivalent to F-mediated DNA transfer in *Escherichia coli*, the molecular and genetic aspects involved in conjugation of Gram-positive bacteria are diverse and markedly different from those of Gram-negatives. The best studied conjugation system in Gram-positive bacteria is that of *E. faecalis*. Molecular studies carried out on certain plasmids of this bacterium revealed the existence of a sex-pheromone mediated conjugative transfer system. Sex pheromones are small, plasmid-specific, hydrophobic peptides produced by cells lacking the corresponding plasmid. The system represents a unique strategy for plasmid acquisition.

Genetic traits of enterococci

Enterococci, like other bacteria, posess a wide variety of elements that facilitate both intra- and interspecies transfer of genetic information, including plasmids (coding for traits such as hemolysins, bacteriocins and antibiotic resistance) and transposons.

According to their preferential location in the intestinal tract and abundance of extrachromosomal elements, enterococci can be considered reservoirs of genetic information available to other intestinal bacteria. The increase of enterococcal infections during the last years, the growing incidence of antibiotic resistance, and the production of bacteriocins and/or hemolysins are leading issues in promoting research on the mechanisms and factors involved in colonization and survival of these bacteria. Enterococcal plasmids have been best characterized in strains of *E. faecalis*, both because of their large plasmid content and because some of them are conjugative.

Although conjugative transfer of chloramphenicol resistance was reported in 1964, the first indication on the role of plasmid DNA came in 1972 (10). Soon after, in 1973, the conjugative determinant of hemolysin production was identified. However, the final demonstration of the existence of a conjugative process in this bacterium came from Jacob and Hobbs in 1974 (30). In the following years, 47 new plasmids were described in *E. faecalis*, of which 29 were conjugative (including several broad host-range elements). At present, the main known types of plasmids in *E. faecalis* are: (a) small, cryptic;

(b) "normal", large, conjugative; and (c) "sexual", pheromone-responding, plasmids.

Conjugative plasmids. The so-called "sexual" plasmids (pheromone-responding), show several distinctive characteristics which make them very different from the usual or "normal" conjugative plasmids of Gram-positive bacteria. They were first described in *E. faecalis* (8).

The two types of conjugative plasmids show different characteristics: (i) "Normal" conjugative plasmids usually carry antibiotic resistance traits, which are much less frequent in "sexual" plasmids. (ii) "Sexual" plasmids induce a prolonged, cell-to-cell contact, whereas the "normal" conjugative plasmids benefit from that activity increasing their frequency of transference, when both plasmids coexist in the same cell. (iii) "Normal" conjugative plasmids have broad host-range, whereas pheromone-responding plasmids are restricted to *E. faecalis*, which is the only known bacterium in which a sex pheromone system has been clearly established. (iv) "Sexual" plasmids can be transferred in liquid media (broth mating), whereas "normal" conjugative plasmids require a solid support (filter mating).

TABLE 1. Characteristics of the pheromone-responding plasmids described in *Enterococcus faecalis*

Plasmid	Size (kb)	Original host	Phenotype*	Pheromone	References
pAD1	58	DS16	Hly/Bac	cAD1	58
pAMγ1	58	DS5	Hly/Bac	cAD1	9
pJH2	58	JH1	Hly/Bac	cAD1	29
pBEM10	71	HH2	Pn^r, Gm^r, Km^r, Tm^r	cAD1	41
pX98	60	–	Hly/Bac	cAD1	31
pPD1	58.9	39-5	Bac	cPD1	64
pMB2	68	S-48	Bac	cPD1	36, 52
pCF10	65	SF-7	Tc^r (Tn*925*)	cCF10	12
pMB1.1	52	T1-4	Bac	cCF10	37, 51, 52
pOB1	68	5952	Hly/Bac	cOB1	47
pYL1	57.5	–	Hly/Bac	cOB1	28
pAM373	37	RC73	–	cAM373	6
pAMγ2	54	DS5	Bac	cAMγ2	9
pAMγ3	54	DS5	–	cAMγ3	9
pAM323	63	HH22	Em^r	cAM323	41
pAM324	56	HH22	–	cAM324	41
pIP1017	58	–	Km^r, Nm^r, Sm^r	–	27
pIP1141	57	–	–	–	49
pIP1438	57	–	Cm^r, Em^r	–	49
pIP1440	91	–	Tc^r,Nm^r, Sm^r	–	49
pIP964	58	–	Hly/Bac	–	3
pMV120	49	–	–	–	4
pHKK100	–	–	–	–	24

* Abbreviations: Hly = hemolysin; Bac = bacteriocin. Cm = chloramphenicol; Em = erythromycin; Gm = gentamycin; Km = kanamycin; Nm = neomycin; Pn = penicillin; Sm = streptomycin; Tc = tetracycline; Tm = tobramycin.

Besides, three new plasmid groups have been described recently, in which transfer frequency is favoured by an aggregation reaction. These are found in *Bacillus thuringiensis* (1), *Lactobacillus plantarum* (53) and *Lactococcus lactis* (59), the latter being phenotypically more similar to *E. faecalis*.

Pheromone-responding plasmids. Up to date, 23 pheromone-responding plasmids have been described (Table 1). There does not seem to be a common phenotype: six of the plasmids carry antibiotic-resistance traits, eleven code for bacteriocins and/or hemolysins, and six remain cryptic.

The sex-pheromone mating system

According to the definition of pheromones proposed by Karlson and Luscher (33) ("substances excreted to the outside of an individual and received by a second individual of the same species in which they release a specific reaction"), several of the peptides secreted by strains of *E. faecalis* can be considered as such. If, indeed, the exchange of genetic material induced by these peptides is considered to be sexual behavior, then they can be called "sex pheromones".

A plasmid-free strain will secrete several (at least, up to five) small, linear peptides of low molecular weight called sex pheromones (9, 18), which are specific for the corresponding conjugative plasmids. When a recipient strain receives a conjugative plasmid, production of the corresponding sex pheromone is arrested, whereas secretion of the remaining pheromones continues.

Cells harboring a conjugative plasmid secrete a linear peptide that behaves as a specific competitive inhibitor of the corresponding pheromone. The inhibitor can prevent induction of clumping by low levels of exogenous pheromone (too low as to yield a productive mating), by small amounts of endogenous pheromone that escape negative control, or by other pheromones that might give cross reaction.

Up to now, five different sex pheromones and four specific inhibitors (Table 2) have been isolated and characterized (38, 39, 40, 42, 44, 52, 56). All are hydrophobic hepta- or octapeptides with a hydroxy terminus that can serve as a modification site for inactivation of endogenous pheromone when the corresponding plasmid is present.

According to the nomenclature proposed by Dunny et al. (18), the different sex pheromones and their inhibitors are designed with the letter "c" (clumping) or "i" (inhibitor), respectively, followed by the name of the corresponding plasmid. For example, cAD1 and iAD1 are the respective pheromone and inhibitor of plasmid pAD1.

It has been postulated that the structural genes responsible for pheromone production are located in the chromosome, because plasmid-free strains do produce sex pheromones. However, no direct evidence has been provided yet.

Main features of the aggregation process

Shortly after induction by the specific sex pheromone (45–60 min), the cells synthesize two surface proteins: the adhesin or aggregation substance (AS), and the surface exclusion protein (ES). The former is responsible for the formation of cell aggregates by attachment to the complementary receptor or binding substance (BS). The prolonged and close cell-to-cell contact between donors and recipients facilitates the conjugative transfer of plasmid DNA. The formation of cell aggregates requires phosphate ions and divalent cations (e.g., Mg^{2+}) (64). The exclusion substance (ES), which is also induced by sex pheromones, avoids DNA transfer between strains carrying homologous plasmids.

TABLE 2. Amino acid sequences of the different sex pheromones and inhibitors described in *Enterococcus faecalis*

Agent*	Sequence†
cCF10	H-Leu-Val-Thr-Leu-Val-Phe-Val-OH
cPD1	H-Phe-Leu-Val-Met-Phe-Leu-Ser-Gly-OH
cAD1	H-Leu-Phe-Ser-Leu-Val-Leu-Ala-Gly-OH
cAM373	H-Ala-Ile-Phe-Ile-Leu-Ala-Ser-OH
cOB1	H-Val-Ala-Val-Leu-Val-Leu-Gly-Ala-OH
iCF10	H-Ala-Ile-Thr-Leu-Ile-Phe-Ile-OH
iPD1	H-Ala-Leu-Ile-Leu-Thr-Leu-Val-Ser-OH
iAD1	H-Leu-Phe-Val-Val-Thr-Leu-Val-Gly-OH
iAM373	H-Ser-Ile-Phe-Thr-Leu-Val-Ala-OH

* See text for explanation of the name of the agent (*c* or *i*).
† The conserved residues of the pheromones and the corresponding inhibitors are underlined.

Cells induced to form aggregates show a fibrillar matrix on their surface, which can be observed under the electron microscope (26, 60, 63). The fibrils extend about 1 nm from the cell surface, at a density of about 1000 fibrils/μm^2.

The distribution and detailed structure of the surface proteins Asc10 and Sec10 (induced by cAD1 pheromone) in four isogenic strains carrying the plasmid pCF10 have been studied by field emission electron microscopy (48). When both proteins are present in the same cell, they are difficult to distinguish because of their intimate association and lack of a specific distribution. However, it seems that Sec10 is responsible for the fibrillar matrix appearing on the cell surface, while Asc10, which is a globular protein, appears as an amorphous material within the Sec10 matrix. At stationary phase, both proteins are distributed homogeneously through the cell surface, but shortly after induction they appear at the old regions of the cell wall in growing cells. Aggregation between donors and recipients also requires a binding substance (BS), which is present on the surface of recipient cells and to which AS binds (18). Recently it has been shown that the aggregation event also requires lipoteichoic acids (LTA) (2). Moreover, addition of low concentrations of exogenous LTA to cells induced to form aggregates is sufficient to inhibit cell clumping, and it has been postulated that the binding substance is LTA (20), known as Lancefield D antigen.

Plasmid transfer. When donor cells are incubated with sex pheromone for 60 min, and then mixed with recipient cells for a short time (10–15 min), the mating frequency increases by 3 to 6 orders of magnitude with respect to uninduced control cells.

Pheromone-mediated plasmid transfer involves several stages (16), as observed for conjugative transfer of pCF10 (Fig. 1):

(i) The recipient cell excretes several small hydrophobic peptides into the growth medium, including the pheromone cCF10.

(ii) The pheromone binds to a receptor on the donor cell and it is internalized via an oligopeptide permease system (43). This triggers new functions in donor cells, such as the synthesis of the aggregation substance (AS) and exclusion surface (ES).

(iii) Binding of AS to binding substance (BS) on the recipient cell promotes close contact that

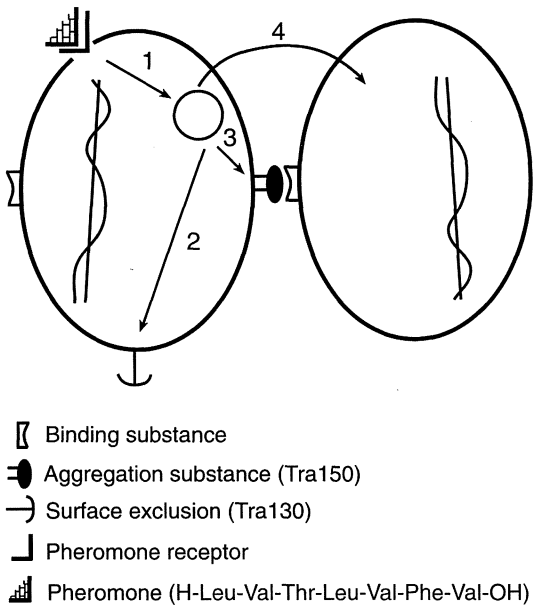

FIG. 1. Model for pheromone induction and transfer as inferred from Dunny (16). Binding of the sex pheromone to its receptor induces a transmembrane signal -1-, that triggers several plasmid-transfer functions such as surface-exclusion -2-, cell-aggregation -3- and DNA transfer -4-. See text for details. (Reproduced with permission of *Molecular Microbiology*.)

facilitates subsequent transfer of plasmid DNA from donor to recipient.

Genetic analysis of several pheromone-responding plasmids

• Plasmid pAD1

This plasmid was initially found in *E. faecalis* DS16, a strain isolated from clinical samples because of its multiple antibiotic resistance (58). It is a large 58 kb plasmid, about half of which encodes for functions related to conjugation; it is inducible by the sex pheromone cAD1 (5, 7, 50), such as the aggregation substance Asa1, and the surface exclusion protein Sea1, as well as genes involved in stabilization of mating aggregates (region G) and DNA transfer (region H).

The aggregation substance Asa1 is a 142 kDa protein with eleven putative glycosilation sites (Asn-Xaa or Asn-Xaa-Thr) within the N-terminal region, which also contains a 43-residue signal peptide (22, 23). The C-terminal portion contains a proline-rich cell-wall-spanning region, followed by a hydrophobic membrane anchor. These regions have been identified according to structural (but not functional) similarity with many cell surface proteins of Gram-positive bacteria (M protein of *Streptococcus pyogenes* or staphylococcal protein A).

The cell surface exclusion protein Sea1 also has a leader peptide (which is absent in the mature protein) and a region that is typical of membrane-anchored proteins. There is also some degree of homology with fragments of the streptococcal M protein and some eukaryotic structural proteins such as myosin and laminin (61). The function of this protein is not known; its extracytoplasmic region might serve to block the formation of the molecular bridges necessary for DNA transfer in cell aggregates.

Regulation of pAD1 response. Besides the structural genes described above, two regulatory sequences have been identified by mutagenesis:

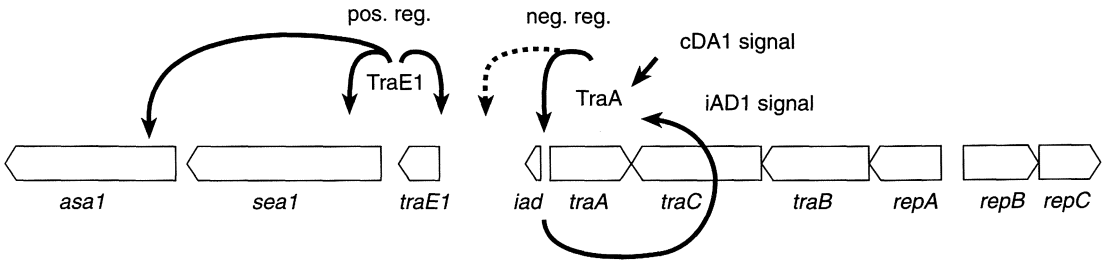

FIG. 2. Map of the pAD1 conjugative region, indicating the structural and regulatory genes involved in pheromone response and a model for its regulation. Abbreviations: pos.reg., positive regulation; neg.reg., negative regulation. (Adapted from Clewell [5], with the permission of *Cell.*)

the transcription positive control *traE1* (able to act in *trans*) and the negative regulator *traA*.

From the studies carried out on gene expression, transcript analysis and transcription initiation points (5, 50, 57), the following model on the regulation of pAD1 and cAD1 has been proposed (Fig. 2):

In the absence of pheromone (presence of extracelular iAD1), TraA only binds between *iad* and *traA,* and plays a major role in the termination of transcription (negative regulator on *iad*). The presence of exogenous pheromone causes the release of the negative effect of TraA on the *iad* promoter and allows increased transcription and read-through into *traE1*. Once the TraE1 product is made, it positively self-regulates at the *traE1* promotor as well as the downstream promoters of *sea1* and *asa1*. This increases the expression of Sea1 protein and allows the start of de novo synthesis of Asa1.

The gene *traC,* located upstream to *traA,* has also been identified (57). It product is a 61 kDa protein (TraC), highly homologous to the oligopeptide-binding proteins of *Escherichia coli, Salmonella typhimurium* and *Bacillus subtilis,* and may be the receptor (or binding protein) for the sex pheromone cAD1. This possibility agrees with its location at the cell surface.

The nucleotide sequence of *traB* predicts a 44 kDa protein (TraB) involved in the shutdown of endogenous cAD1. Adjacent to *traB* there are several open reading frames and a series of direct repeats, which contains the origin of transfer (*oriT*). There is also evidence that this region is involved in plasmid replication and maintenance (5).

• Plasmid pCF10

The 58 kb plasmid pCF10 carries the Tn*125* transposon that encodes tetracycline resistance, and responds to the heptapeptide sex pheromone cCF10 (12). The structural and regulatory genes involved in pheromone response are located in a region of 25 to 30 kb (11, 25, 54).

In this plasmid, the pheromone response genes are known as *prg*. The structural genes *prgA* and *prgB* code for the two cell surface proteins participating in the formation of mating aggregates: Sec10, of 95 kDa (earlier Tra130), and Asc10, of 142 kDa (earlier, Tra150) (32). Finally, *prgQ* codes for the specific inhibitor, iCF10 (45).

Regulation of pCF10 response. Regulation of the transfer fuctions in pCF10 (Fig. 3) appears to be controlled by both positive and negative regulatory circuits, and it seems more complex than pAD1 as there seems to be a *cis*-acting, orientation-dependent positive control (13, 14, 15, 17). There is also a negative control region

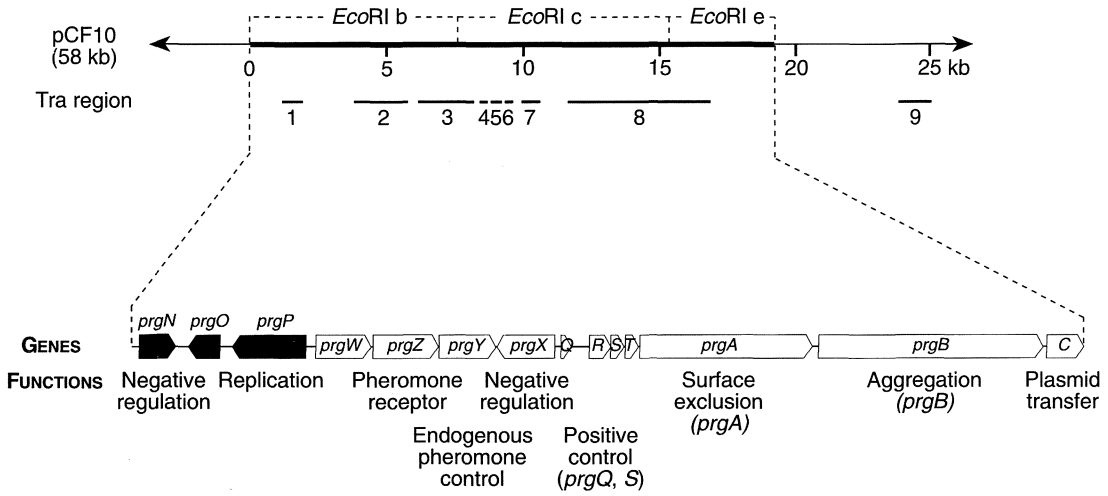

FIG. 3. Physical and genetic maps of the Tra region, encoding pheromone response in the plasmid pCF10. The maps indicate the approximate locations and functions of regulatory and structural genes. (Adapted from Herdberg et al.[25] and Ruhfel et al. [54].)

located in the *Eco*RI fragment b, which is responsible for repression of the expression of Asc10.

Transcription analysis of *prgA* and *prgB* has shown that these genes are transcribed independently. Transcription of *prgB* in cells harbouring pCF10 is inducible (14), while there is a basal expression of *prgA* (and therefore production of Sec10) in wild-type donors in the absence of pheromone. However, production of this protein clearly increases after induction by the specific pheromone (19).

To establish the role of the different genes in activation of *prgB*, the regulatory region has been studied in detail (13). Results indicate that expression of *prgB* is controlled positively by *prgQ* and *prgS*, while other adjacent genes (*prgX*, *prgR*, *prgT*) play no role in positive control.

The gene *prgQ* is essential for expression of *prgA*. Complementation analyses have shown that there is a *cis*-acting regulatory element in the *prgQ* region, and also that at the 3'-end of *prgQ* there are sequences which are essential for positive control but are not translated. Their products are RNA molecules, which are transcribed starting from the promoter of *prgQ* and continuing past the stop codon corresponding to the inhibitor iCF10 (which is also coded by *prgQ*) (15).

Finally, the binding protein PrgZ has been characterized (35). It is similar in sequence to binding proteins (OppA) encoded by oligopeptide permease (*opp*) operons, whose inactivation abolish the response at physiological concentrations of pheromone. The data suggest that processing of the pheromone signal involves recruitment of a chromosomal Opp system by PrgZ, and that signaling occurs by direct interaction of internalized pheromone with intracellular effectors.

Recently, a model for negative regulation of the *prgB* gene has been presented (25), suggesting that the *prgN*, *prgY* and *prgX* genes are involved in negative controls and function as an autonomous replicon. Their data suggest that *prgN* and *prgX* function as negative regulators that may prevent the positive regulatory mechanism from activating *prgB* at wild type levels. Moreover, disruption of *prgY* led to constitutive expresion of *prgB* indicating that PrgY does

function in negative control. Probably, the role for PrgY is to prevent self-induction of donor cells by endogenously produced pheromone, acting in concert with the peptide pheromone inhibitor molecule, iCF10. Likewise, and based on previous studies and on sequence comparisons, *prgW* may be a multifunctional gene, perhaps being involved in the regulation of the expression of *prgZ* and *prgY* as well as being the pCF10 replication initiator. By comparison to the pAD1 system, *prgO* and *prgP* appears to be replication genes. Because constructs with deletion in the *prgO, prgP* and *prgW* genes were not stable in *E. faecalis*, the exact role of these genes is still not fully understood.

According to these results, the following model of cCF10 signaling during pCF10 conjugation has been proposed (35): recipient-secreted pheromone (cCF10) interacts with either the plasmid-encoded specific binding protein PrgZ or the chromosomal binding protein OppA on the donor cell. Pheromone cCF10 is then transported into the cell via the chromosomally encoded oligopeptide permease system. Once inside the cell, the pheromone interacts with intracellular effector molecules, probably displacing iCF10 from the ribonucleoprotein complex and leading to activation of *prgB* and the subsequent conjugative transfer of the plasmid recipients.

• Plasmid pPD1

This plasmid (59 kb) was present in strain 39-5, which was isolated from a subgingival wound and chosen because it produced a bacteriocin (63). Genetic analyses of this plasmid have been carried out to provide comparative data for the regulatory and structural genes known at this moment (21):

– The *asp1* (aggregation substance of pPD1) gene and its product Asp1 show 90% homology with Asc10, and somewhat less with Asa1 (AS of pCF10 and pAD1, respectively). It shows all the features previously reported for aggregation substances: a leader peptide, a cell-wall region, a membrane domain and the conserved amino acid motifs RGD (Arg-Gly-Asp), regions known as recognition sequence for eukaryotic cell receptors (55).

– The gene *sep1* responsible for the surface exclusion protein ORF10p (*sep1*), is located in an *Eco*RI fragment A. It shows a strong homology (91%) with the equivalent genes *sea1* and *prgA* found in the other two plasmid systems.

The physical map of pPD1 has been also determined recently (21) by using a relational clone approach; transposon analysis with Tn*917* and Tn*916* facilitated the location of the bacteriocin-related genes in a segment of about 6.7 kb.

Sequence analysis of the conjugative region (8 kb) showed a number of open reading frames

TABLE 3. Comparison of the pheromone response regions of plasmids pAD1, pPD1 and pCF10 of *Enterococcus faecalis*

Function	Genetic determinants in plasmids		
	pAD1	pPD1	pCF10
Aggregation substance (AS)	*asa1*	*asp1*	*prgB*
Exclusion substance (ES)	*sea1*	ORF10p (*sep1*)	*prgA*
Pheromone inhibitor	*iad*	ORF6 (*ipd*)	*prgQ*
Negative regulatory proteins	*traA*	ORF5	*prgX*
Shutdown of endogenous pheromone	*traB*	ORF4	*prgY*
Pheromone receptor	*traC*	ORF3	*prgZ*
Replication	*repA*	ORF2	*prgW*

(ORF), some of which were named on the basis of homologies with the other pheromone-responding plasmids, pAD1 and pCF10, as indicated in Table 3. Those open reading frames were the following:

– ORF3, designated *traC*, encodes a protein of 60.7 kDa (TraC) that shows a similarity higher than 70 and 87% with TraC and PrgZ of pAD1 and pCF10, respectively. However, Tn*917* insertions in this gene originate a constitutive aggregation phenotype that does not occur in the other plasmid systems. The putative TraC product has a strong similarity to oligopeptide-binding proteins found in other bacterial species and also with the pheromone-binding proteins of pCF10 and pAD1.

– ORF4, designated *traB*, encodes a protein of 43.5 kDa named TraB. This protein has more than 46 and 77% similarity with proteins TraB of pAD1 and PrgY of pCF10, respectively, both of which are involved in shutdown of endogenous pheromone (46).

– ORF2, designated *repA*, was deduced to encode a protein with a molecular mass of 38.6 kDa, with extensive homology with the *prgW*-encoded protein of pCF10 (95.5% identical residues) and significant homology with the *repA*-encoded protein of pAD1 (53.7% identical residues).

– ORF1, designated *repB*, shows 34.1% homology with the first 164 amino residues of the *repB* of pAD1.

– ORF5, designated *traA*, encodes 321 residues (molecular mass of 37.7 kDa), that exhibit significant homology with TraA (36.6%) of pAD1 and PrgX (21%) of pCF10, which are negative regulatory proteins.

– ORF6 was designated *ipd* (competitive inhibitor iPD). It encodes 21 amino acids (TraC) and is located to the right of *traA*.

Finally, a new gene product (TraF) has been found recently, whose interruption does not affect cPD1-induced aggregation, but does reduce the transfer frequency of pPD1 to 2% of the wild-type level (43).

Ecological significance of the sex pheromone system in Gram-positive bacteria

Several conclusions can be drawn on the evolutive and ecological role of the sex pheromone system that has evolved and prevailed in certain strains of *E. faecalis*.

First, let us consider the mode of action of the sex pheromones: small hydrophobic peptides released into the medium, acting on different cells as external signals able to activate silent genes. This represents a sophisticated system that reminds us of the communication language between cells of higher organisms. It has been suggested that sex pheromones can be compared to the competence factors involved in transformation processes of species like *Streptococcus pneumoniae* and *S. sanguis,* as well as to opines secreted by *Agrobacterium*-induced plant tumors, which are able to induce transfer of conjugative Ti plasmids from donors to plasmid-free bacteria.

Second, sex pheromones induce coordinated expression of genes coding for cell surface proteins as well as plasmid DNA transfer between donor and recipient cells. The cell surface proteins are highly conserved in all the plasmid systems studied so far. This seems logical if we consider that the main function of these proteins is adhesion, either to other bacteria during the formation of mating aggregates, or to eukaryotic cells. The latter also contribute to the virulence of enterococci, mostly because aggregation substances can be induced by serum components (62). This would allow the cells to sense if they are living in an eukaryotic environment, in which adhesin synthesis would facilitate adhesion and colonization.

Finally, sex pheromones (particularly

cAM373 and cPD1) are also potent chemotactic agents at submicromolar concentrations, as well as inducers of secretion of lysosomal granules in neutrophyls, although they are not formylated peptides. The reported requirement of a formylmethionine group by neutrophyl receptors seems therefore to be restricted to oligopeptides (di- or tripeptides) but not to larger molecules such as sex pheromones (34).

The aggregation substances are highly conserved and show two well-defined domains: a C-terminal portion that contacts the cell wall and anchors at the cytoplasmic membrane, and the N-terminal region acting as a binding domain for bacterial or eukaryotic cells. The aggregation substances described up to date contain two conserved domains, RGDS and RGDV, at positions 600 and 930, respectively. These domains have been described as recognition sequences for integrins, a family of eukaryotic cell receptors which sense environmental signals and are connected to cytosqueleton proteins (55). It would be of interest to demonstrate that some bacteria can bind to integrins via RGD domains of surface proteins. In fact, cells of *E. faecalis* harboring the plasmid pAD1 can adhere to cultured pig kidney tubular cells to a greater extent than do plasmid-free cells (34). This suggests that RGD sequences of the aggregation substance could serve as binding signals, and hence as virulence factors.

Interactions with recipient bacteria are mediated by the lipoteichoic acids (binding substance), which show a particular and specific structure in enterococci (20). This ensures binding to the correct partner.

Accordingly, the variability of the sex pheromone plasmid system depends on the composition of the five sex pheromones described until now and on the individual positive or negative regulatory systems of each plasmid, but not on the cell surface proteins, which are common.

In this way, the data available on the conjugative systems of Gram-positive bacteria allow a better understanding of the intraspecific genetic transfer:

– First, several plasmid families can be established (pAD1, pCF10, pPD1, pOB1 and pAM373), of which the first three are the best analyzed genetically. The different systems studied show a high degree of homology (higher than 90%) regarding the surface proteins (AS and ES). Nevertheless, they respond to different hydrophobic peptides (sex pheromones) and show particular regulatory mechanisms.

– It is possible that the gene clusters involved in pheromone response (between 25 and 30 kb) may transfer at high frequency and irrespectively of the plasmid (usually of large size, above 56 kb), as suggested by the fact that they are found in different plasmids.

– Intraspecific transfer of these gene clusters would allow a rapid mobilization of other genetic determinants coding for phenotypic characters of ecological advantage, such as antibiotic resistance or the production of hemolysins or antimicrobial substances such as bacteriocins or peptide antibiotics.

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References

1. Andrup, L., Damgaard, J., Wassermann, K. (1993). Mobilization of small plasmids in *Bacillus thuringiensis* subsp. *israelensis* is accompanied by specific aggregation. J. Bacteriol. **175**, 6530–6536.

2. Bensing, B. A., Dunny, G. M. (1993). Cloning and molecular analysis of genes affecting expression of binding substance, the recipient-encoded receptor(s) mediating mating aggregate formation in *Enterococcus faecalis*. J. Bacteriol. **175**, 7421–7429.
3. Borderon, E., Bieth, G., Horodniceanu, T. (1982). Genetic and physical studies of *Streptococcus faecalis* hemolysin plasmids. FEMS Microbiol. Lett. **14**, 51–55.
4. Burdett, V. (1980). Identification of tetracycline resistant R-plasmid in *Streptococcus agalactiae* (Group B). Antimicrob. Agents Chemother. **18**, 753–760.
5. Clewell, D. B. (1993). Bacterial sex pheromone-induced plasmid transfer. Cell **73**, 9–12.
6. Clewell D. B., An, F. Y., White, B. A., Gawron-Burke, C. (1985). *Streptococcus faecalis* sex pheromone (cAM373) also produced by *Staphylococcus aureus* and identification of a conjugative transposon (Tn*918*). J. Bacteriol. **162**, 1212–1220.
7. Clewell, D. B., Pontius, L. T., An, F. Y., Ike, Y., Suzuki, A., Nakayama, J. (1990). Nucleotide sequence of the sex pheromone inhibitor (iAD1) determinant of *Enterococcus faecalis* conjugative plasmid pAD1. Plasmid **24**, 156–161.
8. Clewell, D. B., Weaver K. E. (1989). Sex-pheromones and plasmid transfer in *Enterococcus faecalis*. Plasmid **21**, 175–184.
9. Clewell, D. B., Yagi, Y., Ike, Y., Craig, R. A., Brown, B. L., An, F. Y. (1982). Sex pheromones in *Streptococcus faecalis*: multiple pheromone systems in strain DS5, similarities of pAD1 and pAMγ1, and mutants of pAD1 altered of conjugative properties. *In* Schlessinger, D. (ed.), "Microbiology-1982", pp. 97–100. American Society for Microbiology, Washington.
10. Courvalin, P. M., Carlier, C., Chabbert, Y. A. (1972). Plasmid-linked tetracycline and erythromycin resistance in Group D "Streptococcus". Ann. Inst. Pasteur **123**, 755–759.
11. Christie, P. J., Dunny, G. M. (1986). Identification of regions of the *Streptococcus faecalis* plasmid pCF10 that encode antibiotic resistance and pheromone response functions. Plasmid **15**, 230–241.
12. Christie, P. J. R., Korman, R., Zahler, R. Z., Adsit, J. C., Dunny, G. M. (1987). Two conjugation systems associated with *Streptococcus faecalis* plasmid pCF10: of a conjugative transposon that transfers between *S. faecalis* and *Bacillus subtilis*. J. Bacteriol. **169**, 2529–2536.
13. Chung, J. W., Bensing, B. A., Dunny, G. M. (1995). Genetic analysis of the *Enterococcus faecalis* plasmid pCF10 involved in positive regulation of conjugative transfer functions. J. Bacteriol. **177**, 2107–2117.
14. Chung, J. W., Dunny, G. M. (1992). *Cis*-acting, orientating-dependent, positive control system activates pheromone-inducible conjugation functions at distances greater than 10 kilobases upstream from its target *Enterococcus faecalis*. Proc. Natl. Acad. Sci. USA **89**, 9020–9024.
15. Chung, J. W., Dunny, G. M. (1995). Transcriptional analysis of a region of the *Enterococcus faecalis* plasmid pCF10 involved in positive regulation of conjugative transfer functions. J. Bacteriol. **177**, 2118–2124.
16. Dunny, G. M. (1990). Genetic functions and cell-cell interactions in the pheromone-inducible plasmid transfer system of *Enterococcus faecalis*. Mol. Microbiol. **4**, 689–696.
17. Dunny, G. M., Leonard, B. A. B., Hedberg, P. J. (1995). Pheromone-inducible conjugation in *Enterococcus faecalis*: interbacterial and host-parasite chemical communication. J. Bacteriol. **177**, 871–876.
18. Dunny, G. M., Craig, R. A., Carron, R. L., Clewell, D. B. (1979). Plasmid transfer in *Streptococcus faecalis*: production of multiple sex pheromones by recipients. Plasmid **2**, 454–465.
19. Dunny, G. M., Zimmerman, D. L., Tortorello, M. L. (1985). Induction of surface exclusion by *Streptococcus faecalis* sex pheromones: use of monoclonal antibodies to identify an inducible surface antigen involved in the exclusion process. Proc. Natl. Acad. Sci. USA **82**, 8582–8586.
20. Ehrenfeld, E. E., Kessler, R. E., Clewell, D. B. (1986). Identification of pheromone-induced surface proteins in *Streptococcus faecalis* and evidence of a role for lipoteichoic acid in formation of mating aggregates. J. Bacteriol. **168**, 6–12.
21. Fujimoto, S., Tomita, H., Wakamatsu, E., Tanimoto, K., Ike, Y. (1995). Physical mapping of the conjugation bacteriocin plasmid pPD1 of *Enterococcus faecalis* and identification of the determinant related to the pheromone response. J. Bacteriol. **177**, 5574–5581.
22. Galli, D., Wirth, R. (1991). Comparative analysis of *Enterococcus faecalis* sex pheromone plasmid identifies a single homologous DNA region which codes for aggregation substance. J. Bacteriol. **173**, 3029–3033.
23. Galli, D., Wirth, R. Wanner G. (1989). Identification of the aggregation substance of *Enterococcus faecalis* cells after induction by sex pheromones. Arch. Microbiol. **151**, 486–490.
24. Handwerger, S., Pucci, M. J., Kolokhatis, A.

(1990). Vancomycin resistance is encoded on a pheromone response plasmid in *Enterococcus faecium* 228. Antimicrob. Agent Chemother. **34**, 358–360.

25. Herdberg, P. J., Leonard, E. A. B., Ruhfel, R. E., Dunny, G. M. (1996). Identification and characterization of the genes of *Enterococcus faecalis* plasmid pCF10 involved in replication and in negative control of pheromone-inducible conjugation. Plasmid **35**, 46–57.
26. Hirt, H., Wanner, G., Galli, D., Wirth, R. (1993). Biochemical, immunological and ultrastructural characterization of aggregation substances encoded by *Enterococcus faecalis* sex-pheromone plasmids. Eur. J. Biochem. **211**, 711–716.
27. Horodniceanu, T., Buu-Hoi A., LeBouguenec, C., Bieth, G. (1982). Narrow host range of some streptococcal R plasmid. Plasmid **8**, 199–206.
28. Ike, Y., Clewell, D. B. (1992). Evidence that the hemolysin/bacteriocin phenotype of *Enterococcus faecalis* subsp. *zymogenes* can be determined by plasmids in different incompatibility groups as well as by the chromosome. J. Bacteriol. **174**, 8172–8177.
29. Jacob, A., Douglas, G. I., Hobbs, S. J. (1975). Self-transferable plasmids determining the hemolysin and bacteriocin of *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **121**, 863–872.
30. Jacob, A., Hobbs, S. J. (1974). Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **117**, 360–372.
31. Jett, B., Gilmore, M. S. (1990). The growth-inhibitory effect of the *Enterococcus faecalis* bacteriocin encoded by pAD1 extends to the oral streptococci. J. Dent. Res. **69**, 1640–1645.
32. Kao, S., Olmsted, S. B., Viksnins, A. S., Gallo, J. C., Dunny, G. M. (1991). Molecular and genetic analysis of a region of plasmid pCF10 containing positive control genes and structural genes encoding surface proteins involved in pheromone-inducible conjugation in *Enterococcus faecalis*. J. Bacteriol. **173**, 7650–7664.
33. Karlson, P., Luscher, M. (1959). "Pheromones": a new term for a class of biologically active substances. Nature **183**, 55–56.
34. Kreft, B., Marre, R., Schramamm, U., Wirth, R. (1992). Aggregation substance of *Enterococcus faecalis* mediates adhesion to cultured renal tubular cells. Infect. Immun. **60**, 25–30.
35. Leonard, B. A. B., Podbielski, A., Hedberg, P. J., Dunny, G. M. (1996). *Enterococcus faecalis* pheromone binding protein PrgZ recruits a chromosomal oligopeptide transport system (Opp) to import sex pheromone cCF10 for induction of conjugation. Proc. Natl. Acad. Sci. USA **93**, 260–264.
36. Martínez-Bueno, M., Gálvez, A., Valdivia, E., Maqueda, M. (1990). A transferable plasmid associated with AS-48 production in *Enterococcus faecalis*. J. Bacteriol. **172**, 2817–2818.
37. Martínez-Bueno, M., Valdivia, E., Gálvez, A., Maqueda, M. (1992). Transfer of a plasmid determining bacteriocin Bc-48 production and immunity, and response to sexual pheromones in *Enterococcus faecalis* S-48. Plasmid **28**, 1–8.
38. Mori, M., Sakagami, Y., Narita, M., Isogal, A., Fujino, M. C., Kitada, C., Craig, R., Clewell, D. B., Suzuki, A. (1984). Isolation and structure of the bacterial sex pheromone, cAD1, that induces plasmid transfer in *Streptococcus faecalis*. FEBS Letters **178**, 97–100.
39. Mori, M., Isogal, A., Sakagami, Y., Fujino, M. C., Kitada, C., Clewell, D. B., Suzuki, A. (1986). Isolation and structure of the *Streptococcus faecalis* sex pheromone inhibitor, iAD1, that is excreted by the donor strain harboring plasmid pAD1. Agric. Biol. Chem. **50**, 539–541.
40. Mori, M., Sakagami, Y., Ishii, Y., Isogal, A., Kitada, C., Fujino, M., Adsit, J. C., Dunny, G. M., Suzuki, A. (1988). Structure of cCF10, a peptide sex pheromone which induces conjugative transfer of the *Streptococcus faecalis* tetracycline resistance plasmid, pCF10. J. Biol. Chem. **263**, 14574–14578.
41. Murray, B. E., An, F., Clewell, D. B. (1988). Plasmid and pheromone response of the γ-lactamase producer *Streptococcus (Enterococcus) faecalis* HH22. Antimicrob. Agents Chemother. **32**, 547–551.
42. Nakayama, J., Abe, Y., Ono, Y., Isogal, Y., Suzuki, A. (1995). Isolation and structure of the *Enterococcus faecalis* sex pheromone, cOB1, that induces conjugal transfer of the hemolysin bacteriocin plasmids, pOB1 and pYL1. Biosci. Biotech. Biochem. **59**, 703–705.
43. Nakayama, J., Clewell, D. B., Suzuki, A. (1995). Targered disruption of the PD78 gene (*traF*) reduces pheromone-inducible conjugal transfer of the bacteriocin plasmid pPD1 in *Enterococcus faecalis*. FEMS Microbiol. Lett. **128**, 238–288.
44. Nakayama, J., Ono, Y., Suzuki, A., (1995). Isolation and structure of the sex pheromone inhibitor, iAM373, of *Enterococcus faecalis*. Biosci. Biotech. Biochem. **59**, 1357–1359.
45. Nakayama, J., Ruhfel, R. E., Dunny, G. M., Isogai, A., Suzuki, A. (1994). The *prgQ* gene of the *Enterococcus faecalis* tetracycline resistance plas-

mid pCF10 encodes a peptide inhibitor, iCF10. J. Bacteriol. **176**, 7405–7408.

46. Nakayama, J., Yoshida, K., Kobayashi, H., Isogai, A., Clewell, D. B., Suzuki, A. (1995). Cloning and characterization of a region of *Enterococcus faecalis* plasmid pPD1 encoding pheromone inhibitor (*ipd*), pheromone sensitivity (*traC*) and pheromone shutdown (*traB*) genes. J. Bacteriol. **177**, 5567–5573.
47. Oliver, D. R., Brown, B. L., Clewell, D. B. (1977). Characterization of plasmids determining hemolysin and bacteriocin production in *Streptococcus faecalis* 5952. J. Bacteriol. **130**, 948–950.
48. Olmsted, S. B., Stanley, L. E., Dunny, G. M., Wells, C. J. (1993). High resolution visualization by field emision scanning electron microscopy of *Enterococcus faecalis* surface protein encoded by the pheromone inducible conjugative plasmid pCF10. J. Bacteriol. **175**, 6229–6237.
49. Pepper, K., Horaud, T., LeBoubuenec, C., de Cespedes, G. (1987). Location of antibiotic resistance markers in clinical isolates of *Enterococcus faecalis* with similar antibiotypes. Antimicrob. Agents Chemother. **31**, 1394–1402.
50. Pontius, L. T., Clewell, D. B. (1992). Conjugative transfer of *Enterococcus faecalis* plasmid pAD1: nucleotide sequence and transcriptional fusion analysis of a region involved in positive regulation. J. Bacteriol. **174**, 3152–3160.
51. Quirantes, R., Martín, I., Valdivia, E., Gálvez, A., Martínez-Bueno, M., Maqueda, M. (1994). Bacteriocin plasmid pMB1 of *Enterococcus faecalis:* identification of the cell aggregation substance after induction by sex pheromone. Can. J. Microbiol. **40**, 500–503.
52. Quirantes, R., Gálvez, A., Valvidia, E., Martín, I., Martínez-Bueno M., Méndez, E., Maqueda, M. (1995). Purification of sex pheromones specific for pMB1 and pMB2 plasmids of *Enterococcus faecalis* S-48. Can. J. Microbiol. **41**, 629–632.
53. Reniero, R., Cocconcelli, P., Bottazzi, V., Morell, L. (1992). High frequency of conjugation in *Lactobacillus* mediated by an aggregation-promoting factor. J. Gen. Microbiol. **138**, 763–768.
54. Ruhfel, R. E., Manias, D. A., Dunny, G. M. (1993). Cloning and characterization of a region of the *Enterococcus faecalis* conjugative plasmid, pCF10, encoding a sex pheromone-binding function. J. Bacteriol. **175**, 5253–5259.
55. Ruoslahti, E., Pierschbacher, M. D. (1987). New perspectives in cell adhesion: RGD and integrins. Science **238**, 491–497.
56. Suzuki, A., Mori, M., Sakagami, Y., Isogai, A., Fujimo, M., Kitada, C., Craig, A. R., Clewell, D. B. (1984). Isolation and structure of bacterial sex pheromone cPD1. Science **226**, 849–850.
57. Tanimoto, K., An, F. Y., Clewell, D. B. (1993). Characterization of the *traC* determinant of the *Enterococcus faecalis* hemolysin-bacteriocin plasmid pAD1: binding of sex pheromone. J. Bacteriol. **175**, 5260–5264.
58. Tomich, P. K., An, F. Y., Damle, S. P., Clewell, D. B. (1979). Plasmid related transmissibility and multiple drug resistance in *Streptococcus faecalis* subespecies *zymogenes* strain DS16. Antimicrob. Agent Chemother. **15**, 828–830.
59. van der Lelie, D., Chavarri, F., Venema, G., Gasso M. J. (1991). Identification of a new genetic determinant for cell aggregation associated with lactose plasmid transfer in *Lactococcus lactis.* Appl. Env. Microbiol. **57**, 201–206.
60. Wanner, G., Formanek, H., Galli, D., Wirth, R. (1989). Localization of aggregation substance of *Enterococcus faecalis* after induction by sex pheromones. Arch. Microbiol. **151**, 491–497.
61. Weidlich, G., Wirth, R., Galli, D. (1992). Sex pheromone plasmid pAD1-encoded surface exclusion protein of *Enterococcus faecalis*. Mol. Gen. Genet. **233**, 161–168.
62. Wirth, R. (1994). The sex pheromone system of *Enterococcus faecalis*. More than just a plasmid-collection mechanism? Eur. J. Biochem. **222**, 235–246.
63. Wirth, R., Frisenegger, A., Houraud, T. (1992). Identification of more sex pheromone plasmid in *Enterococcus faecalis*. Mol. Gen. Genet. **233**, 157–160.
64. Yagi, Y., Kessler, R. E., Shaw, J. H., Lopatin, D. E., An, F. Y., Clewell, D. B. (1983). Plasmid content of *Streptococcus faecalis* strain 39-5 and identification of a pheromone (cPD1)-induced surface antigen. J. Gen. Microbiol. **129**, 1207–1215.

MICROBIOLOGÍA SEM **13** (1997), 37–44 **MINIREVIEW**

A dynamic state of a closed ecosystem and its significance to the generation of the Earth's environment

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Summary

The Earth forms a living-system composed of large materially closed ecosystems (CES). We are performing various experiments to study comparable living-systems, especially smaller samples we call "microcosmic type CES". CES have an ability to persist despite perturbation. We show experimental results of the behavior of CES mainly during the transition from a semi-open state to an entirely closed one. We report phenomena on CES instability which may be inherent in them. We suggest that CES possess some properties that may help elucidate the nature of the Earth as a living system. Climatic change on the early Earth, such as oceanic anoxia and its consequences, may have been due to disturbances as modeled in a CES.

Key words: closed ecosystem, living system, instability, regulation, Gaia experiments

Resumen

La Tierra es un sistema vivo compuesto por grandes ecosistemas cerrados (CES). Hemos estudiado varios sistemas vivos comparables, tomando ejemplos de menor tamaño, los "CES microcósmicos", capaces de mantener sus condiciones a pesar de la perturbación. Se muestran los resultados del comportamiento de los CES durante la transición de un estado semiabierto a otro completamente cerrado, y se describen fenómenos de inestabilidad inherentes a los propios sistemas. Se sugiere que poseen propiedades que permiten comprender la naturaleza de la Tierra como un sistema vivo. Los cambios climáticos que se produjeron en la Tierra primitiva, como las anoxias oceánicas y sus consecuencias, podrían deberse a perturbaciones como en el caso de los CES.

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Introduction

With respect to matter but not to solar energy, the Earth is a huge closed ecosystem (CES). The nature of closed ecosystems is crucial to shed some light on the riddles of the Earth.

Ecosystems are materially closed but energetically open. Two types of CES are recognized: CES of a space station type, and CES of a microcosmic type. The actual Earth has never existed as a CES of a space station type, which is controlled and supported for human survival by using mechanical and electric power. The Earth's ecosystem has been developing autonomously and holistically. CES of the microcosmic type are quite suitable for a model of the Earth as a huge CES, although few studies have been published (5–7). CES experiments are significant for the study in miniature of the geophysiological properties of the Earth. We constructed sets of three experimental apparatus of the CES microcosmic type: CES-1, CES-2 and CES-3. The size and shape of the CES containers are illustrated in Fig. 1. Each container is filled halfway with chlorine-free tap water. The container has an atmosphere.

Water plants, aquatic and semi-aquatic animals such as beeshrimps and snails, and pebbles were placed in the container. The container was placed in a water-bath to maintain a nearly constant temperature of 25°C. The system was lit with artificial sunlight. The results here described involve continuous light regimes.

O_2 and CO_2 concentrations in the atmosphere and also dissolved O_2 concentration in the hydrosphere could be measured in real time. These data were entered into computers every second. To estimate the numbers of living and dead bacteria in water, ATP measurements were made dayly by collecting the material of only 100 µl from the hydrosphere. Chemical measurements were also performed to assess water quality, with respect to ions dissolved in water, pH and so on. ATP was measured by the method of luciferin-luciferase bioluminescence. The methods of zirconia solid electrolyte and of nondispersive infrared absorption were used to detect the atmospheric O_2 and CO_2 gases, respectively. To measure dissolved O_2 concentration, fluorescence of a specific organic material quenched by O_2 molecules was quantitated.

In this paper, we show the changes in the CES that appeared during the transition from an "open" system to a "closed" system. The word "open" refers to a CES which has been exposed to air by opening the top part of the container to about a 5 cm-width (4). We experimented with the CES for about one year by using the "open" system. We then closed the container entirely to make it a "closed" state, and compared the behaviors and changes of these two systems.

Regulatory properties of closed ecosystems

It could be thought that a CES will be polluted by the green algae *Rhizoclonium* sp., which outgrows other aquatic organisms. But that is wrong. Whether the CES is "closed" or "open", the water quality in both kinds of ecosystems is rather good. The numbers of living and dead bacteria in the CES, are nearly equal to those in natural potable spring water. The numbers of bacteria in the CES fall below expectations because the organisms do not reach their intrinsic growth rate.

The pH of the hydrosphere in CES is high. The value is over 8.5, whether the CES is "closed" or "open". In the entirely closed state CES pH values are over 9.0. The value of pH in the CES remains nearly constant as long as the ecosystem lasts. These high pH values are due to high consumption of carbonate ion by photosynthetic green algae ($HCO_3^- \rightarrow CO_2 + OH^-$), and also be-

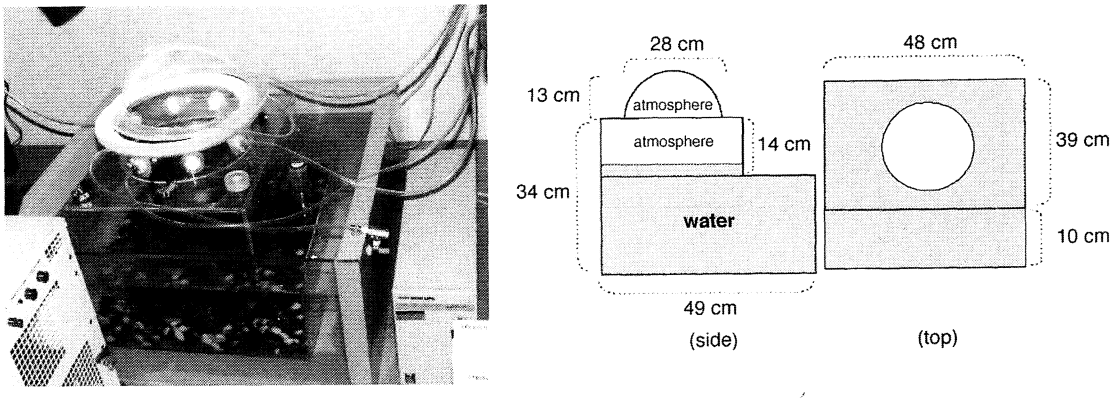

FIG. 1. Photograph of CES (left) and a corresponding sketch of the container (right).

cause of the release of ammonia excreted from the organisms as the decomposition product of protein. Nitrifying bacteria, with peak activity at high pH (pH ≈ 8–9), oxidize ammonia aerobically into nitrite and nitrate. In addition, denitrifying bacteria, which have a pH optimum of 9, release N_2 from nitrite and nitrate. Both the activities of these bacteria which have high pH optima (1) and the limited space in CES contribute to the rise of pH.

The steady state concentrations of the observed ions (for about 12 months) were: NH_3 (~0 mg/l, LDL: [less than the lowest detection limit in the measurement]), NO_2 (0.005 mg/l) and NO_3 (~0 mg/l, LDL). The hydrosphere displays evidence of nitrifying bacteria metabolism. By purifying the hydrosphere in CES by oxidizing ammonia, those bacteria act to retain stability. They are one of the reasons why CES pH remains almost constant against the release of harmful ammonia and represent an example of "living system" regulations.

About the "open" system

Inputs to the "open" CES system include light and CO_2 gas for photosynthesis. As the "open" system is exposed to a large amount of air, organisms may propagate as much as possible. A high increase in the number of organisms is characteristic only of the early stages of the system (Fig. 2). In the mature period, nearly constant populations of organisms are maintained. Supported by a large amount of resources, the "open" system works stably consuming the resources moderately. The numbers of both living and dead bacteria are comparable during the early stages of the system, when their concentrations increased and decreased out of phase with each other (Fig. 2). This regulating behavior stabilized the young system. During the semi-open period (day ca.100 to 135), the number of bacteria was at least 5 times larger than during the mature period. In this period, whether the system was "open" or "closed", the living bacteria always outnumbered the dead ones, except at the times of "crises" (Fig. 2).

Transition from "open" to "closed" system

The behavior of the CES after complete closure is instructive. The dissolved O_2 concentration in the hydrosphere dropped abruptly just after closure (Fig. 3A). Near the bottom of the container (25 cm below the water surface) it de-

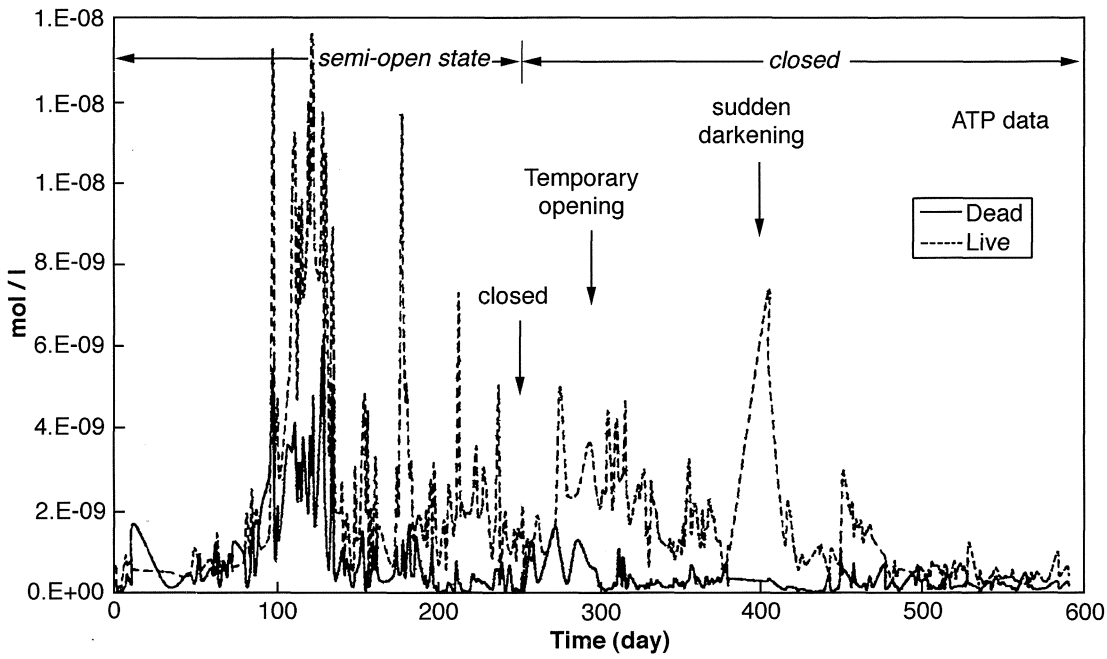

FIG. 2. ATP data of CES from experimental onset (July 19, 1994). The dotted and solid lines correspond to the number of living and dead organisms, respectively. The dates of some experiments are shown with the arrows.

creased rapidly from 12 ppm to 0–0.8 ppm in only 4 days. The concentration was restored gradually and became 10 ppm in a month. An anoxic state can be induced easily in the CES hydrosphere by some disturbance.

The O_2 concentration in the atmosphere of the CES also decreased sharply to about 1%, but it recovered again in 3 days. The CO_2 atmospheric gas concentration decreased rapidly together with a decrease in O_2 concentration, ranging from 0.02% to near 0%, i.e., the lowest limit of the measuring instrument. All the CO_2 released into the closed space is used immediately for photosynthesis.

The regulating behavior in response to the abrupt disturbance was clearly recorded by variations of oscillating periods of atmospheric O_2 and CO_2 concentration. Fourier analyses of the data indicate 0.5–1.5 days was the most prominent oscillation period in the "open" system. After closure, it shifted quickly to shorter periods of 6–7 h and of 1.5 h. We interpret these as regulating responses of the system to the disturbance caused by the abrupt closure of the CES.

Instability of closed ecosystems

In spite of gas and pH regulation, the CES studied were unstable. The following supports this suggestion:

(i) We had assumed that the temporary opening of the "closed" system would not seriously damage it, because the previous "closed" system maintain a near-constant status for 46 days after closure. Over 46 days the atmospheric O_2 concentration remained almost constant at ca. 21%, comparable to open air. A temporary opening of the system, with all other circumstances unchanged, and without other disturbances, was predicted to cause minimal effects.

However, only one hour after we opened the

CES, the O_2 concentrations both in the atmosphere and dissolved in the hydrosphere began to drop dramatically. The atmospheric O_2 concentration dropped from around 21% to about 15% in 40 days. Photosynthetic processes of the system seem to have been impaired. We have not yet been able to explain this effect, which is likely not to be so simple. The green algae population which had vigorously covered the water surface decreased sharply. This decrease probably in-

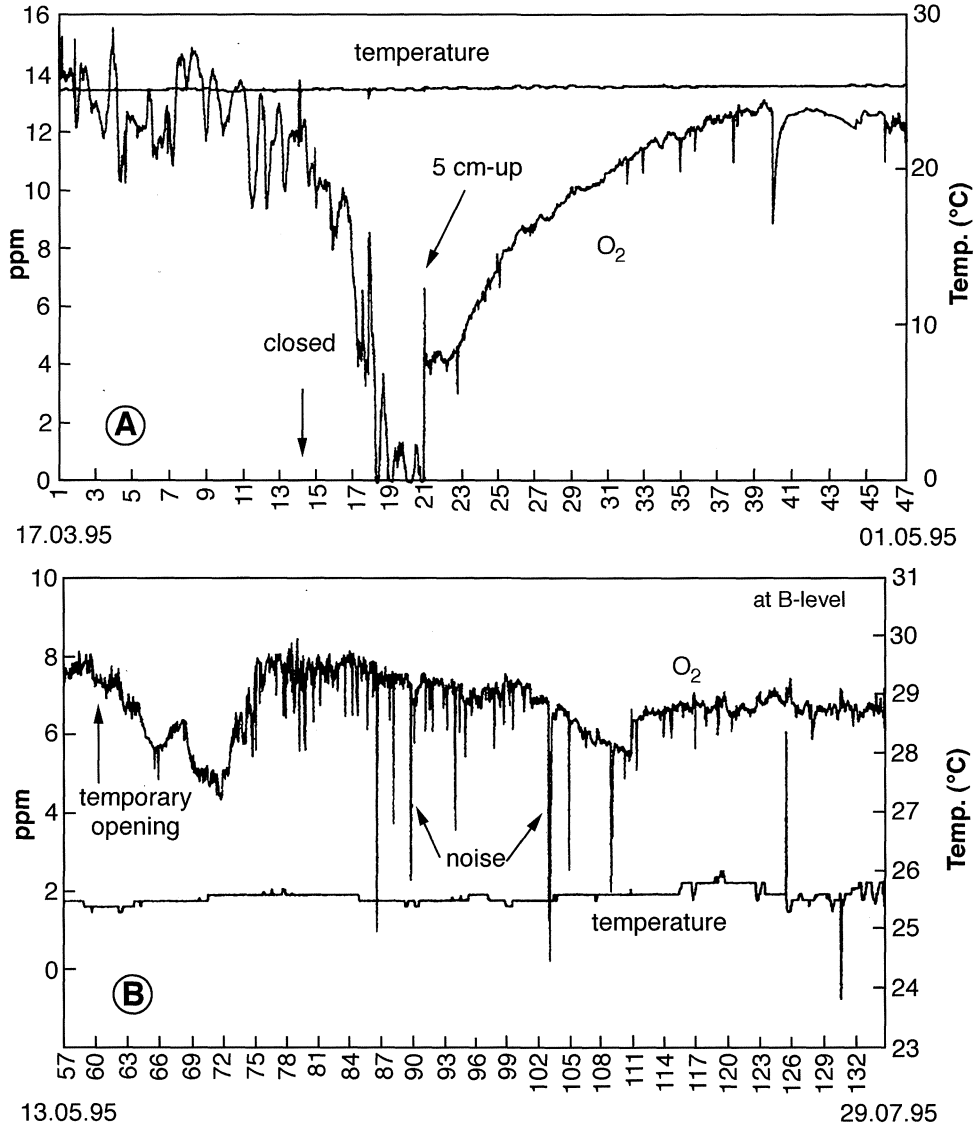

FIG. 3. Concentration of O_2 dissolved in the hydrosphere. The origin of the abscissa is March 17, 1995. (A) Before and after complete closure. 'Temperature' indicates that of the water near the O_2 sensor. '5 cm-up' means lifting the O_2 sensor up 5 cm from near the bottom of the container. Before and after, measurements were made near the bottom and at the '5 cm-up' point, respectively. In the stable-state periods, the O_2 values at both points are nearly equal. (B) Before and after the temporary opening. The data are at the '5 cm-up' level. (Numbers in abscissas are the days elapsed since the begining of the experiment.)

duced the sharp drop of atmospheric O_2 concentration in the CES.

The dissolved O_2 in the hydrosphere of the CES showed different behavior from that of the atmospheric O_2. Although the latter varied increasing and decreasing, the dissolved O_2 concentration showed a net rapid decrease from around 10 ppm to about 6.5 ppm in only 10 days. Yet it recovered to a value of 9 ppm two weeks after the temporary opening (Fig. 3B). Green algae recovered very well in the hydrosphere except at the surface. They covered the bottom of the container with vigor. The two-week delayed response to the one-hour removal of the top and exposure to room air deserves careful attention.

(ii) Another abrupt and unexpected change of the state of the CES occurred three months after the above change, when a light was turned off for one day. The stable state of the system of long standing was destroyed almost immediately. O_2 concentration in the atmosphere decreased from about 19% to 12% in 10 days, whereas the CO_2 concentration increased from about 0% to 1.7% in 12 days (Fig. 4A). Other unexpected changes in dissolved O_2 concentration in the hydrosphere were noted. In just one day, O_2 concentrations had dropped sharply from 9.7 ppm to a mere 1.5 ppm (Fig. 4B). The green algae which had extensively covered the hydrosphere were badly damaged. Only some small patches of green algae remained in the bottom of the hydrosphere. Most snails died.

The ATP data correlated with that sudden darkening (Fig. 2). The concentration of living bacteria became ten times larger than before the one-day darkness episode. The concentration of dead bacteria remained unchanged. The pH value declined from 9.5 to 7.5.

The results of the "darkness flash" demonstrate the influence of photosynthetic living organisms. As they died, the nature of the system changed from an oxic one to one with little O_2. The leading organisms supporting the system must have been "rearranged": both methane bacteria and methane-oxidizing bacteria became more numerous and influential, and they began to emit CO_2 from the decomposition of organic matter like green algae.

These results suggest that analogous mechanisms might cause climatic changes: anoxia events would break out suddenly in the hydrosphere and they would propagate slowly into the atmosphere. The system lacking photosynthetic organisms would have an environment enriched with CO_2 gas, as proposed by Lovelock (2, 3). The responses to the temporary opening and the short-time light-loss tell us that the CES which looks superficially stable might actually be unstable. Even slight disturbances can transfer the system toward another unexpected state. The universality of rapid responses needs further detailed investigation.

Recovery

The CES has been shown to have a latent regulating ability against perturbance. In the case (ii), 18 days after the abrupt decrease in dissolved O_2 concentration, the O_2 concentration in the hydrosphere began suddenly to increase. The behavior of the CES (Fig. 4B) is noted. The O_2 concentration, constant at 1.5 ppm for 18 days, showed no oscillations. After this "sleep", the whole system, including green algae and snails, seemed to have returned to life. The O_2 concentration increased in the hydrosphere fairly rapidly, with oscillatory motions. By day 195, the system had completely recovered the O_2 concentration: the hydrosphere contained 14.7 ppm (Fig. 4B), and the atmospheric CO_2 concentration had decreased from 1.7% at maximum to 0.01% (Fig. 4A). The pH value had risen to 9.2.

The atmospheric O_2 data after day 165 of

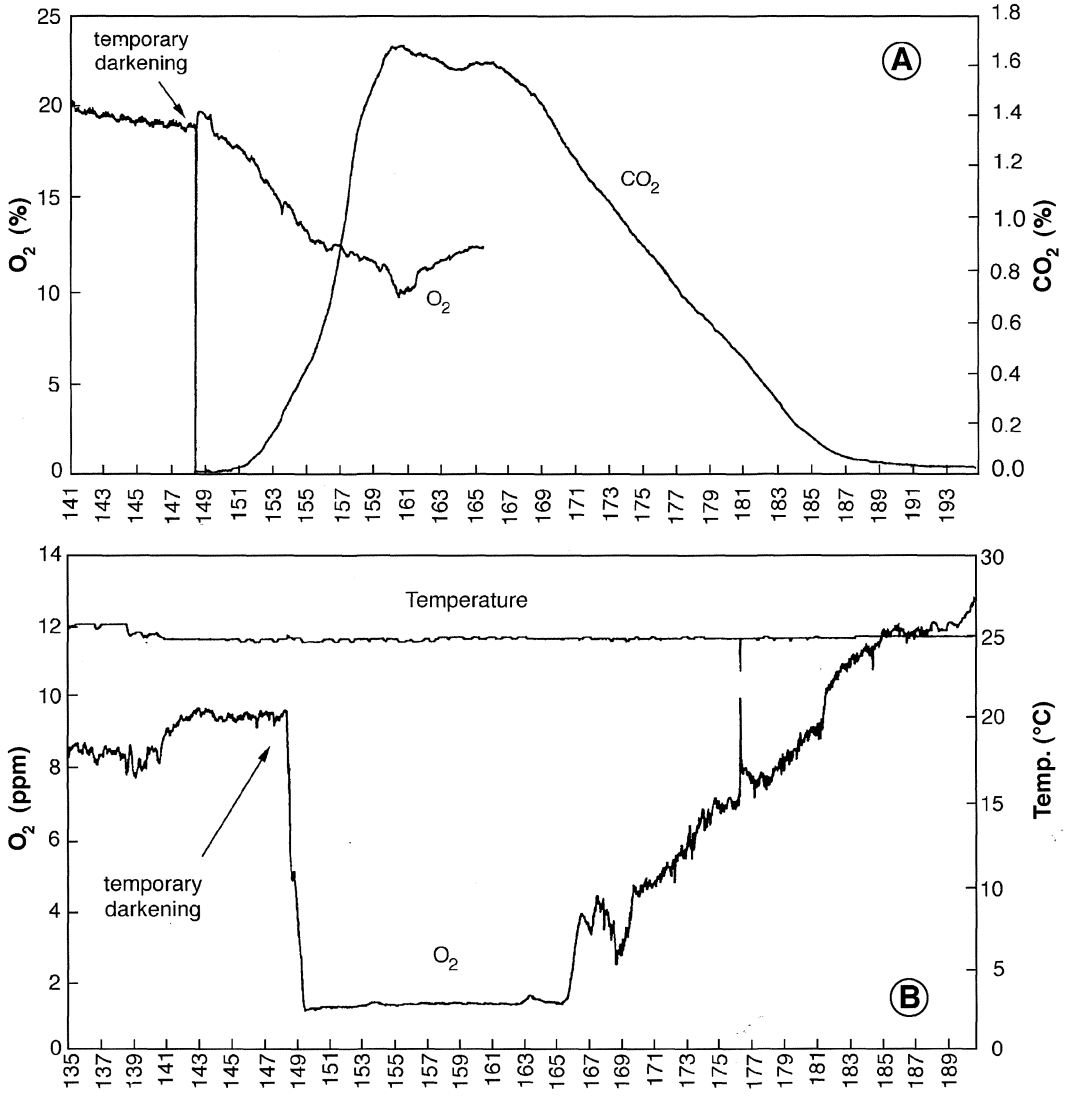

FIG. 4. Instability and recovery of CES at the temporary darkening. The origin of the abscissa is March 17, 1995. (A) Changes of atmospheric O_2 and CO_2 gas concentrations before and after the temporary darkening, and recovery of atmospheric CO_2 gas. (B) Change of O_2 concentration in the hydrosphere before and after the temporary darkening, and its recovery. (Numbers in abscissas are the days elapsed since the begining of the experiment.)

Fig. 4A were lost due to problems in the measuring device. However, after resumption of the measurement (50 days after day 165), atmospheric O_2 concentration in the the CES held around 21%, which is comparable to that in the Earth's atmosphere, although the details of regulation in both macrocosmic and microcosmic scales elude us. The nature of the CES may resemble the mechanisms of climatic changes. Slight disturbances may change the Earth's state drastically, although the significance of differences in scale (volume) of about 10^{22} is inestimable. The anoxic state in the ocean could have emerged not by external forcing but by small amplification of the inherent nature of the CES itself. Oceanic anoxia which occurred on the

Permotriassic boundary (250 million years ago) has been explained by changes of oceanic currents and of biotic activities linked with super continental formation. These explanations are based on mechanical responses of the Earth system for the given external and powerful perturbations.

Earth may not need such external, mechanical causes for the appearance of ocean anoxia, because the inherent instability of the CES itself can induce an anoxic state. As our experiments indicate, slight disturbances on the Earth may suffice to bring widespread anoxia which then impacts the atmosphere. Besides, CES, large or small, have strong regulatory abilities against perturbation, i.e., a robust ability for recovery.

The above matters need further study. It is crucial to estimate the significance of total and proportional volume and species composition to the stability properties of the system.

Conclusions

We believe further research into the stability and perturbability behavior of CES, as living systems, are required to understand the nature of the actual Earth's huge CES. Even in microcosms, CES tend to regulate their gas composition and pH features. If the results of CES are extrapolated to the Earth, then life and its environment on the Earth form a cooperative system. They are never separated from each other.

CES operate with their own dynamism and oscillations. This may be analogous to climatic variations. The CES has a possibility for sudden change by slight perturbation. It also has a persistently powerful restoring ability. Abrupt anoxia events occurring in the hydrosphere should be noted the most. This indicates that, despite an apparent stable state, there is some kind of instability in the CES. Current theories for the mechanism of climatic changes do not acknowledge the Earth as a CES.

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References

1. Alexander, M. (1961). Introduction to Soil Microbiology. John Wiley & Sons, Inc., New York.
2. Lovelock, J. E. (1979). Gaia: A New Look at Life on Earth. Oxford University Press, Oxford and New York.
3. Lovelock, J. E. (1991). Gaia: The Practical Science of Planetary Medicine. Gaia Books Ltd., London.
4. Moriyama, S., Nomoto, G., Kogami, S. (1993). Evolution of the earth's environment and closed ecosystem: Proposition of geophysiology. CELSS J. **5**, 17–25.
5. Obenhuber, D. C., Folsome, C. E. (1984). Eucaryote/procaryote ratio as an indicator of stability for closed ecological systems. BioSystems **16**, 291–296.
6. Obenhuber, D. C., Folsome, C. E. (1988). Carbon recycling in materially closed ecological life support systems. BioSystems **21**, 165–173.
7. Takano, C. T., Folsome, C. E., Karl, D. M. (1983). ATP as a biomass indicator for closed ecosystems. BioSystems **16**, 75–78.

MICROBIOLOGÍA SEM **13** (1997), 45–56

Structure and composition of freshwater microbial mats from a sulfur spring ("Font Pudosa", NE Spain)

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Summary

Different types of microbial mats developing on the wall of a non-thermal sulfur freshwater spring have been studied. Both, light and electron microscopy as well as HPLC analysis of photosynthetic pigments revealed their structure and composition. Prokaryotic chlorophylls and carotenoids helped in the taxonomical assignment of the main photosynthetic groups. "Inverted position" mats (Mat-I) were dominated by Chromatiaceae; they were located close to the water outlets (0.3 mM sulfide). "Normal position", that is, cyanobacterial-covered mats (Mat-II and Mat-IV), developed elsewhere on the stone walls at lower sulfide concentrations. A third type of mat (Mat-III), covered by chemolithotrophic bacteria, was distinguishable at the water-air interface, strongly attached to the walls of the spring. Up to six physiological types of microorganisms have been recognized: cyanobacteria, Chromatiaceae, purple nonsulfur bacteria, Chlorobiaceae, Chloroflexaceae, and chemolithotrophic bacteria. Cyanobacteria *Lyngbya*-like, *Oscillatoria*-like and *Pseudanabaena* sp. were found. The diversity of Chromatiaceae (six morpho-/pigment types of the genus *Chromatium*, plus two non identified Chromatiaceae, named PB1 and PB2 were observed) was noticeable. Chemolithotrophic bacteria were represented by the genera *Beggiatoa* and *Thiothrix*. Finally, small numbers of *Chloroflexus*-like bacteria and *Chlorobium limicola* were found in all the studied mats.

Key words: microbial mats, non-thermal sulfur spring, cyanobacteria, Chromatiaceae/ Chlorobiaceae, *Chloroflexus*-like

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Resumen

Se han estudiado los tapetes microbianos de las paredes de una fuente sulfurosa de aguas templadas. Mediante técnicas de microscopía (óptica y electrónica) y de análisis de pigmentos fotosintéticos por HPLC se ha podido conocer su estructura y composición. Las diferentes clorofilas procarióticas y los carotenoides han facilitdo la determinación taxonómica de los principales grupos fotosintéticos. Los tapetes de "posición invertida" (Mat-I) están dominados por Chromatiaceae, y se encuentran próximos a las salidas de agua (sulfuro: 0,3 mM). Los tapetes de "posición normal" (Mat-II y Mat-IV) están dominados por cianobacterias y se desarrollan en diversos lugares en las paredes de piedra, con concentraciones de sulfuro más bajas. Un tercer tipo de tapete (Mat-III), cubierto por bacterias quimiolitotrofas, se localizaba en las interfases aire-agua, fuertemente adherido a las paredes de la fuente. Se observaron hasta seis tipos fisiológicos diferentes de microorganismos: cianobacterias, Chromatiaceae, bacterias rojas no del azufre, Chlorobiaceae, Chloroflexaceae y bacterias quimiolitotrofas. Se encontraron cianobacterias tipo *Lyngbya*, tipo *Oscillatoria* y *Pseudanabaena* sp. Es notable la diversidad de Chromatiaceae (se observaron seis morfo-/pigmentotipos de *Chromatium*, más dos Chromatiaceae no identificadas, denominadas PB1 y PB2). Las bacterias quimiolitotrofas estaban representadas por *Beggiatoa* y *Thiothrix*. En todos los tapetes se apreciaban pequeñas cantidades de formas tipo *Chloroflexus* y de *Chlorobium limicola*.

Introduction

Microbial mats are commonly laminated communities, basically composed of phototrophic and chemotrophic prokaryotes. The vertical stratification of the community is a response of the organisms to the gradients of light, oxygen, sulfide and pH, according to their physiological requirements. Mats have been extensively studied in marine intertidal, in other hypersaline environments (1) and in hot springs (4, 7). Few studies have been published concerning non-thermal freshwater microbial mats others than epilithic structures from streams and treatment plants of urban wastewater (8, 24–26).

Although the existence of microbial mats in "Font Pudosa" (i.e., Pudosa Spring [Banyoles, NE Spain]) has been previously reported (6), so far no intensive studies have been carried out. Sulfide (0.3 mM) is directly supplied by the spring water as in thermal sulfur springs. Mats grew up on the spring walls, therefore stratification occurred parallel to the vertical walls of the spring instead of the sediment or bottom. Three main types of microbial mats were distinguishable: "inverted position" mats (Mat-I); "normal position" mats (Mat-II and Mat-IV) and chemolithotrophic sulfur bacteria-dominated mats (Mat-III).

This work is aimed at describing and characterizing the microbial mats of Pudosa Spring through the observation and analysis of their communities. From the basis that accurate pigment information is essential for the identification of phototrophic microorganisms, reverse-phase HPLC pigment studies are a useful tool (16, 17). We have carried out a high precision separation of prokaryote photosynthetic chlorophylls (Chl *a*; BChls *a*, *b*, *c*, *d*, and *e*) and carotenoids as β- and γ- carotenes (Car), okenone (Oke), spirilloxanthin (Spr), chlorobactene (Cbt), rhodopinal (Rhl) and rhodopin + lycopene (Rhp

+ Lyp). Previous pigment studies of mats, made by spectrophotometry of different colored layers extracts (23) were usually focused on chlorophylls, both Chl *a* and Bchls *a*, *b*, *c*, *d*, and *e*, but were seriously handicapped by the coincidence of their absorption maxima. An accurate pigment analysis, together with morphological and ultrastructural microscopic observations, has provided an approach to the microbial phototrophic communities living in the studied mats.

Material and methods

Physical and chemical measurements. The microbial mats hereby studied were located in a temperate-freshwater, sulfur spring located in the karstic lacustrine area of Banyoles (NE Spain), which is characterized by sulfate-enriched waters (2 mM SO_4^{2-}). The water outlet is channelled off through four principal vents at the base of the spring body, but it also flows all around the base of the column through fissures in the rock blocks. Water fills a circular reservoir (diameter, 1.2 m) surrounding the spring and flows away through an irregular outlet stream. Fig. 1 shows the structure of the spring and the location of the mats studied.

Sulfide was analyzed by the methylene-blue method of Pachmayr. Oxygen concentration was determined by iodometric methods (9), after previous precipitation of sulfide with zinc acetate (1% v/v). Conductivity and temperature were measured using a conductimeter WTW LF model 191, with a built-in temperature probe. For redox potential measurements a platinum combined Metrohm 6,041,100 electrode was used. Physical and chemical variables at the main outlets and at different points of the spring were monitored bimonthly (n = 9).

The mats were chosen according to their position and macroscopical aspect. Mat-I, was light-purple and it was located on the column of the spring itself, close to the main jets. Mat-II and Mat-IV were both green, but clearly differed in thickness: 1.8 mm and 4–8 mm, respectively. Mat-II was on the column of the spring but it was not continuously covered by water. Mat-IV was located on the body of the spring, submerged in the reservoir. Finally, Mat-III was white and was on the wall of the circular channel, just at the air-water interface facing the principal vents (see Fig. 1).

Microscopy. Smears of fresh samples at different depths were studied by light microscopy (phase contrast and epifluorescence), immediately after sampling in order to obtain a first insight into the organization of the mat community.

The study of the undisrupted structure of the mats was performed by light and electron microscopy. For this purpose, samples were fixed in situ with 2.5% glutaraldehyde in 0.1 M cacodylate buffered spring water (pH 7.4) and kept for 24 h at 4°C in the laboratory. Prior to further processing, fixed samples were cut in 1-mm fragments. Next, they were washed in buffer and postfixed in 1% osmium tetroxide in cacodylate buffer for 2 h at 4°C. After washing again, samples were stained with 1% uranyl acetate for 1 h and washed in malate (pH 5.2) and cacodylate (pH 7.4) buffers. Dehydration was made in an ascending series of acetone to 100% before final embedding in 100% Spur resin. Samples were placed in such a way that transversal sections of the resin blocs showed the vertical stratification of the mat. Semi-thin sections were stained with toluidine blue and used for light microscopy study. For electron transmission microscopy, ultrathin sections were stained with uranyl acetate and lead citrate.

HPLC pigment analyses. For pigment analysis, the differently colored layers of each sample were sliced under a dissection microscope. Mat-III, dominated at the upper part by chemo-

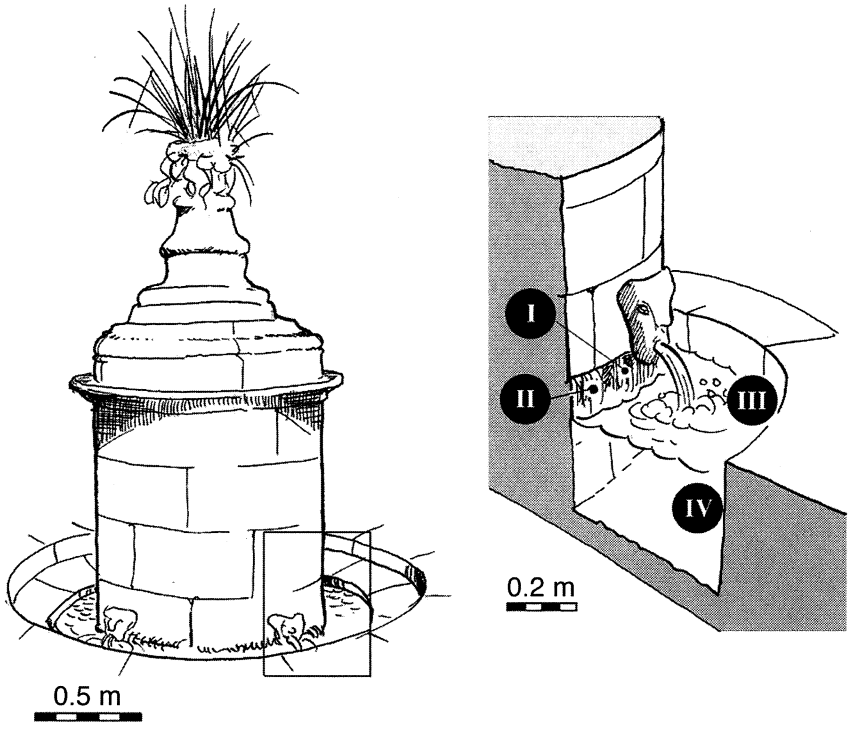

FIG. 1. Diagram of the structure of the Pudosa spring. Emplacements of the mats here studied are in the inlet.

lithotrophic bacteria, was not analyzed. Fresh samples were embedded in 5% agar to ease cutting. Pigments from slices were extracted in 90% acetone at –20°C for 24 h. Extracts were then separated and analyzed by reversed-phase HPLC (3). Relative abundance of each pigment per layer was calculated as a percentage of the total peak area at a given detection wavelength corrected by the molar extinction coefficients found in literature (Montesinos, E. 1982, Ph. D. Thesis, Autonomous University of Barcelona) (16).

Taxonomic assignment was made following Rippka (21) for cyanobacteria, and Pfennig and Trüper (18, 19, 27) for sulfur bacteria. Morphology, biometrics, ultrastructure and bacterial chlorophylls and carotenoids were used for the identification of phototrophic sulfur bacteria.

Results

At the water outlet, physical and chemical variables such as temperature (16.7 ± 0.45°C), pH (6.7 ± 0.1), conductivity (1696 ± 78 μS cm^{-1}), redox potential (–318.5 ± 30.7 mV), and sulfide (0.3 ± 0.03 mM) concentrations were fairly constant all year round. Oxygen concentration increased with the exposure time to the atmosphere. The incoming water was anoxic, whereas 0.9 to 2.1 ppm O_2 could be measure immediately after it was poured, reaching values about 3.5 ppm O_2 in the outlet channel. In addition to atmospheric oxygen dissolution, some oxygen was biologically produced at the cyanobacterial layers, as deduced from the bubbles retained in the surface of the mats.

TABLE 1. Some general characteristics of the studied mats. Color of layers are in brackets

	Mat-I	Mat-II	Mat-III	Mat-IV
Location	Walls	Walls	Walls (water-air interface)	Walls (submerged)
Thickness (mm)	1.2	1.4	1.8	4
Color	Light purple	Green	White	Green
Consistency	Loose	Loose	Filamentous/Compact	Compact
Layers distinguishable	3	4	3	5
Layer divisions (mm)				
#1	0 to 0.7 [light purple]	0 to 0.2 [green]	0 to 0.9 [white]	0 to 0.8 [green]
#2	0.7 to 1.0 [dark purple]	0.2 to 0.9 [light purple]	0.9 to 1.6 [green]	0.8 0 to 1.8 [purple]
#3	1.0 to 1.2 [black]	0.9 to 1.3 [dark purple]	1.6 to 1.8 [black]	1.8 to 2.0 [bright purple]
#4	–	1.3 to 1.4 [black]	–	2.0 to 3.5 [yellow-green]
#5	–	–	–	3.5 to 4.0 [black]

Some general properties of the studied mats are summarized in Table 1. The pigment composition (chlorophylls and carotenoids) of each layer is shown in Table 2. Okenone, spirilloxanthin and carotenoids of the rhodopinal series were identified as purple sulfur bacterial carotenoids. Although it was poorly represented, the presence of chlorobactene reveals the presence of green sulfur bacteria which have been scarcely reported in microbial mats so far (4, 14). In addition to the pigment composition, morphology, biometrics and ultrastructure facilitated the identification of the main phototrophic and chemolithotrophic bacterial species.

Up to six taxonomic and physiological types have been recognized: cyanobacteria, Chromatiaceae, purple nonsulfur bacteria, Chlorobiaceae, Chloroflexaceae, and chemolithotrophic bacteria. The presence of *Lyngbya*-like, *Oscillatoria*-like and *Pseudanabaena* sp. is a feature common to all studied mats. A remarkable finding is the diversity of *Chromatium*: six different morpho-/pigment types were identified. The purple sulfur bacterial group was completed by a non identified Chromatiaceae named PB1 which showed a tubular photosynthetic system, and a clump-forming, heavily ensheated Chromatiaceae named PB2. Chemolithotrophic bacteria were represented by the genera *Beggiatoa* and *Thiothrix*. Other groups, such as the non sulfur purple bacterium *Rhodopseudomonas* and spirochetes, were found as minor components of the microbial community.

Microscope observations of the undisrupted community revealed the vertical stratification of the mat forming microbiota. Idealized diagrams of the structures of mats are shown in Fig. 2. As a general feature, cyanobacteria were found mainly at the top of the "normal position" mats; small Chromatiaceae were generally located above larger Chromatiaceae. Finally, green sulfur bacteria did not form any distinguishable layer. Instead, they were found in unevenly distributed microcolonies, deep enough to avoid oxygen. In

TABLE 2. HPLC pigments study of the differently colored layers in mats of type I, II and IV

Layer	Pigments	Mat-I	Mat-II	Mat-IV
#1	Chlorophylls	BChl *a* (83.7)* BChl *c* (8.0)	Chl *a* (69.8) BChl *a* (8.0) BChl *c* (3.9)	Chl *a* (71.2)
	Carotenoids	Cbt (0.8) Oke (0.6) Rhp + Lyp (4.8) Rhl (1.1) Other† (1.0)	β-Car (15.4) Cbt (1.3) Rhl (0.7) Spr (0.9)	β-Car (13.5) Other (13.5)
#2	Chlorophylls	Chl *a* (0.7) BChl *a* (89.7) BChl *c* (1.1)	BChl *a* (80.3) BChl *c* (7.7)	Chl *a* (41.1) BChl *a* (13.8) BChl *c* (18.3) BChl *d* (11.2)
	Carotenoids	Cbt (0.8) Rhp + Lyp (2.1) Rhl (4.7) Spr (0.9)	β-Car (0.6) Cbt (1.2) Rhp + Lyp (7.2) Rhl (2.1) Other (0.9)	β-Car (12.0) γ-Car (12.0) Oke (0.2) Other (2.0)
#3	Chlorophylls	Not determined	Chl *a* (0.5) BChl *a* (90) BChl *c* (1.0)	Chl *a* (16.3) BChl *a* (18.9) BChl *c* (25.9) BChl *d* (10.9)
	Carotenoids	Not determined	Cbt (0.6) Rhp + Lyp (2.3) Rhl (4.7)	β-Car (8.8) γ-Car (1.7) Oke (0.8) Other (6.7)
#4	Chlorophylls	–	Not determined	Chl *a* (6.7) BChl *a* (13.6) BChl *c* (47.0)
	Carotenoids	–	Not determined	β-Car (10.5) Cbt (1.0) Oke (0.5) Spr (1.9) γ-Car (3.6) Other (15.2)
#5	Chlorophylls	–	–	Chl *a* (21.5) BChl *a* (43.6) BChl *c* (6.3) BChl *d* (1.7)
	Carotenoids	–	–	β-Car (10.7) γ-Car (0.4) Oke (0.5) Rhp + Lyp (3.7) Other (6.7)

* Percentage of total pigments in parenthesis.
† Non-photosynthetic pigments.

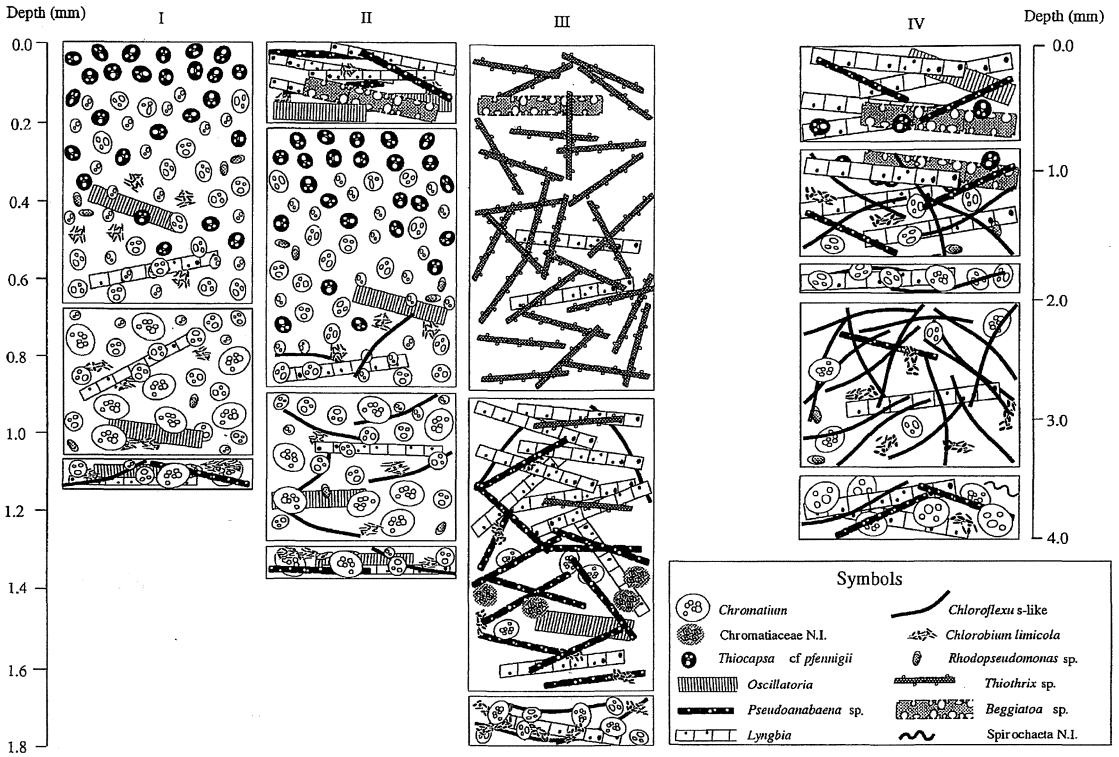

FIG. 2. Schematic representation of the organization of microbial mats from Pudosa Spring. Layering, from the surface to the bottom, follows the macroscopically different colored layers.

spite of these common aspects, each mat had a different organization depending on both the place where it was found and the microbial composition. Both optical and electron micrographs of mat forming microbiota are composed in Fig. 3.

Mat-I. Purple sulfur bacteria were dominant in this mat. Filamentous cyanobacteria (Fig. 3 D–E) formed a minor group located mainly at the innermost layer. No chemolithotrophic sulfur bacteria were observed. Microcoonies of the green sulfur bacterium *Chlorobium limicol*a (Fig. 3 I) could occasionally be distinguished below 0.4 mm.

Considering morphology, biometrics and carotenoids, dominant purple sulfur bacteria in the first layer were tentatively identified as *Chromatium minutissimum* and *C. minus*. Finally, the unidentified Chromatiaceae PB1 was observed. It had the tubular photosynthetic system typical of *Thiocapsa pfennigii*, but neither BChl *b* nor tetrahydrospirilloxanthin were detected.

Dominant Chromatiaceae were identified as *Chromatium warmingii* and *C. minutissimum.*

Mat-II. Although it essentially had the same structure as Mat-I, two remarkable features are characteristic of Mat-II. First, a superficial 0.2 mm thick, green layer and second, the presence of a multicellular filamentous green bacterium *Chloroflexus*-like from 0.7 mm depth to the bottom.

Microscope studies showed the uppermost green layer to be composed mainly of cyanobac-

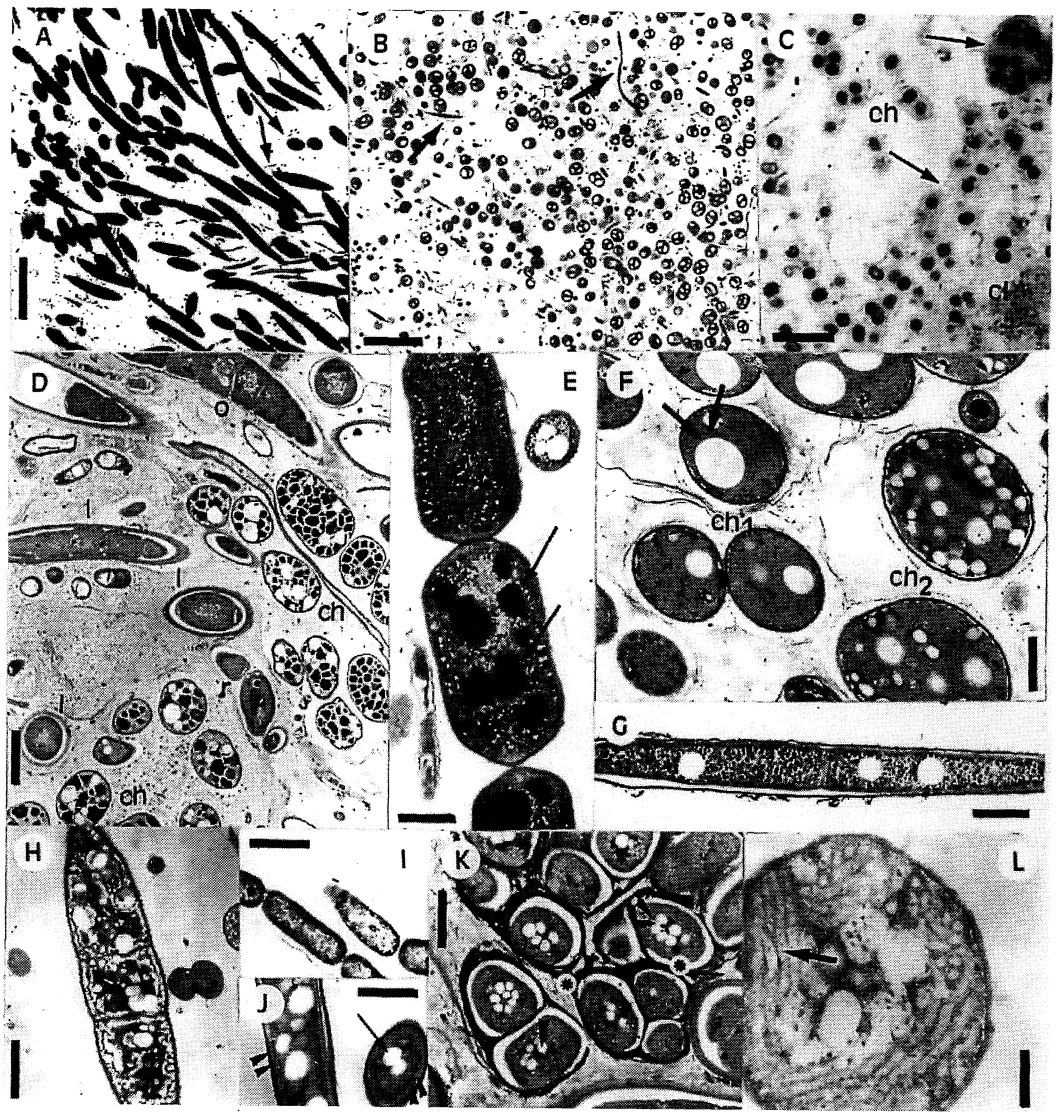

FIG. 3. Microphotograph of the microbial community at 1 mm depth of Mat-IV. **A**: The cyanobacterium *Lyngbya* shares dominance with the filamentous sulfur bacterium *Chloroflexus*-like (thin arrows) (mostly in cross section). Arrows indicate the chemiolithotrophic sulfur bacterium *Thiothix*. Bar: 5.6 μm. **B**: Microphotograph of the microbiota of Mat-IV in layer 3: purple sulfur bacteria, with abundant sulfur inclusions, and filaments of *Chloroflexus*-like, cross and longitudinal sectioned (arrows). Bar: 8.9 μm. **C**: Purple sulfur bacteria and *Chlorobium limicola* at the innermost layer of Mats. Thick slime capsules (arrows) can be appreciated. cl: *Chlorobium limicola*, ch: Chromatiaceae. Bar: 5.3 μm. **D**: Electron micrograph of the community in Mat-IV at the layer 4. Cyanobacteria, purple sulfur bacteria and purple nonsulfur bacteria coexist. I: *Lyngbya*; o:*Oscillatoria*; ch: *Chromatium*; r: *Rhodopseudomonas*. Bar: 2.5 μm. **E**: Longitudinal section of *Pseudanabaena*. Arrows denote glycogen inclusions. Bar: 0.3 μm. **F**: Purple sulfur bacteria observed in layer 4 of the Mat-IV. ch_1: *C. vinosum*; ch_2: *C. weissei*. Bar: 0.85 μm. **G, H**: Longitudinal sections of filamentous chemolithotrophic bacteria: G: *Thiotrix* from Mat-III, and *Beggiatoa* from Mat-II and Mat-IV. Bars: G: 0.9 μm; H: 1,75 μm. **I**: Microcolonies of *Chlorobium limicola* showing chlorosomes (arrows). Bar: 1 μm. **J**: Cross and longitudinal section of the *Chloroflexus*-like filamentous sulfur bacterium, showing PHB inclusions (arrows), and chlorosomes similar to those of *C. limicola*. Bar: 0.4 μm. **K**: Unidentified ensheathed purple sulfur bacterium found at 1.5 mm depth in Mat-III. Sulfur inclusions (arrow heads) and sheath (*) are indicated. Bar: 0.9 μm. **L**: Section of a *Thiocapsa*-like purple sulfur bacterium with a tubular photosynthetic system (arrows).

teria. In addition, the chemolithotrophic sulfur bacterium of the genus *Beggiatoa* (Fig. 3 H), the Chromatiaceae PB1 (Fig. 3 L) and the green sulfur bacterium *Chlorobium limicola* were also observed. These observations agree with the pigment profile of this layer (Table 2). In the inner dark purple layer γ-carotene, possibly from a *Chloroflexus*-like organism, was also detected.

Mat-III. White and filamentous, no pigment study was made for this Mat-III in which chemolithotrophic bacteria widely dominated the upper part. A two layered, green inner part, was strongly wrapped to the stone wall. The white surface layer was loose and clearly dominated by the chemolithotrophic sulfur bacterium *Thiothrix* (Fig. 3 G).

Typical rosettes were observed in vivo. *Beggiatoa*, and the cyanobacterium *Lyngbya*-like, were poorly represented. The next two layers were dominated by cyanobacteria and phototrophic sulfur bacteria. The upper part was dominated by a *Lyngbya*-like cyanobacterium, whereas *Pseudanabaena* sp. was enriched between 1.2 and 1.4 mm depth. Two types of aggregates of purple sulfur bacteria were observed, which consisted of *Chromatium*, and the unidentified and heavily ensheathed Chromatiaceae, PB2 (Fig. 3 K). Microcolonies of *Chlorobium limicola* were found to be associated to the cyanobacteria at the upper part of this layer and free at the deepest part.

The third dark-green layer was composed of two types of purple sulfur bacteria of the genus *Chromatium* and the green bacteria *Chlorobium*. The *Lyngbya*-like cyanobacterium, and the multicellular filamentous green bacterium *Chloroflexus*-like were also found.

Mat-IV. It was characterized by high cyanobacterial density at the surface and increasing significance of multicellular filamentous green bacteria in depth. Purple sulfur bacteria occasionally organized in bright purple spots visible with the naked eye, whereas green sulfur bacteria aggregates were visible only under the microscope.

The green surface layer was mainly composed of the *Lyngbya*-like and *Pseudanabaena* sp. cyanobacteria. The chemolithotrophic *Beggiatoa* and the Chromatiaceae PB1 were also observed between the end of this layer and the beginning of the next one. In the second layer, cyanobacteria shared dominance with a multicellular filamentous green bacterium *Chromatium* and *Chlorobium* were also observed.

In the 1.8–2 mm layer, macroscopic bright purple spots were observable. These spots were composed of two different purple sulfur bacteria of the genus *Chromatium*, probably *C. minus* and *C. weissei*, after considering biometrics and carotenoids. Cyanobacteria and *Chloroflexus*-like filamentous green bacteria were also present.

The 2–3.5 mm yellowish-green layer was dominated by the *Chloroflexus*-like filamentous green bacterium (Fig. 3 J), which reached the highest densities at the upper part. Cyanobacteria, purple sulfur bacteria (*C. minus, C. weissei, C. vinosum*) and *Chlorobium limicola*, were also observed.

Finally, in the innermost layer, all microbial chlorophylls were detected. A relative enrichment of cyanobacteria was also observed. In addition, the presence of *Chromatium okenii* and *C. warmingii* were deduced from biometrics and carotenoids.

Discussion

Distinctive features of the mats studied herein can be summarized in: (i) their development on vertical walls, in contrast to the commonly described horizontal emplacement on sediments and (ii) the non-thermal, sulfide-containing freshwater environment where they are found.

Gravity and continuous water erosion frequently detach mats from walls, making them thinner than those described elsewhere. Mat-I and Mat-II (1.2, 1.4 mm thick respectively) seem to be two different stages of development of the same mat, which organized differently depending on the sulfide water supply. Mat-I is continuously covered by sulfide containing water, whereas Mat-II grows under an intermittent water flow. Mat-III resisted the continuous water surface agitation through the filamentous matrix formed by both the chemolithotrophic community (*Thiothrix*) and the underlying cyanobacteria. This situation is commonly found in continuously flowing, sulfide-containing environments (2). Only Mat-IV could develop up to 8 mm in thickness due to the leathery texture conferred by cyanobacteria.

Two types of microbiota in depth have been described in mats: the generally described as "normal position", that is, cyanobacteria at the surface and phototrophic sulfur bacteria underneath (1, 10, 13, 14, 22), and the "inverted position", with purple sulfur bacteria at the upper part (8). In Pudosa Spring, Mat-I agrees with a "inverted position", whereas Mat-II and Mat-IV can be considered as "normal-position" mats. Mat-III must be considered apart.

In a mat considered to have a "normal position", both oxygen depletion and sulfide production occur with depth. In contrast, in sulfur springs, oxygen and sulfide coexist in a steady state and cyanobacteria must be sulfide tolerant. In Pudosa environment cyanobacteria perform oxygenic photosynthesis, as the presence of oxygen bubbles retained at the top of Mat-IV demonstrates. Moreover, according to redox potential profiles, some oxidation was observed at the upper part in Mat-IV (dominated by cyanobacteria). Values around –60 mV at the cyanobacterial layer contrasted with those measured in the covering water and at 2 mm to the bottom (–94 mV and –140 mV, respectively). (J. Figueras, unpublished data.)

The inverted position in mats are usually related to the sufficient primary sulfide in the spring source water (sulfur springs) or to seasonal high rates of both sulfate reduction and sulfide diffusion to the surface from deeper layers. *Chloroflexus* (20), *Ectothiorhodospira* (15), *Thiocapsa*, *Chlorobium* (4) and *Chromatium* (12) have been found at the surface of mats from sulfur environments. In hot sulfur springs, the anoxygenic sulfur bacterium that usually develops at the surface of the "inverted position" mats is the filamentous *Chloroflexus*. Cyanobacteria compete poorly in these conditions due to the fact that thermophile strains are inhibited by sulfide (7).

The only "inverted position" mat found in Pudosa Spring, Mat-I, was thoroughly dominated by purple sulfur bacteria. In this mat, surface cyanobacterial growth was limited by overlying sulfide, although they were found in deeper positions. Jørgensen and Nelson (11) reported the presence of cyanobacteria in inverted mats like Mat-I, as a consequence of sulfide depletion from the photosynthetic activity by *Chloroflexus*.

As a general feature, in Pudosa Spring, cyanobacteria were more abundant as sulfide was depleted and the oxygen concentration increased with the atmosphere exposure. This makes "normal-position" mats which have cyanobacteria at the uppermost layer, as found in Mat-II and Mat-IV. Mat-III, in contrast, could not be assigned to either "normal" or "inverted" position. In fact, it is a bacterial aggregate growing at the water-air interface, attached to the wall, and widely dominated by sulfide-oxidating chemolithotrophic bacteria.

The green sulfur bacterium *Chlorobium limicola* was widely found in Pudosa Spring as microcolonies next to purple sulfur bacteria or cyanobacteria. Benthic green sulfur bacteria,

have been previously reported (4, 5, 14), whereas *C. limicola* had not been reported in microbial mats so far.

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References

1. Bauld, J. (1981). Occurrence of benthic microbial mats in saline lakes. Hydrobiologia **81**, 87–111.
2. Bland, J. A., Staley, J. T. (1978). Observations on the biology of *Thiothrix*. Arch. Microbiol. **117**, 79–87.
3. Borrego, C. M., Garcia-Gil, L. J. (1994). Separation of Bacteriochlorophyll homologues by reversed-phase HPLC. Photosynth. Res. **41**, 157–164.
4. Castenholz, R. W. (1987). The green sulfur and non sulfur bacteria of hotsprings. *In* Olson, J. M., Ormerod, J. G., Amesz, J., Stackebrandt, E., Trüper, H. G. (ed.), Green Photosynthetic Bacteria, pp. 243–255. Plenum Press, New York.
5. Clavero, E., Grimalt, J. O., Merino, V., Hernández-Mariné, M. (1993). Structure of the sediment at deposital saline environments. *In* Stal, L. J., Caumette, P. (ed.), Microbial Mats. Structure, development and environmental significance, pp. 91–96. Springer-Verlag (NATO ASI Series G: Ecological Sciences, vol. 35), Berlin.
6. Esteve, I., Martínez-Alonso, M., Mir, J., Guerrero, R. (1992). Distribution, typology and structure of microbial mat communities in Spain: a preliminary study. Limnetica **8**, 185–195.
7. Giovannoni, S. J., Revsbech, N. P., Ward, D. M., Castenholz, R. W. (1987). Obligately phototrophic *Chloroflexus*: primary production in anaerobic hot spring. Arch. Microbiol. **147**, 80–87.
8. Haak, T. K., McFeters, G. A. (1982). Microbial dynamics of an epilithic mat community in a high alpine stream. Appl. Environ. Microbiol. **43**, 702–707.
9. Ingvorsen, K., Jørgensen, B. B. (1979). Combined measurement of oxygen and sulfide in water samples. Limnol. Oceanogr. **24**, 390–393.
10. Javor, B. J., Castenholz, R. W. (1981). Laminated microbial mats in Laguna Guerrero Negro, Mexico. Geomicrobiol. J. **2**, 237–273.
11. Jørgensen, B. B., Nelson, D. C. (1988). Bacterial zonation, photosynthesis, and spectral light distribution in hot spring microbial mats of Iceland. Microbiol. Ecol. **51**, 408–417.
12. Madigan, M. T. (1986). *Chromatium tepidum* sp. nov., a thermophilic photosynthetic bacterium of the family Chromatiaceae. Int. J. Syst. Bacteriol. **36**, 222–227.
13. Mir, J., Martinez-Alonso, M., Esteve, I., Guerrero, R. (1991). Vertical stratification and microbial assemblage of a microbial mat in the Ebro Delta (Spain). FEMS Microbiol. Ecol. **86**, 59–68.
14. Nicholson, J. A. M., Stolz, J. F., Pierson, B. K. (1987). Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiol. Ecol. **45**, 343–364.
15. Oren, A., Keesel, M., Stackebrandt, E. (1989). *Ectothiorhodospira marismortui* sp. nov., an obligately anaerobic moderately halophilic purple sulfur bacterium from a hypersaline sulfur spring on the shore of the Dead Sea. Arch. Microbiol. **151**, 524–529.
16. Palmisano, A. C., Cronin, S. E., Des Marais, D. J. (1988). Analysis of lipophilic pigments from a phototrophic microbial mat community by high performace liquid chromatography. J. Microbiol. Meth. **8**, 209–217.
17. Palmisano, A. C., Cronin, S. E., D'Amelio, E. D., Munoz, E., Des Marais, D. J. (1989). Distribution of lipohilic pigments in a laminated microbial mat community near Guerrero Negro, Mexico. *In* Cohen, Y., Rosenberg, E. (ed.), Microbial Mats. Physiological Ecology of Benthic Microbial Communities. pp. 138–152. American Society for Microbiology, Washington.
18. Pfennig, N., Trüper, H. G. (1989). Anoxigenic phototrophic bacteria. *In* Staley, J. T., Bryant, M. P., Pfennig, N., Holt, J. G. (ed.), Bergey's Manual of Systematic Bacteriology, vol. 3, pp. 1635–1709. Williams & Wilkins, Baltimore.
19. Pfennig, N., Trüper, H. G. (1992). The family Chromatiaceae. *In* Balows, A., Trüper, H. G., Dworkin, M., Harder, W., Schleifer, K. H. (ed.),

The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, vol. 4, pp. 3200–3221. Springer-Verlag, New York.

20. Pierson, B. K., Castenholz, R. W. (1974). A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus auriantiacus*, gen. and sp. nov. Arch. Microbiol. **100**, 5–24.
21. Rippka, R. (1988). Recognition and identification of cyanobacteria. Meth. Enzymol. **167**, 28–67.
22. Stal, L. J., Krumbein, W. E. (1985). Isolation and characterization of cyanobacteria from a marine microbial mat. Botanica Marina **28**, 351–365.
23. Stal, L. J., van Gemerden, H., Krumbein, W. E. (1984). The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. J. Microbiol. Meth. **2**, 295–306.
24. Stock, M. S., Ward, A. K. (1989). Establishment of a bedrock epilithic community in a small stream: Microbial (algal and bacterial) metabolism and physical structure. Can. J. Fish. Aquat. Sci. **46**, 1874–1883.
25. Strzeszewski, B. (1913). Beitrag zur Kenntnis der Schwefelflora in der Umgebung von Krakau. Bulletin de l'Academie des Sciences de Cracovie. Serie B, 309–334.
26. Szafer, W. (1910). Zur Kenntnis de Schwefelflora in der Umgebug von Lemberg. Bulletin de l'Academie des Sciences de Cracovie. Serie B, 161–167.
27. Trüper, H. G., Pfennig, N. (1992). The family Chlorobiaceae. *In* Balows, A., Trüper, H. G., Dworkin, M., Harder, W., Schleifer, K. H. (ed.), The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, vol. 4, pp. 3583–3592. Springer-Verlag, New York.
28. van Gemerden, H., Tughan, C. S., de Wit, R., Herbert, R. A. (1989). Laminated microbial ecosystem on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbiol. Ecol. **62**, 87–102.

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α-Tubulin of *Histriculus cavicola* (Ciliophora; Hypotrichea)

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Summary

An α-tubulin gene fragment amplified by PCR from the hypotrichous ciliate *Histriculus cavicola* has been sequenced. This fragment, 1,182 bp long, contains an in-frame "stop" codon (UAA), which in other hypotrichous species codes for a glutamine residue. The comparison of the α-tubulin genes from several ciliates classes have revealed amino acid positions which could serve to distinguish these taxonomic groups.

Key words: *Histriculus cavicola*, α-tubulin, UAA codon, phylogeny, hypotrichs

Resumen

Se ha secuenciado un fragmento de un gen de la α-tubulina, amplificado por PCR, del ciliado hipotrico *Histriculus cavicola*. Este fragmento, de 1182 pb, contiene un codón de terminación (UAA) en la fase de lectura, que en otros ciliados hipotricos codifica la glutamina. La comparación de los genes de la α-tubulina de varias clases de ciliados ha mostrado que algunas posiciones de aminoácidos pueden servir para distinguir estos grupos taxonómicos.

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Introduction

Microtubules are present in all eukaryotic cells as a major component of the cytoskeleton. They are involved in many cellular functions, such as motility, cell division, location of the Golgi and endoplasmic reticulum (ER), and establishment of cell polarity (8).

The major protein of microtubules is tubulin, a heterodimer of two distinct polypeptides designated as α and β. Each subunit has a molecular mass of about 50,000 Da and consists of different isotypes which are highly conserved in eukaryotic evolution. Both are generally post-translationally modified (18).

In higher eukaryotes, α- and β-tubulins are encoded by complex gene families. Tubulin heterogeneity therefore can be attributed both to primary genetic diversity and to a variety of post-translational modifications (5, 6, 12, 13).

Ciliates have a large variety of microtubular networks with functional and morphological differences. At least 13 distinct microtubular arrays have been described in *Paramecium* (7). In spite of their microtubular diversity, both α-tubulins and β-tubulins of ciliates are encoded by a small number of strikingly similar genes. In fact, most differences in the properties of the diverse microtubular arrays can be attributed to post-translational modifications and/or to interactions with accesory proteins (4).

Genes coding for α-tubulin have been sequenced in fifteen species belonging to six classes of the phylum Ciliophora: *Stylonychia lemnae*, *Oxytricha granulifera*, *Euplotes octocarinatus*, *Euplotes vannus*, *Euplotes aediculatus* (Class Hypotrichea), *Tetrahymena pyriformis*, *Tetrahymena thermophila*, *Frontonia* sp. (Class Oligohymenophorea), *Zosterograptus* sp. (Class Nassophorea), *Stentor coeruleus*, *Condylostoma magnum* (Class Heterotrichea), *Spathidium* sp., *Epidinium* sp., *Entodinium* sp. (Class Litostomatea) and *Loxodes striatus* (Class Karyorelictea).

We sequenced a 1,182 bp fragment of an α-tubulin gene from *Histriculus cavicola*, a hypotrichous ciliate that forms resting cysts under adverse conditions. This sequence contains a canonical in-frame "stop" codon (UAA) that instead codes for a glutamine residue. Comparison of the amino acid sequences of *Histriculus* α-tubulin with α-tubulins from other ciliates indicates that some amino acids are characteristic for each of the ciliate classes studied. In addition, our results support taxonomic relationships based on rRNA sequences.

Material and methods

H. cavicola was grown at a temperature of 20°C in Pringsheim's solution and fed with the green algae *Chlorogonium* sp. DNA was isolated from starved vegetative cells following conventional methods (14). *Histriculus* α-tubulin gene was amplified with Taq polymerase (Boehringer) from genomic DNA using degenerate oligonucleotides as primers. The sense and antisense primers, based on the conserved CLEHGIQ (position 25–31 respect to *Tetrahymena pyriformis* published α-tubulin, standard and referred gene in this paper) and MEEGEF (position 413–418) amino acid sequences, were:

5' TGYYTKGAGCAYGGTATYCAACC 3'
5' SAGAAYTCTCCTTCYTCCAT 3'

PCR amplification conditions were as follows: denaturation at 94°C for 30 s, annealing at 55°C for 30 s and polymerization at 72°C for 1 min (35 cycles). The amplified product was isolated and blunt-end ligated into the *Eco*RV site of the pBluescript II KS vector (Stratagene, La Jolla, CA, USA). The insert was sequenced

using the DIG-Taq sequencing kit (Boehringer) and the direct blotting electrophoresis (MWG-Biotech). The sequence is in the EMBL database, with the accession number Y10035.

The amino acid sequences were aligned with the following α-tubulin sequences taken from the GenEMBL database (with their respective accession numbers): *Stylonychia lemnae* (X01746 and X12365); *Oxytricha granulifera* (Z11763); *Euplotes octocarinatus* (X69466); *Euplotes vannus* (Z11769); *Euplotes aediculatus* (Z49851); *Tetrahymena pyriformis* (X12767); *Tetrahymena thermophila* (M86723); *Zosterographtus* sp. (Z49855); *Stentor coeruleus* (Z49853); *Condylostoma magnum* (Z49850); *Spathidium* sp. (Z49848); *Epidinium* sp. (Z49856); *Entodinium* sp. (Z49849); *Loxodes striatus* (Z49852). The sequence of *Frontonia* sp. was kindly provided by Anne Baroin-Tourancheau (Orsay, France).

Computer analyses were made through the Wisconsin Sequence Analysis Package (Genetic Computer Group), version 8.1. The alignment was performed using PILEUP program.

Results and discussion

PCR reactions gave a single band of about 1.1 kb. No bands were observed in those reactions lacking template. The amplified DNA was cloned and several positive selected. Among the positive clones, one was completely sequenced in both strands. The fragment contained 1,182 nucleotides.

The nucleotide and deduced amino acid sequence is presented in Fig. 1. This DNA fragment contains most (90%) of the coding region of a standard α-tubulin gene, e.g., *Tetrahymena pyriformis*, and does not contain introns.

Four putative GTP binding sites (15) were present: VPRCVFLDLEP (aa. 62–72) and RFDGAL (aa. 243–248), both involved in base binding; GGGTGSG (aa. 142–148), implicated in ribose binding; and AVVEPYN (aa. 180–186), involved in phosphate binding. The fragment has a lysine residue located at position 40, which may serve as a substrate for a post-translational acetylation (16).

The sequence contains a UAA codon in frame (nucleotide 526–528) that, as described in other hypotrichs (2), is used as a sense codon for a glutamine residue.

The G+C content of the fragment was 49.6%. The codon usage for this gene fragment is showed in Table 1. Of the 61 existing codons, only 38 are used. Three of them are used once, and six are used twice. In addition to methionine and tryptophan, five amino acids are codified by only one codon (arg/AGA, asn/AAC, cys/TGC, phe/TTC, gln/CAA). The nucleotide third-position preference can be summarized as follows: C(44.5%)>T(23.3%)>A(21.7%)>G(10.5%). Of the codons used, 45% have A or T in the third position.

Codon bias correlates with the level of the gene expression in a wide range of organisms (3). In general, genes coding for highly expressed proteins show a strong bias for codons complementary to the major isoacceptor tRNA species.

Sequence analyses of additional *Histriculus* genes will be required to verify whether the bias in the α-tubulin gene is representative of the codon subset used to encode highly expressed *Histriculus* proteins.

The *Histriculus* gene fragment shows a great degree of sequence similarity with other ciliates. The nucleotide homology between this fragment (taking met-32 to gly-412) and other ciliate α-tubulins is shown in Table 2. The *Histriculus* fragment is most similar to other hypotrichous ciliate tubulin genes, e.g., *Oxytricha* (92.5%) and *Stylonychia* (93.9–93.4%). The percentage of identity with the euplotids ranges from 87.7% to 85.4%. In descending order of similarity, the

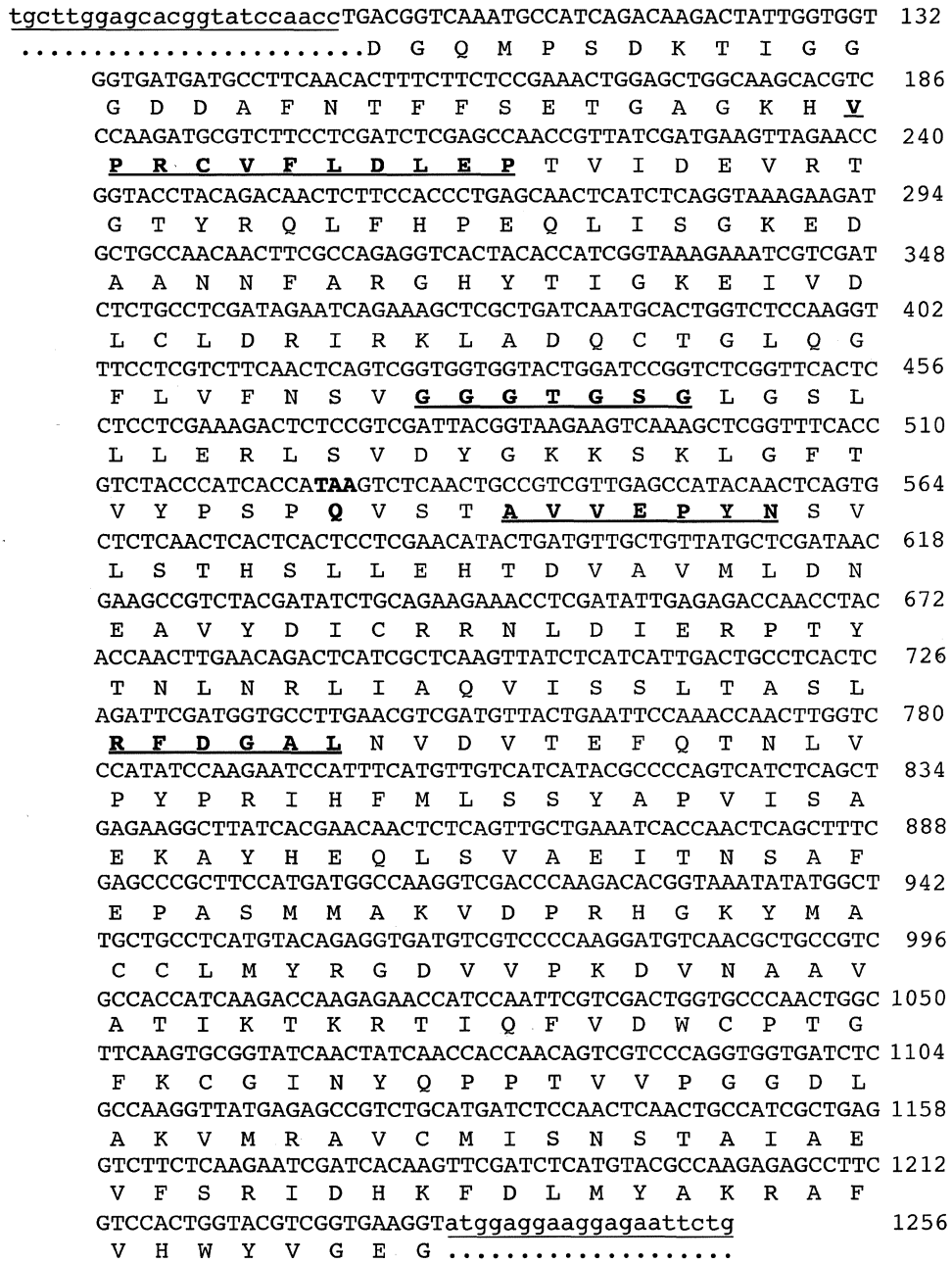

FIG. 1. Nucleotide and predicted amino acid sequence (one code letter) of the amplified fragment from *Histriculus* α–tubulin. The sequence of the synthetic primers used for PCR are underlined. The in-frame UAA "stop" codon and the corresponding gln are in bold letters. The GTP-binding domains are in bold and underlined. The numbering scheme refers to the nucleotides of the published *Tetrahymena pyriformis* α-tubulin gene.

TABLE 1. Codon usage of the amplified fragment of *Histriculus* α-tubulin. Underlined codons denote that they are the most frequently or nearly equally used for a given amino acid. The numbers are the total number of occurrences of each codon. The in-frame "stop" codon is in bold letter

Codon	aa.	No.									
TTT	phe	–	TCT	ser	–	TAT	tyr	4	TGT	cys	–
TTC	phe	19	TCC	ser	5	TAC	tyr	11	TGC	cys	10
TTA	leu	–	TCA	ser	20	**TAA**	**gln**	**1**	TGA	OPA	–
TTG	leu	6	TCG	ser	–	TAG	AMB	–	TGG	trp	2
CTT	leu	–	CCT	pro	2	CAT	his	2	CGT	arg	–
CTC	leu	26	CCC	pro	2	CAC	his	9	CGC	arg	–
CTA	leu	–	CCA	pro	15	CAA	gln	11	CGA	arg	–
CTG	leu	–	CCG	pro	–	CAG	gln	–	CGG	arg	–
ATT	ile	2	ACT	thr	12	AAT	asn	–	AGT	ser	–
ATC	ile	18	ACC	thr	12	AAC	asn	15	AGC	ser	–
ATA	ile	–	ACA	thr	1	AAA	lys	3	AGA	arg	19
ATG	met	11	ACG	thr	–	AAG	lys	15	AGG	arg	–
GTT	val	9	GCT	ala	13	GAT	asp	20	GGT	gly	26
GTC	val	25	GCC	ala	16	GAC	asp	4	GGC	gly	2
GTA	val	–	GCA	ala	–	GAA	glu	13	GGA	gly	3
GTG	val	1	GCG	ala	–	GAG	glu	9	GGG	gly	–

percentage of identities are: *Tetrahymena*, 86.7–86%; *Frontonia*, 86.5%; *Stentor*, 85%; *Zosterographus*, 81.4%; *Loxodes*, 78.8%; litostomes (*Epidinium*, *Entodinium* and *Spathidium*), 78.8–76.9%; and *Condylostoma*, 77.9%.

As expected, nucleotide sequence homology was lower than the corresponding amino acid sequence homology (Table 2). Percentages of similarity between the deduced amino acid sequence of α-tubulin of *Histriculus* and those of the ciliates listed above ranged from 99.5% (*Stylonychia*) to 93.4% (*Spathidium*).

The alignment of the *Histriculus* α-tubulin amino acid sequence with those of other ciliates reveals some interesting points (Fig. 2). Several traditional taxonomic groups differ in the identity of amino acids present at a few key positions in the α-tubulin protein sequence. Table 3 summarizes the characteristic amino acid substitutions at several positions in the α-tubulin of litostomes, oligohymenophorans, pseudohypotrichs (Euplotia) and euhypotrichs (Oxytrichia).

The litostomes and the euplotids contain nine and five characteristic amino acids, respectively. These amino acid changes strongly support the uniquely derived status of these clades. The euhypotrichs show two characteristic amino acids and their α-tubulin sequences contain at the position 526–528 an in-frame UAA codon which codifies for glutamine 176. The oligohymenophorans contain asparagine at position 300 and serine at position 316.

Our sequence analysis indicates that a deep split exits between pseudohypotrichs, such as *Euplotes*, and the euhypotrichs, such as *Histriculus*, *Stylonychia* and *Oxytricha*. The data agree with the results of Baroin-Tourancheau et al. (1), who reported a large divergence be-

TABLE 2. Nucleotide (a) and amino acid (b) homology (% identity) between the *Histriculus cavicola* α-tubulin and the α-tubulin from other ciliates

Ciliate	a	b
Class Hypotrichea		
Subclass Oxytrichia (Euhypotrichs)		
Stylonychia lemnae 1	93.9	99.5
Stylonychia lemnae 2	93.4	98.2
Oxytrichia granulifera	92.5	97.0
Subclass Euplotia (Pseudohypotrichs)		
Euplotes octocarinatus	87.7	96.8
Euplotes aediculatus	87.5	95.8
Euplotes vannus	85.4	94.7
Class Oligohymenophorea		
Tetrahymena pyriformis	86.7	96.3
Tetrahymena thermophila	86.0	96.0
Frontonia sp.	86.5	95.8
Class Heterotrichea		
Stentor coeruleus	85.0	95.0
Condylostoma magnum	77.9	97.4
Class Nassophorea		
Zosterograptus sp.	81.4	96.0
Class Karyorelictea		
Loxodes striatus	78.8	95.8
Class Litostomatea		
Epidinium sp.	78.8	93.7
Entodinium sp.	77.6	93.5
Spathidium sp.	76.9	93.4

tween *Euplotes* and the euhypotrichs based on rRNA sequence analysis.

Surprisingly, the single representative of nassophoreans, *Zosterograptus*, contains, as do the euhypotrichs, glutamine at position 128 and leucine at position 317. Additional α-tubulin sequences are required to test the association of the nassophoreans with the euhypotrichs.

In the heterotrichs (*Stentor*, *Condylostoma* and *Blepharisma* [data not shown]) an asparagine residue is replaced by a glutamine at position 215. The same amino acid substitution is also observed in the karyorelictid *Loxodes* and in the litostomes. Recently, both Baroin-Tourancheau et al. (1) and Hammerschmidt et al. (10), used rRNA sequences to demostrate that the karyorelicteans are the sister group of the heterotrichs.

To sum up, the α-tubulin genes are good markers for the inference of relationships among ciliates. The comparative analysis of α-tubulin sequences in general supports classical taxonomic groups based on ultrastructural and other morphological characters. In addition, the analysis reinforces phylogenetic relationships derived from comparisons of rRNA sequences, for example the split between euplotids and euhypotrichs, and the similarity between *Loxodes* and the heterotrichs.

```
                          40         50         60         70         80         90
H. cavicola          DGQMPSDK TIGGGDDAFN TFFSETGAGK HVPRCVFLDL EPTVIDEVRT GTYRQLFHPE
O.granulifera        -------- ---------- ---------- ---------- ----D----- ----------
S. lemnae (1)        -------- ---------- --------E- ---------- ---------- ----------
S. lemnae (2)        ------N- ---------- -------T-- ---------- ---------- ----------
Zosterograptus sp.   -------- ---------T ---------- Y-----M--- ---------- ----------
E. vannus            -------- ---------- ---------- ----A-LV-- ----C--I-- ----------
E. aediculatus       -------- ---------- ---------- ----A----- ----C----- ----------
E. octocarinatus     -------- ---------- ---------- ----A----- ----C----- ----------
Entodinium sp.       -------- ---------- ---------- ----S----- ----V----- ----------
Epidinium sp.        -------- ---------- ---------- ----S----- ---------- ----------
Spathidium sp.       -------- ---------- ---------- ---------- ----V----- ----------
T. pyriformis        -------- ---------- ---------- ----A----- ---------- ----------
T. thermophila       -------- ---------- ---------- ----A----- ---------- ----------
S. coeruleus         -------- ---------- ---------- ----A----- ---------- ----------
C. magnum            -------- ---------- ---------- ----A----- ----V----- --I-------
L. striatus          -------- ---------- ---------- ----S----- ---------- ----------
                         100        110        120        130        140        150
H. cavicola          QLISGKEDAA NNFARGHYTI GKEIVDLCLD RIRKLADQCT GLQGFLVFNS VGGGTGSGLG
O. granulifera       ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)        ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (2)        ---------- ---------- ---------- G--------- ---------- ----------
Zosterograptus sp.   ---------- ---------- ---------- ---------P ---------- ----------
E. vannus            -I-------- ---------- -------V-- -------N-- -----IG-H- ----------
E. aediculatus       ---------- ---------- ---------- ---R---N-- -----IG-H- ----------
E. octocarinatus     ---------- ---------- ---------- -------N-- -----IG-H- ----------
Entodinium sp.       ---------- --Y---YC-- ----I--A-- -------N-- ---------A ----------
Epidinium sp.        ---------- --Y---YC-- ----I--A-- -------N-- ---------A ----------
Spathidium sp.       ---------- --Y---YC-- ----I--A-- -------N-- ---------A ----------
T. pyriformis        ---------- ---------- ---------- -------N-- ---------- ----------
T. thermophila       ---------- ---------- ---------- -------N-- ---------- ----------
S. coeruleus         ---T------ ---------- ---------- -------N-- ---------- ----------
C. magnum            ---------- ---------- ---------- -------N-- ---------- ----------
L. striatus          ---------- ---------- ---------- -------N-- ---------A ----------
                         160        170        180        190        200        210
H. cavicola          SLLLERLSVD YGKKSKLGFT VYPSPQVSTA VVEPYNSVLS THSLLEHTDV AVMLDNEAVY
O. granulifera       ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)        ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (2)        ---------- ---------- I--------- ---------- ---------- ----------
Zosterograptus sp.   ---------- ---------- I--------- ---------- ---------- --------I-
E. vannus            ---------- ---------- I--------- ---------- ---------- ----------
E. aediculatus       ---------- -------T-- I--------- ---------- ---------- ----------
E. octocarinatus     ---------- -------T-- I--------- ---------- ---------- ----------
Entodinium sp.       ---------- ---------- ------I-N- -------I-- ---------- --------I-
Epidinium sp.        ---------- ---------- ------I-N- -------I-- ---------- --------I-
Spathidium sp.       ---------- ---------- I-----I-N- -------I-- ---------- --------I-
T. pyriformis        ---------- ---------- I--------- -------I-- ---------- --------I-
T. thermophila       ---------- ---------- I--------- -------I-- ---------- --------I-
S. coeruleus         ---------- ---------- I--------- ---------- ---------- ----------
C. magnum            ---------- ---------- IH-------- ---------- ---------- ----------
L. striatus          ---------- ---------- ---------- ---------- ---------- --------I-
                         220        230        240        250        260        270
H. cavicola          DICRRNLDIE RPTYTNLNRL IAQVISSLTA SLRFDGALNV DVTEFQTNLV PYPRIHFMLS
O. granulifera       ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)        ---------- ---------- ---------- ---------- ---------- ---SVIIR--
S. lemnae (2)        ---------- ---------- ---------- ---------- ---------- ----------
Zosterograptus sp.   ---------- ---------- -S-------- ---------- -I-------- ----------
E. vannus            ---------- ---------- ---------- ---------- -I-------- ----------
E. aediculatus       ---------- ---------- ---------- ---------- -I-------- ----------
E. octocarinatus     ---------- ---------- ---------- ---------- ---------- ----------
Entodinium sp.       -----Q----- ---------- ---------- ---------- -M-------- ----------
Epidinium sp.        -----Q----- ---------- ---------- ---------- -M-V------ ----------
Spathidium sp.       -----Q----- ---------- ---------- ---------- ---V------ ----------
T. pyriformis        ---------- ---------- ---------- ---------- -I-------- ----------
T. thermophila       ---------- ---------- ---------- ---------- -I-------- ----------
S. coeruleus         -----Q----- ---------- ---------- ---------- ---------- ----------
C. magnum            -----Q----- ---------- ---------- ---------- ---------- ----------
L. striatus          -----Q----- ---------- ---------- ---------- ---------- ----------
```

FIG. 2. Comparison of the deduced amino acid sequence of *Histriculus* α-tubulin is shown on the upper line. Amino acid identities are dotted, while differences are denoted by the corresponding single letter, indicating the substituted amino acid. The numering refers to the corresponding deduced amino acid sequence of the published *Tetrahymena pyriformis* α-tubulin gene. (For complete names of the genera, see text.)

The structure of ciliate phylogeny has traditionally rested on ultrastructural features of the cortex and of somatic and oral ciliature. The absence of derived morphological characters that unite groups and the difficulty in distinguishing between ancestral and derived morphologies make this kind of phylogeny assessment somewhat speculative. Recently, phylogenetic relationships within the phylum have been investigated by using 18S (9, 11,17) and 28S rRNAs (1). We believe extensive analyses of the α-tubulin sequences of ciliates should lead to the construction of convincing molecular trees. These results can be compared to rRNA trees and to phylogenies inferred from traditional morphological features. This approach will test the con-

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                          280        290        300        310        320        330
H. cavicola               SYAPVISAEK AYHEQLSVAE ITNSAFEPAS MMKAVDPRHG KYMACCLMYR GDVVPKDVNA
O. granulifera            ---------- ---------- ---------- ----C----- ---------- ----------
S. lemnae (1)             ----STP--- ---------- ---------- ----C----- ---------- ----------
S. lemnae (2)             ---------- ---------- ---------- ----C----- ---------- ----------
Zosterographus sp.        ---------- ---------- ----VY---- ----C----- ---------- --A-------
E. vannus                 --G------- ---------- ----S----- ----C----- ------M-F- ----------
E. aediculatus            ---------- ---------- ----S----- ----C----- ------M--- --------D-
E. octocarinatus          ---------- ---------- ----S----- ----C----- ------M--- ----------
Entodinium sp.            --R-I----- ---------- ---------- ----C----- ------M--- ----------
Epidinium sp.             ----I----- --D------- ---------- ----C----- ------M--- ----------
Spathidium sp.            ----I----- ---------- ----S----- ----C----- ------M--- ----------
T. pyriformis             ----I----- ---------- ---------N ----C----- -----SM--- ----------
T. thermophila            ----I----- ---------- ---------N ----C----- -----SM--- ----------
S. coeruleus              ----I----- ---------- ---------- ----C----- ------M--- ----------
C. magnum                 ----I----- ---------- ----T----- ----C----- ------M--- ----------
L. striatus               ----I----- ---------- ----V----- S-V-C----- ------M--- ----------
                          340        350        360        370        380        390
H. cavicola               AVATIKTKRT IQFVDWCPTG FKCGINYQPP TVVPGGDLAK VMRAVCMISN STAIAEVFSR
O. granulifera            ---------- ---------- ---------- ---------- ---------- ----------
S. lemnae (1)             ---------- ---------- ---------- ----S--P-- ---------- ----------
S. lemnae (2)             ---------- ---------- ---------- ----S----- ---------- ----------
Zosterographus sp.        ---------- ----N----- ---------- A--------- ---------- ----------
E. vannus                 ---------- ------S--- --V------- ---------- ---------- ----------
E. aediculatus            ---------- ------S--- ---------- ---------- ---------- ----------
E. octocarinatus          ---------- ------S--- ---------- ---------- ---------- ----------
Entodinium sp.            S--------- ---------- ---------- ---------- ---------- ----------
Epidinium sp.             S--------- ---------- --------FL ---------- ---------- ----------
Spathidium sp.            ---------- ---------- ---------- ---------- ---------- ----------
T. pyriformis             SI-------- ---------- --V------S ---------- ---------- ----------
T. thermophila            SI-------- ---------- --V------- ---------- ---------- ----------
S. coeruleus              ---------- ---------- ---------- -------H-- ---------- ----------
C. magnum                 ---------- ---------- ---------- ---------- ---------- ----------
L. striatus               S--------- ---------- ---------- ---------- ---------- T---------
                          400        412
H. cavicola               IDHKFDLMYA KRAFVHWYVG EG
O. granulifera            ---------- ---------- --
S. lemnae (1)             ---------- ---------- --
S. lemnae (2)             ---------- ---------- --
Zosterographus sp.        ---------- ---------- --
E. vannus                 ---------- ---------- --
E. aediculatus            ---------- ---------- --
E. octocarinatus          ---------- ---------- --
Entodinium sp.            L--------T -------F-- --
Epidinium sp.             L--------T -------F-- --
Spathidium sp.            L--------T -------F-- --
T. pyriformis             L--------- ---------- --
T. thermophila            L--------- ---------- --
S. coeruleus              ---------- ---------- --
C. magnum                 ---------- ---------- --
L. striatus               ---------- ---------- --
```

FIG. 2. (*Continued.*)

TABLE 3. Characteristic amino acids in the α-tubulin of some ciliate groups. Characteristic amino acids and their position are written in bold. Amino acids inside the parenthesis refer to those normally found outside the group

Group	Species	Marker
Class Litostomatea	*Entodinium* sp.	**tyr-103** (phe); **tyr-107** (his)
	Epidinium sp.	**cys-108** (tyr); **ile-115**(val)
	Spathidium sp.	**ala-118** (cys/val); **ile-177**(val)
		asn-179 (thr); **thr-400** (ala)
		phe-408 (tyr)
Subclass Euplotia (Pseudohypotrichs)	*Euplotes aediculatus*	**cys-75** (ile/asp); **ile-136** (leu)
	Euplotes vannus	**gly-137** (val); **his-139** (asn)
	Euplotes octocarinatus	**ser-347** (cyst)
Subclass Oxytrichia (Euhypotrichs)	*Oxytrichia granulifera*	**gln-128** (asn)
	Stylonychia lemnae	**leu-317** (met)
	Histriculus cavicola	
Class Oligohymenophorea	*Tetrahymena pyriformis*	**asn-300** (ser)
	Tetrahymena thermophila	**ser-316** (cys)
	Frontonia sp.	

gruencies/incongruencies between two molecular markers along with more classical approaches.

Acknowledgements

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References

1. Baroin-Tourancheau, A., Delgado, P., Perasso, R., Adoutte, A. (1992). A broad molecular phylogeny of ciliates: Identification of major evolutionary trends and radiations within the phylum. Proc. Natl. Acad. Sci. USA **89**, 9764–9768.
2. Baroin-Tourancheau, A., Tsao, N., Klobutcher, L. A., Pearlmann, R. E., Adoutte, A. (1995). Genetic code deviations in the ciliates: Evidence for multiple and independent events. EMBO J. **14**, 3262–3267.
3. Bennetzen, J. L., Hall, B. D. (1982). Codon selection in yeast. J. Biol. Chem. **257**, 3026–3031.
4. Callen, A. M., Adoutte, A., Andreu, J. M., Baroin-Tourancheau, A., Bré, M. H., Calvo Ruiz, P., Clerot, J. C., Delgado, P., Fleury, A., Wolf, R. J., Viklicky, V., Villalobo, E., Levilliers, N. (1994). Isolation and characterization of libraries of monoclonal antibodies directed against various forms of tubulin in *Paramecium*. Biol. Cell. **81**, 95–119.
5. Cleveland, D. W. (1987). The multitubulin hypothesis revisited. What have we learned. J. Cell Biol. **104**, 381–383.
6. Cleveland, D. W., Sullivan, R. F. (1985). Molecular biology and genetics of tubulin. Annu. Rev. Biochem. **54**, 331–365.
7. Cohen, J., Beisson, J. (1988). The cytoskeleton. *In* Gortz, H. D. (ed.), *Paramecium*, pp. 363–392. Springer-Verlag, Berlin-Heidelberg.
8. Dustin, P. (1984). Microtubules. Springer-Verlag. New York.
9. Greenwood, S. J., Sogin, M. L., Lynn, D. H. (1991). Phylogenetic relationships within the class Oligohymenophorea, phylum Ciliophora, inferred from the complete small subunit rRNA gene se-

quences of *Colpidium campylum*, *Glaucoma chatoni* and *Opisthonecta henneguyi*. J. Mol. Evol. **33**, 163–174.

10. Hammerschmidt, B., Schlegel, M., Lynn, D. H., Leipe, D. D., Sogin, M. L., Raikov, I. B. (1996). Insights into the evolution of nuclear dualism in the ciliates revealed by phylogenetic analysis of rRNA sequences. J. Euk. Microbiol. **43**, 225–230.
11. Leipe, D. D., Bernhard, D., Schlegel, K. M. (1994). Evolution of 16S-like ribosomal RNA genes in the ciliophoran taxa Litostomatea and Phyllopharyngea. Eur. J. Protistol. **30**, 354–361.
12. Ludueña, R. F. (1993). Are tubulin isotypes functionally significant? Mol. Biol. Cell **4**, 445–457.
13. Mandelkow, E. M., Herman, M., Ruhl, U. (1985). Tubulin domains probed by limited proteolysis and subunit-specific antibodies. J. Mol. Biol. **185**, 311–327.
14. Maniatis, T., Fritsch, E. F., Sambrook, J. (1982). Molecular cloning: A Laboratory Manual. Cold Springs Harbor Laboratory, Cold Spring Harbor, New York.
15. McRae, T. H., Langdon, C. M. (1989). Tubulin synthesis, structure, and function: what are the relationships? Biochem. Cell Biol. **18**, 770–790.
16. Piperno, G., Fuller, M. T. (1985). Monoclonal antibodies specific for an acetylated form of α-tubulin recognize the antigen in cilia and flagella from a variety of organisms. J. Cell Biol. **101**, 2085–2094.
17. Schlegel, M. (1991). Protist evolution and phylogeny as discerned from small subunit ribosomal RNA sequence comparisons. Europ. J. Protistol. **27**, 207–219.
18. Sullivan, K. F. (1988). Structure and utilization of tubulin isotypes. Annu. Rev. Cell Biol. **4**, 687–716.

MICROBIOLOGÍA SEM **13** (1997), 67–70

The genus *Rhodosporidium*: a potential source of β-carotene

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Summary

Four wild-type species of the genus *Rhodosporidium* have been studied as possible sources for the industrial production of β-carotene. HPLC-based studies showed that their carotenoid composition consisted of almost pure β-carotene at concentrations ranging from 226 to 685 μg/g of dried yeast biomass. These results are consistent with those obtained by spectrophotometry at 480 nm.

Key words: *Rhodosporidium*, β-carotene, HPLC, quantification, extraction

Resumen

Se han estudiado cuatro cepas silvestres del género *Rhodosporidium* como posibles fuentes para la producción industrial de β-caroteno. Estudios de cromatografía líquida de alta resolución (HPLC) han puesto de manifiesto una composición de β-caroteno prácticamente puro a concentraciones entre 226 y 685 μg/g de peso seco de levadura. Estos resultados concuerdan con los que se han obtenido por medio de espectrofotometría a 480 nm.

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Introduction

The interest of β-carotene and other carotenoids lies not only in their role as provitamin A and in their use as food colorants (2). They have also antioxidant properties (10), photoprotection activity (9), as well as capacity to act as defense systems and possible anticarcinogenic properties (4). These substances can be synthesized only by plants and microorganisms, which are the natural sources for the pigmentation of certain animals. Many carotenoid-producing microorganisms have been extensively studied to evaluate their possible industrial interest (1), but to the best of our knowledge only the halophilic alga *Dunaliella* is commercially produced as a source of β-carotene (3). Although yeasts are among the most efficient tools for the production of natural molecules in biotechnology, until now, the only economically valuable carotenoid production process using yeasts is the one used to produce of astaxanthin by *Phaffia rhodozyma* (7, 8). In recent years, species of *Rhodosporidium* have aroused increasing interest because they produce lipids of commercial interest or compounds with potential application in medicine, such as the phenylalanine ammonia-lyase (PAL) enzyme (6). In this paper, we report the study of four wild-type species of the genus *Rhodosporidium*; the results suggest that they could be efficient microbiological sources of β-carotene.

Materials and methods

Strains and medium. The *Rhodosporidium* strains used were: *Rh. toruloides* (CBS 349); *Rh. sphaerocarpum* (CBS 5941); *Rh. paludigenum* sexual type A_1 (CBS 6567) and *Rh. paludigenum* sexual type A_2 (CBS 6566).

The medium used for yeast growth and maintenance was YM (yeast extract: 3 g/l; malt extract: 3 g/l; peptone: 5 g/l; glucose: 10 g/l; plus agar [30 g/l] if solid media was to be used).

Reagents and chemicals. All solvents were HPLC-grade from Romil Chemicals, but from the dimethylsulfoxide (DMSO) used for the carotenoid extraction, which was from Merck.

Sample preparation for HPLC. The four strains of *Rhodosporidium* were grown in YM broth on a rotary shaker at 200 rpm and 30°C for three days. In some experiments the growth temperature was increased to 35°C to check both yeast ability to grow and to synthetize β-carotene. Then, cells from 40 ml samples were harvested by centrifugation at 4000 rpm for 5 min, washed with sterile water and resuspended in 10 ml of DMSO. Afterwards, the biomass was heated at 55°C and vortexed for 30 s (11). Following this, 1 ml of 0.1 M phosphate buffer pH 7.0 and 10 ml of hexane-fraction from petroleum were added and mixed by vortexing for an additional minute. Finally, samples were filtered through 0.22 μm Gelman glass-fiber membranes and stored at –20°C until analyzed.

Carotenoid analysis and standards. Chromatographic separations were performed by high performance liquid chromatography (HPLC) on a Beckman System Gold chromatograph with an Ultrasphere silica 5 μm, 250 × 4.6 mm column (Teknokroma), protected by an Ultrasphere 5 μm, 45 × 4.6 mm guard column (Teknokroma). The eluting solvent was the hexane-fraction from petroleum/ethyl acetate 1/1 (v/v), and flow rate was 1 ml/ min. The eluant was monitored at 480 nm. β-Carotene (Sigma) was used as the standard and its concentration in the different samples was calculated in relation to the linear calibration curve (peak area vs. concentration) of the standard stock solutions.

Dry weight estimation. Dry weight estimation was performed by taking 1 ml samples with different absorbance at 600 nm and cells precipitated in 10 ml of 10% trichloroacetic acid (TCA)

at 0°C. After 5 min, the cells were filtered through a previously dried and weighed 0.22 μm Gelman glass-fiber membrane. Afterwards, the filter was dried at 80°C for 3 h and weighed again. The difference between both mesurements was the dried weight of yeast contained in 1 ml of culture with that optical density.

Results and discussion

Fig. 1 shows typical chromatograms of the carotenoids extracted from the four species of *Rhodosporidium*. β-carotene is the main compound, representing up to 98% of the total carotenoid content in all strains. The quantitative results from HPLC-analysis are summarized in Table 1. We also observed that the four *Rhodosporidium* species did not have special nutritional requirements and could even grow and become pigmented in poor culture media. The optimal growth temperature ranges from 30 to 35°C, depending on the species, which is higher than the optimal temperature for other industrial yeasts (e.g. 22–24°C for *Phaffia rhodozyma*). In fact, *Rh. toruloides* and *Rh. sphaerocarpum* may grow faster and carry out β-carotene synthesis efficiently (Fig. 2) at 35°C, with no apparent loss in their carotenoid content. All these observations suggest that the genus *Rhodosporidium* would be a good microbiological source of β-carotene, since all chromatograms so far analyzed show a main peak of β-carotene whose area corresponds to 96–98% of the total peak-area. The amount of this pigment is high enough for the genus *Rhodosporidium* to be considered as an alternative source of β-carotene of possible industrial application. Moreover, it has to be pointed out that the results reported here correspond to wild-type strain. The carotenoid content may be easily increased (up to 10 times) by the optimization of the culture medium, the addition of certain precursors such as mevalonic acid (5), and finally by the obtention of hyperproducing mutants such as those obtained in *P. rhodozyma*.

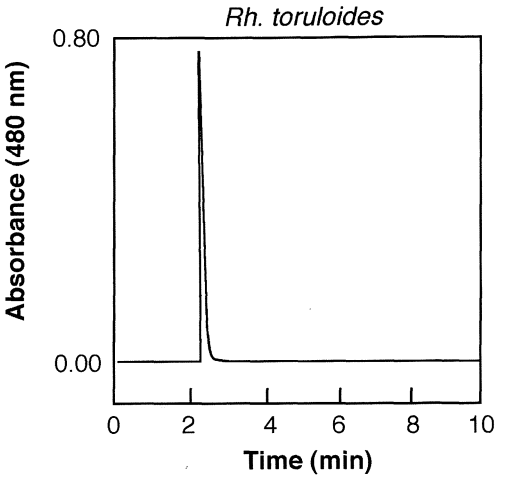

FIG. 1. HPLC-carotenoid profiles of intracellular extracts from *Rhodosporidium toruloides*. The main peak, with a retention time of 2.15 min, corresponds to β-carotene.

The advantages of using *Rhodosporidium* spp. as a commercial source of β-carotene may be summarized as follows: (i) β-carotene is produced as a primary metabolite and hence no induction technique is required, which is an advantage in relation to the industrial production by *D. salina*; (ii) the purity of the β-carotene

TABLE 1. β-carotene quantification (μg/g dry weight) in the four *Rhodosporidium* species assayed

	Rh. toruloides	*Rh. sphaerocarpum*	*Rh. paludigenum* A_1	*Rh. paludigenum* A_2
Total	226.4	685.7	254.3	246.4
β-carotene	222.0	672.0	249.2	241.5

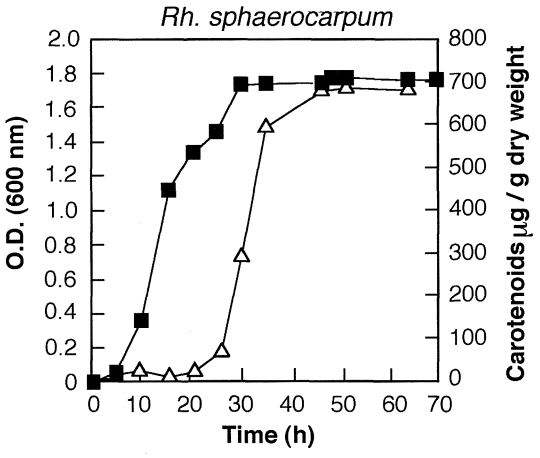

FIG. 2. Typical one-step growth (■) of *Rhodosporidium sphaerocarpum* and its onset of carotenogenesis (Δ). As shown, maximal levels are attained at 52 h.

extracted is about 98% of the total carotenoid content; (iii) the growth speed is relatively high, as it is the optimum growth temperature, which allows to attain the maximum production rate in a few days, and may reduce the refrigeration costs of industrial fermenters; and (iv) all technological equipment and updated related aspects of the industrial production of baker's yeast may be easily adapted to the production of *Rhodosporidium*.

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References

1. Armstrong, G. A., Alberti, M., Hearst, J. E. (1990). Conserved enzymes mediate the early reactions of carotenoid biosynthesis in non-photosynthetic and photosynthetic prokaryotes. Proc. Natl. Acad. Sci. USA **87**, 9975–9979.
2. Bauernfeind, J. C. (1981). Carotenoids as Colorants and Vitamin A Precursors. Academic Press, New York.
3. Ben-Amotz, A., Avron, M. (1980). Glycerol, β-carotene and dry algal meal production by commercial cultivation of *Dunaliella*. *In* Shelef, G., Soeder, C. J. (ed.), Algae Biomass. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 603–610.
4. Bendich, A. (1991). β-carotene and the immune response. Proc. Nutr. Soc. **50**, 363–374.
5. Calo, P., de Miguel, T., Velázquez, J. B., Villa, T. G. (1995). Mevalonic acid increases trans-astaxanthin and carotenoid biosynthesis in *Phaffia rhodozyma*. Biotech. Lett. **17**, 575–578.
6. Hodgins, D. S. (1971). Yeast phenylalanine ammonia-lyase: purification, properties and the identification of catalytically essential dehydroalanine. J. Biol. Chem. **246**, 2977–2985.
7. Johnson, E. A., Villa, T. G., Lewis, M. J., Phaff, H. J. (1978). Simple method for the isolation of astaxanthin from the basidiomycetous yeast *Phaffia rhodozyma*. App. Environ. Microbiol. **35**, 1155–1159.
8. Johnson, E. A., Schreiber, D., Ho, K. P., Hall, W. T., Yang, H., Geldiay-Tuncer, B. (1991). Process for in-vivo production of astaxanthin and *Phaffia rhodozyma* yeast of enhanced astaxanthin content. International Patent Cooperation Treaty (PCT) application no. WO 91/020060.
9. Krinsky, N. I. (1989). Antioxidant function of carotenoids. Free Radical. Biol. Med. **7**, 617–635.
10. Moore, M. M., Breedveld, M. W., Autor, A. P. (1989). The role of carotenoids in preventing oxidative damage in pigmented yeast *Rhodotorula mucilaginosa*. Arch. Biochem. Biophys. **280**, 419–431.
11. Sedmak, J. J., Weerasinghe, D. K., Jolly, S. O. (1990). Extraction and quantitation of astaxanthin from *Phaffia rhodozyma*. Biotechnology Techniques **4**, 107–112.

MICROBIOLOGÍA SEM **13** (1997), 71–74

Transport of phosphate into vacuoles of *Saccharomyces cerevisiae*

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Summary

Isolated vacuoles of the yeast *Saccharomyces cerevisiae* accumulated orthophosphate at pH 6–7. This accumulation was inhibited by MgATP, and was insensitive to protonophores. Triton X-100 blocked this process. The accumulation increased linear by any phosphate concentrations employed (from 0.2 to 10 mM). It is proposed that phosphate is transported into yeast vacuoles via a channel transport system independent of the electrochemical proton gradient on the vacuolar membrane.

Key words: *Saccharomyces cerevisiae*; transport system, phosphate, vacuoles, lysosomes

Resumen

Las vacuolas aisladas de levaduras del género *Saccharomyces cerevisiae* acumulan ortofosfato a valores de pH entre 6 y 7. Esa acumulación es inhibida por el MgATP, y no se ve afectada por los protonóforos. El Tritón X-100 bloquea este proceso. La acumulación aumenta linealmente con cualquiera de las concentraciones de fosfato ensayadas (de 0,2 a 10 mM). Se concluye que el fosfato es transportado al interior de las vacuolas de levadura por medio de un sistema de canales, independiente del gradiente electroquímico de protones en la membrana vacuolar.

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Introduction

Recently a wide variety of genes encoding proteins, which probably ensure transmembrane solute transport, was identified by the analysis of data on systematic genome sequencing (1). The functions and localization of many relevant proteins are still unknown. Therefore, the investigation of particular transport systems is of great interest.

Yeast vacuole is a storage compartment of amino acids (13), ions (9) and other compounds (6). The vacuolar membrane contains H^+/substrate antiporters for amino acids (11). Ca^{2+} (8), Zn^{2+} (12) and uniporters for citrate and ketoglutarate (5). All these transport systems use the energy of electrochemical proton gradient on the vacuolar membrane. They are MgATP–stimulated and protonophore–sensitive.

Vacuoles are the main compartment of orthophosphate in the yeast cell (9), but the mechanism of transport of this anion into vacuoles is still unknown. Our attempts to demonstrate MgATP-dependent phosphate accumulation in isolated yeast vacuoles has not been successful up to date. Recently, several transport systems (for dipeptides, K^+, NO_3^-) independent of H^+-pumps and inhibited by MgATP were observed in plant vacuoles (3, 4). These data aroused our interest in the study of the mechanism of phosphate transport in yeast vacuoles.

Materials and methods

The yeast *Saccharomyces cerevisiae* VKM-1147 was grown on glucose-peptone medium (2% glucose, 1% peptone, 0.5% yeast extract) up to mid logarithmic phase, as described (5). Vacuoles were isolated as in reference 5. Protein was determined accordingly as in reference 2.

The accumulation of [^{32}P]-orthophosphate (Obninsk, Russia) was measured as described earlier (5). The incubation medium for transport experiments contained 0.3 M sorbitol and 10 mM MOPS-Na, pH 7.2. Orthophosphate was added as Na salt. Addition of other compounds is mentioned in Table 1.

Results

The uptake of phosphate in the isolated *S. cerevisiae* vacuoles in amedium with 0.3 M sorbitol, 10 mM MOPS-Na, pH 7.2, without other compounds added, increased for 5 min (Fig. 1). Accumulated phosphate was retained in the vacuoles for at least 15 min. This uptake was linearly dependent upon phosphate concentration at all concentrations tested (Fig. 2).

Phosphate transport in the isolated yeast vacuoles was stimulated by ATP (Table 1). The degree of stimulation was low, in comparison with the stimulation of dipeptide transport in plant vacuoles (4).

MgATP as substrate of vacuolar H-ATPase stimulates transport of many compounds into

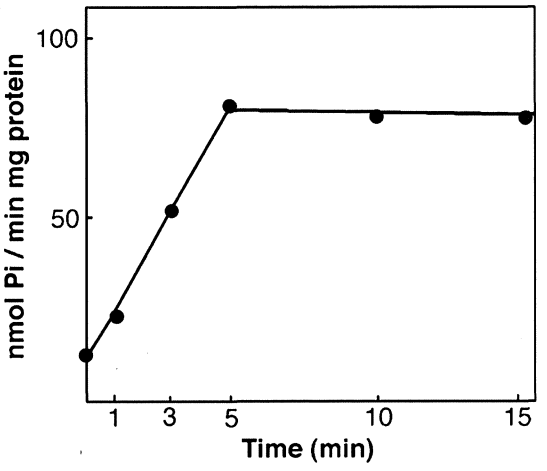

FIG.1. Time dependence of phosphate transport in isolated vacuoles of *Saccharomyces cerevisiae*.

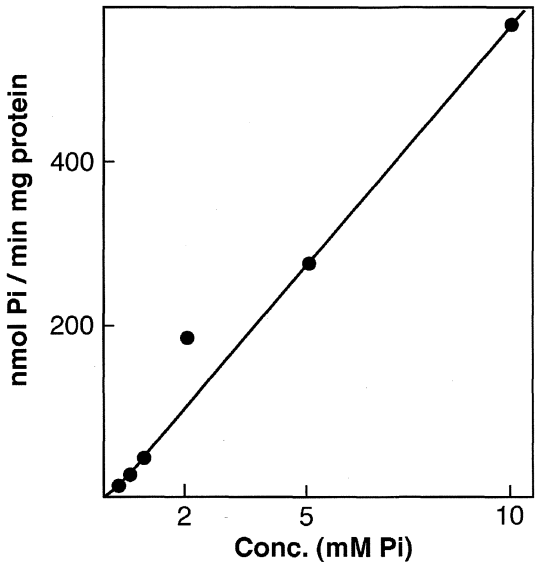

FIG. 2. Concentration dependence of phosphate transport in isolated vacuoles of *Saccharomyces cerevisiae*.

isolated yeast vacuoles, i.e. amino acids, Ca^{2+} and organic acids. In contrast, MgATP inhibits phosphate transport by 66% (Table 1). This inhibition can be only partially explained by Mg^{2+}–phosphate complex formation. Mg^{2+} at the same concentration inhibits phosphate transport only by 40%. Inhibition by MgATP and low sensitivity to protonophore FCCP (Table 1) allows us to conclude that orthophosphate transport in the isolated yeast vacuoles is independent of the electrochemical proton gradient on the vacuolar membrane.

TABLE 1. Effect of some compounds on orthophosphate uptake in isolated vacuoles of *Saccharomyces cerevisiae*

Additions	Uptake rate nmol/mg of protein·min
Experiment N1 (0.4 mM Pi)	
control	15
5 mM ATP	20
1 mM orthovanadate	19
0.2% Triton X-100	0
0.2% Triton X-100 + 5 mM ATP	0
Experiment N2 (10 mM Pi)	
control	420
5 mM Mg SO_4	250
5 mM Mg SO_4 + 5 mM ATP	140
0.005 mM FCCP	340

Solubilization of vacuoles with Triton X-100 led to a complete lack of phosphate uptake, both in the presence and in the absence of ATP. Orthovanadate used as a phosphate analog did not inhibit phosphate uptake but stimulated it. The pH optimum for the uptake was 6–7 (Table 2).

Discussion

We concluded that isolated yeast vacuoles are able to accumulate orthophosphate. This process is inhibited completely by detergent, Triton X-100; therefore intact vacuolar membranes are necessary. At the same time, uptake is decreased by MgATP and shows low sensitivity to protonophore FCCP. This transport is independent of the electrochemical proton gradient on the vacuolar membrane, in contrast to vacuolar transport systems for Ca^{2+}, amino acids and organic acids.

Phosphate transport into isolated yeast vacuoles is probably similar to the mechanism of dipeptide transport in plant vacuoles (4). We observed two differences between properties of these transport systems. On one hand, the ATP stimulation of phosphate transport was lower than the stimulation of dipeptide transport. On the other hand, phosphate uptake into isolated yeast vacuoles had linear kinetics, while dipeptide uptake into plant vacuoles had Michaelis kinetics (4).

Ortophosphate concentration in the yeast cytoplasm (1.5 mM) (9) is sufficient for the activity of this uptake system. Cytosolic pH in the yeast cell (7) is near the pH optimum for this

TABLE 2. Dependence on pH of orthophosphate uptake into isolated yeast vacuoles

pH of incubation medium	Uptake rate nmol/min mg of protein
5.0	0
6.0	380
7.0	420
8.0	240
9.0	0

tranport. Hence the phosphate transport process observed in the isolated vacuoles may occur in vivo.

Ortophosphate accumulated in vacuoles may remain partly in free form (9), although at the same time phosphate could be involved in polyphosphate synthesis. Yeast vacuoles contain a pool of condensed phosphate-inorganic polyphosphates. Orthophosphate incorporates into long chains of inorganic polyphosphates within lysosomes of human fibroblasts (10). Lysosomes of animal cells and vacuoles of yeast cells have similar functions. Therefore, synthesis of polyphosphates from orthophosphate might also occur in yeast vacuoles.

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References

1. Andre, B. (1995). An overview of membrane transport protein in *Saccharomyces cerevisiae*. Yeast **11**, 1575–1613.
2. Bensadoun, A., Weinstein, D. (1976). Assay of proteins in the presence of interfering materials. Analyt. Biochem. **70**, 241–250.
3. Dietz, K. J., Klughammer, B., Hang, B., Thume, H. (1994). Solute transport across the tonoplast of barley mesophyll vacuoles: Mg^{2+} determines the specificity and ATP and lipophylic amino acids tye activity of amino acids transport carrier. J. Membr. Biol. **137**, 151–158.
4. Jamai, A., Gaillard, C., Delrot, S., Martinoia, E. (1995). Dipeptide transport in barley mesophyll vacuoles. Planta **196**, 430–434.
5. Kulakovskaya, T. V., Matys, S. V., Okorokov, L. A. (1991). Transport of organic acid anions and guanosine into vacuoles of *Saccharomyces pastorianus*. Yeast **7**, 495–501.
6. Matile, P. (1982). Vacuoles come of age. Physiol. Veg. **20**, 303–310.
7. Nicolay, K., Scheffers, W. A., Bruinenberg, P. M., Kaptein, R. (1982). Phosphorus-31 nuclear magnetic resonance studies of intracellular pH, phosphate compartmentation and phosphate transport in yeast. Arch. Microbiol. **133**, 83–89.
8. Ohsumi, Y., Anraku, Y. (1983). Calcium transport driven by proton motive force in a vacuolar membrane vesicles of *Saccharomyces cerevisiae*. J. Biol. Chem. **258**, 5614–5617.
9. Okorokov, L. A., Lichko, L. P., Kulaev, I. S. (1980). Vacuoles: main compartment of potassium, magnesium and phosphate ions in *Saccharomyces carlsbergensis* cells. J. Bacteriol. **144**, 661–665.
10. Pisoni, R. L., Lindley, E. R. (1992). Incorporation of [^{32}P] orthophosphate into long chains of inorganic polyphosphate within lysosomes of human fibroblasts. J. Biol. Chem. **267**, 3626–3631.
11. Sato, T., Ohsumi, Y., Anraku, Y. (1984). Substrate specificity of active transport systems for amino acids in vacuolar-membrane vesicles of *Saccharomyces cerevisiae*. Evidence of seven independent proton/amino acid antiport systems. J. Biol. Chem. **259**, 11505–11508.
12. White, C., Gadd, G. M. (1987). The uptake and cellular distribution of zinc in *Saccharomyces cerevisiae*. J. Gen. Microbiol. **133**, 727–737.
13. Wiemken, A., Durr, M. (1974). Characterization of amino acids pools in the vacuolar compartment of *Saccharomyces cerevisiae*. Arch. Microbiol. **101**, 45–57.

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Genomic polymorphism in herpes simplex virus keratitis

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Summary

Viruses are the major infectious agents causing conjunctivitis and keratoconjunctivitis. Two antigenic types, herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), can be recognized in which numerous genomic variants or genotypes have been described. HSV-1 infection is one of the major causes of blindness. Different strains can induce different types of ocular lesions. Twelve patients with acute herpetic epithelial keratitis were studied by monoclonal antibodies and restriction enzymes, and nine different HSV-1 genomic variants were identified. This study confirms that a variety of HSV-1 strains is associated to this kind of infection.

Key words: herpes simplex virus (HSV), keratitis, ocular lesions, polymorphism, fingerprinting

Resumen

Los virus son los principales agentes infecciosos causantes de conjuntivitis y queratoconjuntivitis. Se conocen dos tipos antigénicos de virus herpes simplex (HSV), los HSV-1 y los HSV-2, de los cuales se han identificado numerosos genotipos o variantes genómicas. La infección por HSV-1 es una de las principales causas de ceguera. Se conocen diferentes tipos de lesión ocular producidas por distintas cepas. En este trabajo se presentan los resultados obtenidos al estudiar, mediante anticuerpos monoclonales y enzimas de restricción, doce pacientes con queratitis epitelial herpética aguda. Se

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identificaron nueve variantes genómicas de HSV-1, confirmando que existen diferentes cepas de HSV-1 asociadas a este tipo de infección.

Introduction

Herpes simplex virus type 1 (HSV-1) is the most common infectious causing agent responsible for blindness in developed countries. The clinical manifestations of herpetic ocular infections are heterogeneous. The symptomatic presentation may correspond to a primary HSV infection or to a viral recurrence, due to the HSV ability to become latent in the regional sensitive ganglia of infected persons. In both cases—primary infection and recurrence—the infection can be either symptomatic or asymptomatic. About 20% of herpetic ocular infections have recurrences in the two years following the first episode. This percentage rises to 43–68% after the first recurrence (11)

The incidence of herpetic keratitis ranges from 5.9 to 20.7 episodes/100,000 persons per year (4). Herpes simplex virus (HSV) belongs to the Herpesviridae family, morphologically characterized by a diameter of 150 to 200 nm, an icosahedral capsid that contains a 150 kbp DNA genome, and a lipid envelop. Tegument proteins have been identified between the capsid and the viral envelope. Two antigenic types of HSV are recognized (HSV-1 and HSV-2), of which genomic variants have been described (3, 8). The HSV genome consists of a linear, double stranded DNA made by two covalently bonded segments, each one flanked by inverted-repeat regions; in each replicative cycle, four populations of genomic isomers are originated. There are extensive sequence homologies between HSV-1 and HSV-2 involving around 50% of the genome. Differences can be observed by means of restriction endonuclease analyses. Differences exist also between sequences in the same HSV types, which account for the different genotypes described. The use of endonucleases has shown that such differences exist (8). Different HSV strains can induce different types of ocular lesions, which can compromise epithelial and/or estromal tissue, thus leading to different outcomes of the herpetic disease (5). Animal models have confirmed that certain viral strains cause severe ocular damage, whereas others result only in mild epithelial lesions (1, 7).

Restriction endonucleases have been widely used to identify HSV genomic polymorphism in different human infections. Those technical procedures have proven to be useful for the genomic identification of epidemiologicaly related strains (9).

In this study we describe both antigenic and genomic HSV types isolated from corneas of patients who suffered acute episodes of herpetic keratitis with epithelial compromise.

Materials and methods

From August 1991 to January 1993, patients with viral conjunctivitis or keratoconjunctivitis were studied through viral isolation in cellular cultures. It was confirmed that 4.5% of the infections were HSV keratitis (10).

Samples were obtained from each patient by swabbing their corneal lesions with a sterile cotton swab, and placing it in a tube with transport medium, as previously described (10). 0.2 ml of each sample was inoculated into culture tubes with Vero cells (ATCC strain CCL 81) monolayers and maintained at 37°C until the cytopathic effect characteristic for herpes virus was detected (6). Positive cultures were typed by direct

immunofluorescence, using commercial monoclonal antibodies (Kallestad, Austin, TX, USA). The viral isolates obtained were compared by restriction enzyme to electrophoretical patterns. Viral DNA was extracted by using a commonly available phenol/chloroform protocol (Sigma, St. Louis, MO, USA) and precipitated with ethanol (6). Fingerprinting analysis was performed by using restriction enzymes *Hin*dIII initially and then *Bgl*II with those strains that have similar *Hin*dIII restriction patterns (BioLabs, Beverly, MA, USA), and 0.7% agarose gel electrophoresis.

Lambda DNA 301-1S (BioLabs) was the molecular marker and digestion assay control. Prototypes HSV-1 (strain F) and HSV-2 (strain G) were provided by Dr. Bernard Roizman (2). Isolate identity was based on the presence or absence of known cleavage sites (9).

Results and discussion

Twelve positive cultures were obtained. Eleven HSV-1 isolates and one HSV-1/HSV-2 reactive isolate were identified by using monoclonal antibodies. Restriction enzyme analyses identified all of the twelve DNAs as HSV-1. Within this type, we identified nine different genotype profiles, all of which differed from the

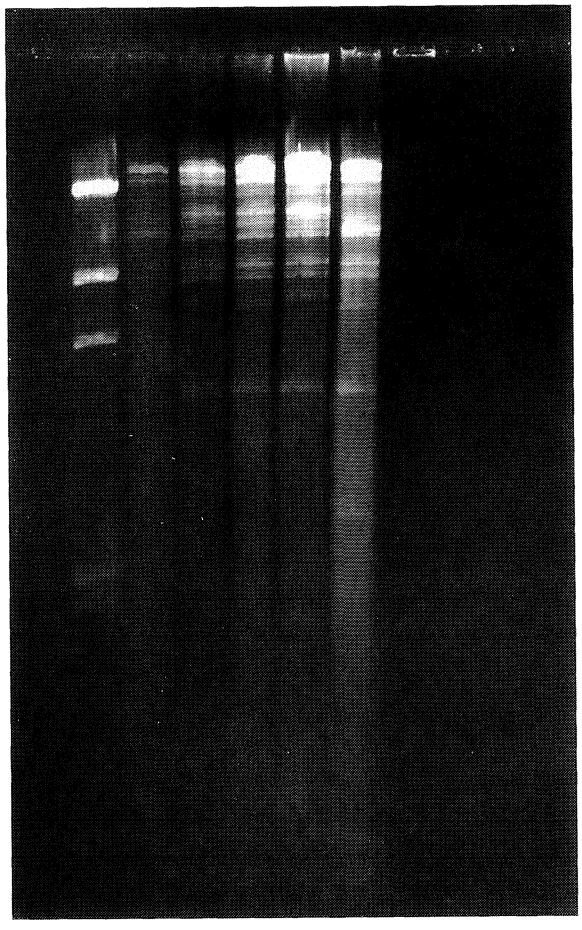

FIG. 1. Four different genotypes obtained with *Hin*dIII, corresponding to five HSV isolates.

electrophoretical pattern of the standard HSV-1 strain (strain F). Fig. 1 shows four genotypes obtained with *Hin*dIII, which correspond to five HSV isolates. Herpes keratitis can have diverse clinical outcomes. In the last few years, efforts have focused on the identification of viral characteristics that may be associated to a more severe evolution. To our knowledge, only one previous study on the disease related genotypes of herpes simplex virus to their clinical pictures in HSV keratoconjunctivitis (12). Our study confirms that individuals with herpetic ephithelial lesions can harbor a variety of HSV-1 genomic variants. Further studies will be needed to define if a specific variant may be associated to a more severe clinical manifestation.

Acknowledgments

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References

1. Centifanto-Fitzgerald, Y. M., Fenger, T., Kaufman, H. E. (1982). Virus proteins in herpetic keratitis. Exp. Eye Res. **25**, 425–441.
2. Ejercito, P., Kieff, E., Roizman, B. (1968). Characterization of herpes simplex virus strains differing in their effects on social behaviour of infected cells. J. Gen. Virol. **2**, 357–364.
3. Hayward, G., Frenkel, N., Roizman, B. (1975). Anatomy of herpes simplex virus DNA: strains differences and heterogeneity in the locations of restriction endonuclease cleavage sites. Proc. Nat. Acad. Sci. USA **72**, 1768–1772.
4. Kaye, S. B., Baker, K. (1996). Herpes simplex keratitis. J. Med. Microbiol. **45**, 3–5.
5. Liesegang, T. (1992). Biology and molecular aspects of herpes simplex and varicella zoster virus infections. Ophthalmology **99**, 781–797.
6. Martínez, M. J., Saavedra, T., Ojeda, J. M., Suárez, M. (1996). Caracterización antigénica y genómica de virus herpes simplex aislados de dobles infecciones genitales. Rev. Med. Chil. **124**, 153–159.
7. Newell, C. K., Martin, S., Sendele, D. (1989). Herpes simplex virus-induced stromal keratitis: role of T-lymphocyte subsets in immunopathology. J. Virol. **63**, 769–775.
8. Roizman, B. (1990). Herpesviridae: a brief introduction. *In* Fields, B. N. (ed.), Virology. Lippincott-Raven Publisher, 2nd ed., pp. 1787–1789.
9. Roizman, B., Tognon, M. (1982). Restriction enzyme analysis of herpes-virus DNA: stability of restriction endonuclease patterns. Lancet **1**, 667.
10. Vogel, M., Martínez, M. J., Traipe, L., Srur, M., Suárez, M. (1992). Caracterización clínica, antigénica y genómica de cepas de virus herpes simplex (HSV) aisladas de pacientes con patología ocular. Arch. Chil. Oftal. XLIX **1**, 40–44.
11. Vogel, M., Martínez, M. J., Suárez, M. (1992). Rol de los virus en las conjuntivitis y queraconjuntivitis. An. Oftal. IV **1**, 16–21.
12. Wang, H. L., Shen, M. M., Huang, W. L., Chen, C. W., Chou, L. C., Lin, K. H. (1995). A molecular study of ocular herpes simplex infection in Kaohsiung, Taiwan. Part I: Cleavage pattern analysis. Kao Hsiung I Hsueh Ko Hsueh Tsa Chih. **11**, 507–513.

MICROBIOLOGÍA SEM **13** (1997), 79–82 **PERSPECTIVAS**

Enrique Montoya (1928–1996), una vida dedicada a la docencia y a la investigación

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Escribir sobre los méritos docentes y científicos del Prof. Enrique Montoya Gómez (Córdoba, 24.04.1928–Granada, 09.12.1996) es hablar de su vida misma, que no ha sido otra que la entrega a la docencia y a la investigación, sacrificando muchas veces otras obligaciones.

Estudia el bachillerato en el colegio de los Hermanos Maristas, en Lucena y en Córdoba. Cursa el primer año de universidad en Sevilla y, en 1946, se traslada a Granada para estudiar Farmacia, título que obtiene, con la calificación de sobresaliente, en 1951. En 1953 obtiene el grado de Doctor en Farmacia por la Universidad Complutense (en esa época todas las tesis doctorales debían presentarse en Madrid), con la máxima calificación.

Enrique Montoya ha sido maestro de universitarios. Proyectó su vocación de magisterio con gran derroche de generosidad, tanto en la docencia como en la investigación, sin olvidar su labor académica y en la administración. Su carrera docente se inicia en los años 50: Ayudante honorario de la Facultad de Farmacia y de la de Ciencias; Profesor encargado de la extensión de Fermentaciones; Profesor encargado de la extensión de Fitopatología; Ayudante de clases prácticas; Adjunto honorario en la Facultad de Farmacia; Diplomado en Sanidad. Obtiene por oposición la Cátedra de Microbiología de la Facultad de Ciencias de la Universidad de Sevilla en 1967, donde participa en la puesta en marcha de la Sección de Biología. Coincide en esa etapa con Manuel Losada, Emilio Fernández Galiano, Salvador Peris y Enrique Cerdá, todos los cuales comparten la ilusión por la tarea a realizar, junto a un elevado espíritu de compañerismo. En esos años reside en el Colegio Mayor Hernando Colón, de Sevilla, etapa de grato recuerdo que perdura en los que eran estudiantes y en los profesores.

Vuelve a Granada, en 1970, para ocupar la Cátedra de Microbiología en la Sección de

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Biológicas, que empieza a funcionar. Imparte clases de Biología General, Microbiología y Virología e Inmunología. Más tarde, de Bacteriología, Microbiología Industrial y Fitopatología. En estas dos últimas le ayudan José Olivares y Pedro Ramos, sus jóvenes colaboradores en la Estación Experimental del Zaidín, del CSIC. Participa en la consolidación de la Sección de Ciencias Biológicas, de la que sería director. Vicedecano de la Facultad de Ciencias de 1971 a 1973. Vicerrector de la Universidad de Granada de 1973 a 1976. Director del Departamento interfacultativo Farmacia-Ciencias de 1973 a 1986, y del Departamento de Microbiología (constituido según la LRU) desde 1990 hasta su fallecimiento.

Enrique Montoya ha enseñado Microbiología a una treintena de promociones de estudiantes y ha "enseñado a enseñar" a docenas de profesores, repartidos hoy por toda la geografía española. Tuvo siempre un empeño didáctico, conciso, claro y actualizado al máximo, con una metodología impecable. El mejor homenaje lo recibió en 1993 de los alumnos de Microbiología General; al terminar el curso, y ante la incertidumbre de la edad de jubilación a los 65 años, los alumnos pretextaron una clase extra a final de curso para rendirle un sencillo homenaje.

Pero si extensa ha sido su labor docente, tanto o más cabe decir de la investigación. Desarrolló líneas de investigación en Microbiología del Suelo (*Azotobacter, Rhizobium,* bacterias del fósforo, micorrizas), levaduras (metabolismo, genética, toxohormona, supresividad, transformación, factores "killer", fusión celular) y fisiología bacteriana (*Rhizobium* y mixobacterias). Se inicia en las tareas de investigación en 1951, bajo la dirección del Prof. Vicente Callao —su admirado maestro—, en la Estación Experimental del Zaidín, en Granada, casi recién creada. Becario (1951–53), becario con estancia en Pavía (1953), ayudante de Sección (1954), Colaborador Científico (1956), Investigador (1962), becario de la Fundación Juan March (1963) y Profesor de Investigación (1967). Esta primera etapa coincide con directores del Zaidín como Enrique Gutiérrez Ríos, Ángel Hoyos de Castro y Luis Recalde Martínez, y con colegas como Manuel Lachica, Fermín Capitán y, poco más tarde, Enrique Hernández y José Olivares.

Sus primeros trabajos, en la línea de investigación del Zaidín, se centran en la importancia y aprovechamiento del orujo de la aceituna. Realiza su tesis sobre la utilización de este subproducto como materia prima para fermentaciones, tema que influyó en posteriores trabajos sobre productos agrícolas y del suelo. Inicia una nueva línea sobre toxohormona de levaduras como modelo de célula cancerosa, que culmina en artículos de gran impacto en *Science* en 1961, 1963 y 1967, que le valen reconocimiento nacional e internacional. Esta línea se ampliaría a distintos aspectos de las levaduras. En tanto, seguían apareciendo sus publicaciones sobre Microbiología del Suelo. En el año 1965 aborda un tema inédito: la importancia de las mixobacterias en la Microbiología del Suelo, que retomaría a su vuelta a Granada en los años 70, incluyendo estudios bioquímicos y moleculares sobre diversas actividades enzimáticas, producción de antibióticos, morfogénesis, diferenciación celular y papel de las mixobacterias en la biomineralización. Esta línea se constituyó en Grupo de Investigación y se sigue trabajando en ella.

Durante su estancia en la Universidad de Sevilla estructura la investigación del Departamento de Microbiología y se rodea de un grupo de jóvenes becarios. Es nombrado Jefe de la Sección de Microbiología y Bioquímica del Centro de Biología Aplicada y Edafología de la Estación del Cuarto de Sevilla, del CSIC (1969–70). A su vuelta a Granada tiene nuevamente que partir de cero y montar los laboratorios de investigación. Los becarios que le acompañan tienen

que recurrir al Zaidín para poder continuar sus tareas de investigación. Es nombrado Director del Departamento de Microbiología del Zaidín (1973–79) y vinculado como Director adherido al claustro en el período de reestructuración organizativa del CSIC.

Enrique Montoya es autor de numerosos artículos científicos y de un libro. Dirigió un gran número de tesis y tesinas, y contribuyó a la creación de diversos grupos de investigación, en el CSIC y en la universidad. Si su labor en las aulas universitarias y en los laboratorios de investigación ha sido la columna que ha vertebrado su trayectoria profesional, dedicó también su tiempo y capacidad a la administración universitaria. Con honestidad y sentido del deber, sirvió en puestos de responsabilidad de la Facultad y en el Rectorado. Así mismo, en el Consejo de Administración y Presidencia de la Caja Provincial de Ahorros de Granada.

En la Sociedad Española de Microbiología fue Vocal de la Junta Directiva durante varios años, y Presidente del Congreso Nacional de Microbiología celebrado en Granada en 1973. También presidió y organizó otros congresos internacionales. Fue Presidente de la Academia de Ciencias Físico-Químicas, Naturales y Matemáticas de Granada durante seis años y estaba en posesión de varias condecoraciones, entre las que destaca la Encomienda con Placa de la Orden Civil de Alfonso X el Sabio.

Enrique Montoya, como persona, podía aparecer a primera vista serio y frío e infundir respeto; pero su afecto se manifestaba pronto, porque era hombre sencillo, afectuoso y cordial. Como científico, destacaba por tener una sólida y amplia formación y un espíritu exigente, crítico y apasionado en su trabajo. Como docente, encarnaba la sencillez y espíritu vocacional. Como funcionario, una gran humanidad y responsabilidad. Como intelectual, era culto y fuera de toda petulancia. Como jugador, apostaba a ganar.

FIG. 1. Enrique Montoya (1928–1996).

Estas líneas no pretenden dar un retrato perfilado de las realizaciones y personalidad de Enrique Montoya; sólo un breve recorrido por la vida docente e investigadora de un hombre que hasta el día antes de dejarnos estuvo en el aula dando clase. Es justo decir que ha muerto enseñando.

Principales trabajos científicos

Montoya, E., Bolcato, V. (1955). Isolation of butyraldehyde in the butyl alcohol fermentation. Naturwissenschaften **6**, 157.

Bolcato, V., Boschetti, E., Montoya, E. (1956). Formation of formaldehyde and formyl-compounds during the respiration of acetate and glycolate by *Escherichia coli*. Antonie van Leeuwenhoek **23**, 131–138.

Callao, V., Montoya, E. (1960). The use of dyes to distinguish between species of the genus *Azotobacter*. J. Gen. Microbiol. **22**, 657–661.

Montoya, E. (1961). Actividad catalásica de mutantes de *E. coli* y *S. aureus* con deficiencia respiratoria. Microbiología Española **14**, 65–71.

Callao, V., Montoya, E. (1961). Toxohormone-like factor from microorganisms with impaired respiration. Science **134**, 2041–2042.

Callao, V., Olivares, J., Montoya, E. (1963). Toxohormone inhibitory effect on the growth of an instable strain of yeast. Science **142**, 1668–1669.

Callao, V., Alvarado, R., Sedano, A., Olivares, J., Montoya, E. (1966). Efecto antagónico del *Myxococcus xanthus* sobre *Azotobacter*. Microbiología Española **19**, 45–51.

Olivares, J., Callao, V., Montoya, E. (1967). Toxohormone from normal tissues. Science **157**, 327–328.

Olivares, J., Costa, V., Montoya, E. (1973). Selective inhibitory effect of toxohormone on mice catalase forms. FEBS Letters **36**, 343–346.

Barea, J. M., Navarro, E., Palomares, A., Montoya, E. (1974). A rapid microbiological assay method for auxin, gibberellic acid and kinetin using yeast. J. Appl. Bacteriol. **37**, 171–174.

Ocampo, J. A., Barea, J. M., Montoya, E. (1975). Interaction between *Azotobacter* and phosphobacteria and their establishment in the rhizosphere affected by soil fertility. Can J. Microbiol. **21**, 1160–1165.

Olivares, J., Montoya, E. (1977). Estudios genéticos en *Rhiozobium*. Conjugación y fijación de nitrógeno. *In* Portolés, A., Baquero, F. (ed.), Aspectos Actuales en la Transmisión de Información Genética de Microorganismos, 251–265. Monografías Básicas de la Sociedad Española de Microbiología.

Barea, J. M., Navarro, E., Montoya, E. (1976). Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria. J. Appl. Bacteriol. **40**, 129–134.

Olivares, J., Montoya, E., Palomares, A. (1977). Some effects derived from the presence of extracromosal DNA in *Rhizobium meliloti*. *In* Newton, W., Posgate, J. K., Rodríguez Barrueco, C. (ed.), Recent Development in Nitrogen Fixation, pp. 375–385. Academic Press, New York.

Corral, E., Montoya, E., Olivares, J. M. (1978). Sensitivity to phages in *Rhizobium meliloti* as a plasmid consequence. Microbios Lett. **5**, 77–80.

Arias, J. M., Rodriguez, C., Montoya, E. (1979). Purification and partial characterization of an antibiotic produced by *Myxococcus coralloides*. J. Antibiotics **32**, 205–211.

Extremera, A. L., Martin, I., Montoya, E. (1982). A new killer toxin produced by *Saccharomyces cerevisiae*. Current Genetics **5**, 17–19.

Martínez, J., Valdivia, E., Maqueda, M., Ortega, J. M., Montoya, E. (1983). Regulation of catalase and peroxidase in *Saccharomyces cerevisiae*: a plausible site of action of an intracellular factor. FEMS Microbiol. Lett. **18**, 19–24.

Arias, J. M., Fernández Vivas, A., Montoya, E. (1983). Evidences of an activating substance related to autolysis in *Myxococcus coralloides*. Arch. Microbiol. **134**, 164–166.

Valdivia, E., Martínez, J., Ortega, J. M, Montoya, E. (1983). Control of catalase and peroxidase biosynthesis by carbon source and oxygen in the yeast *Saccharomyces cerevisiae*. Can. J. Microbiol. **29**, 1200–1204.

Fernández Vivas, A., Arias, J. M., Montoya, E. (1985). Lysis induced by uncoupling genes in *Myxococcus coralloides*. FEMS Microbiol. Lett. **27**, 99–102.

Gálvez, A., Maqueda, M., Valdivia, E., Quesada, A., Montoya, E. (1986). Characterization and partial purification of a broad spectrum bacteriocin produced by *S. faecalis* subsp. *liquefaciens*. Can. J. Microbiol. **32**, 765–771.

González, F., Arias, J. M., Montoya, E. (1987). Phosphatase activities in the life cycle of *Myxococcus coralloides* D. J. Gen. Microbiol. **133**, 2327–2332.

González, F., Muñoz, J., Arias, J. M., Montoya, E. (1989). Polyphosphate and ortophosphate content during the life cycle of *Myxococcus coralloides* D. FEMS Microbiol. Lett. **65**, 105–108.

González, F., Montoya, M. D., Fárez, E., Arias, J. M., Montoya, E. (1990). Effects of phosphate on antibiotic and extracellular protein production by *Myxococcus coralloides*. Appl. Microbiol. Biotechnol. **33**, 78–80.

Arias, J. M., Montoya, E. (1990). Mixobacterias: Un modelo para el estudio de la diferenciación celular. *In* Casadesús, J., Ruiz Berraquero, F. (ed.), Microbiología 1990, pp. 79–88. Publicaciones de la Universidad de Sevilla.

González, M. T., Montoya, E., Arias, J. M., Rodríguez-Gallego, M. (1993). Struvite production by *Myxococcus coralloides*. Chemosphere **26**, 1881–1887.

Montoya, M. D., Gálvez, A., Arias, J. M., Montoya, E. (1994). Autolytic effect of the antibiotic produced by *Myxococcus coralloides*. Microbiología SEM **10**, 395–402.

González, F., Fárez-Vidal, E., Arias, J. M., Montoya, E. (1994). Partial purification and biochemical properties of acid and alkaline phosphatases from *Myxococcus coralloides*. J. Appl. Bacteriol. **77**, 567–573.

Montoya, E. Lecciones de Bacteriología. I, Parte General. Vol. I (1987). II, Parte Especial. Vol. II (1992).

MICROBIOLOGÍA SEM **13** (1997), 83–84 **PERSPECTIVAS**

Jacobo Cárdenas (1940–1996): un amigo

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Hace pocas semanas, el 28 de octubre de 1996, falleció de forma repentina Jacobo Cárdenas Torres, cuando se encontraba de viaje en Guadalajara (Méjico).

Jacobo Cárdenas había nacido en Lebrija (Sevilla), el 31 de enero de 1940. Si queremos resumir en breves palabras su personalidad noble, apasionada y luchadora, diríamos que Jacobo fue un "torrente de vida" desbordado prematuramente. Alguien estos días ha comentado que Jacobo llenaba mucho espacio; de hecho, siempre lo llenaba todo, porque rebosaba vida, simpatía y actividad, sazonada con cierta socarronería.

Trabajador sin horas, lector asiduo y rápido, preocupado por la comunicación de los resultados de la investigación, intentó y consiguió llenar huecos en la bibliografía, en materias que consideraba que había que desarrollar para completar la formación científica. A ese fin se dirigen sus libros *Reading and Writing Science* (1990) y *Glosario de Biología Molecular* (1996), publicados por la Universidad de Córdoba. Tuvo también una activa participación en la creación y desarrollo de la Colección *Ciencia Hoy* (Ediciones Pirámide), de la que era codirector. Una actividad intelectual en la que conjugaba sus conocimientos científicos con sus dotes didácticas, en un deseo de poner a disposición del público todo cuanto pudiera contribuir a que la ciencia ejerciera en otros la atracción que había ejercido en él.

Alrededor de sí creó una escuela de amigos incondicionales, con los que más de una vez he comentado que había que aceptar su rica personalidad en bloque. Todos somos una suma algebraica de valores y desvalores; en esto consiste la grandeza y la pequeñez humana. La suma algebraica en este caso daba un saldo muy favorable, Jacobo era una persona con la que se podía contar. En la Sociedad Española de Bioquímica (SEB) trabajó en la Junta Directiva, contribuyendo a la creación del activo

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FIG. 1. Jacobo Cárdenas (1940–1996).

grupo de metabolismo del nitrógeno en plantas. Últimamente había organizado el XIX Congreso de la SEB, celebrado en Córdoba. Era miembro de la Sociedad Española de Microbiología desde el año 1984.

Terminó la Licenciatura en Químicas en 1968, en Sevilla, y obtuvo el Premio Real Maestranza de Caballería. Perteneciente a la primera hornada de discípulos del Prof. Manuel Losada, realizó su tesis doctoral, que defendió en 1972, sobre la nitrito reductasa. Tras una larga estancia postdoctoral en Estados Unidos, donde trabajó bajo la dirección del Prof. Mortenson en Purdue (Indiana), da por terminada su formación bioquímica. Trabaja en nitrato y nitrito reductasas de cianobacterias, metabolismo de aminoácidos, ciclo glutamina sintetasa–glutamato sintasa, pasando a estudiar, en los últimos años, los genes que modulan el proceso de maduración de frutos. Sus raíces (aunque de familia lebrijana, se educó en Huelva) le hacen volver a la zona de la fresa, buscando una aplicación social a su trabajo de tantos años. Su labor científica se ha plasmado en un gran número de artículos publicados en revistas internacionales de primera línea.

En año 1977 obtiene la plaza de Profesor Adjunto de Bioquímica, y en 1980 la Agregaduría. El curso 1980–1981 lo pasó en Málaga, donde fundó el Departamento de Bioquímica en la Facultad de Ciencias. Desde 1981 era catedrático en Córdoba. El año 1979 recibió la primera amenaza seria contra su salud: antes de cumplir cuarenta años tuvo un infarto de miocardio; pero la recuperación fue rápida y nunca cedió ante el miedo o la aprensión. Durante una estancia en Estados Unidos en el verano del 1987 tuvo un segundo aviso, lo que le obligó a someterse a una dura operación de doble "by-pass", de la que quedó francamente recuperado. Estaba convencido de que moriría joven (más de una vez lo había comentado con sus amigos y compañeros), y vivía en plena actividad. Quiso morir de pie, como los árboles, y así ha sido.

Sus convicciones personales religiosas eran profundas y definitivas. Había ingresado en la Compañía de Jesús cuando tenía 16 años y se ordenó de sacerdote en 1970. Tenía muy claro que en su vida intelectual había que hacer un esfuerzo de diálogo entre la Teología y la Ciencia, para lo cual leía afanosamente y asimilaba todo lo que se publicaba sobre el diálogo Ciencia-Fe. También tuvo siempre muy definido el convencimiento de que era prioritario ayudar a las personas cercanas, y por ellas estaba dispuesto a luchar. Con buen humor a prueba de exabruptos breves y superficiales, nos dejó un buen amigo y compañero y algo se murió en el alma... Pero habría que cambiar la letrilla de la sevillana: "Cuando un amigo se va / algo renace en el alma." Nos ha renacido en el alma el recuerdo de la amistad, la humanidad, la esperanza y el sentido de la vida. Gracias, Jacobo, por tu última lección.

MICROBIOLOGÍA SEM **13** (1997), 85–88 **PERSPECTIVES**

Engineers and microbiologists: a future together in bioremediation

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The awareness of a polluted environment is in the minds of people having to live with the consequences of the industrialized society. The Industrial Revolution in England during the last century resulted in a fast improvement in living standards. This improvement was directly bound to the discovery of new, useful materials, easier and cheaper to make. We ate and dressed better, traveled faster, and fought wars more effectively but, in doing so, we did not take into consideration the dangers coming from the toxic derivatives originated, until they started to affect us negatively. The realization that something had to be done came when these compounds had already deeply affected a large portion of the other life forms. It was realized, in the 1960s and 70s in the United States, and later in other industrialized countries, that recalcitrant xenobiotics had become widely distributed throughout the environment. The widespread pollution of soils, sediments and groundwater created hazardous conditions that had to be dealt with.

In the last ten years, major advances have been made in the development of techniques for the clean-up of polluted environments. Environmental engineers have developed physical and chemical methods to remove recalcitrant and/or toxic compounds from soils and waters. Examples of groundwater cleaning techniques are the adsorption of hydrophobic pollutants to activated carbon and air stripping of volatile compounds. Soil has been remediated by incineration or by extraction with solvents. Many pollutants that are widespread contaminants in Europe and the United States have been removed from contaminated sites by these and other methods. These contaminants include non-chlorinated hydrocarbons, such as BTEX compounds (benzene, toluene, ethylbenzene and xylene) and PAHs (polyaromatic hydrocarbons), and chlorinated ones, like PCBs (polychlorinated biphenyls), lindane (hexachlorocyclohexane) and chlorinated solvents such as PCE (tetrachloroethene) and TCE (trichloroethene). However, these techniques are very expensive and have different drawbacks. Soil incineration perturbs the environment, and adsorption and stripping techniques do not destroy pollutants.

A third method which has a large potential and which may have more benefits than the physical and chemical ones, is the biological method. The principle of this method is that

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microorganisms degrade organic pollutants and, in many cases, can use these compounds as growth substrates. By doing so, they detoxify or mineralize the pollutants to innocuous products such as water and CO_2 (Fig. 1).

Microorganisms are ubiquitous in the environment. In the upper layers of the soil, one million to one hundred million bacteria per gram of soil can be found. Most of these bacteria live and grow by degrading organic matter which originates from plants and animals. Many molecules in this organic matter show a large structural resemblance to pollutants, like aliphatic and aromatic hydrocarbons, which are present in petroleum derivatives. There is a wide array of bacteria capable of degrading these contaminants. However, these abilities in polluted environments may be limited by such factors as nutrient limitations, lack of oxygen, predation of bacteria by protists and/or limited availability of the pollutant, and consequently the rates of biodegradation in nature are often low. The engineered process by which conditions are optimized so that specific populations of microorganisms will increase and, therefore, the rates of pollutant degradation will increase as well, is called bioremediation.*

Bioremediation has already been applied successfully on beaches in Alaska by Prof. Ronald M. Atlas (University of Louisville, Kentucky) after the Exxon Valdez oil spill of 1989. Inorganic nutrients like nitrogen and phosphorous in an oil-soluble form were added to the polluted sites to stimulate oil degradation by indigenous microorganisms. Biodegradation processes have also been applied successfully in Germany and the Netherlands, where a technique called "landfarming" was developed. In this technique, soil contaminated with oil is excavated and isolated with plastic. Ploughing the soil to provide oxygen, addition of specific nutrients and use of temperatures above common environmental levels are ways to increase the microbial activity and thus the degradation rate of the hydrocarbons in the soil.

Even though in the above examples engineers and microbiologists had little cooperation, enough knowledge about the microbial aspects of the particular degradation processes had already been gathered by microbiologists over a long period of time. Knowledge of the bacteria involved, their nutrient requirements and degradation pathways, the intermediates and end products formed, and the optimal growth and degradation conditions had been elucidated by microbiologists in the laboratory. These existing data enabled engineers, then, to apply the techniques in a successful manner in the field.

Chlorinated hydrocarbons are more toxic and more persistent in the environment than the above discussed hydrocarbons. Their main use as solvents and degreasing agents (from dry cleaning to the degreasing of machinery and military equipment) has resulted in a widespread release of these compounds into the environment due to indiscriminate use, spills and improper disposal. Even though their inherent health hazards make their degradation process a high priority, the application of bioremediation techniques to treat chlorinated pollutants is almost non-existent. Why? On one hand, physical and chemical methods presently utilized to remove these compounds from the environment are reliable (even though they are very expensive and have disadvantages). On the other hand, although more and more is known about biological transformations of chlorinated compounds, the bioremediation methods have not been sufficiently developed yet and, furthermore, it is difficult to replace a system that works with a system that, as of yet, has not proven its efficacy. Laboratory studies have shown that chlorinated pollutants can be

* The English term "bioremediation" has been previously translated into Spanish as "biorremediación" or "biorrestauración". We believe the term "biodescontaminación" is more appropriate and conveys better the intrinsic meaning of the English equivalent ("bioremediation", which in itself is also different from "biodegradation").

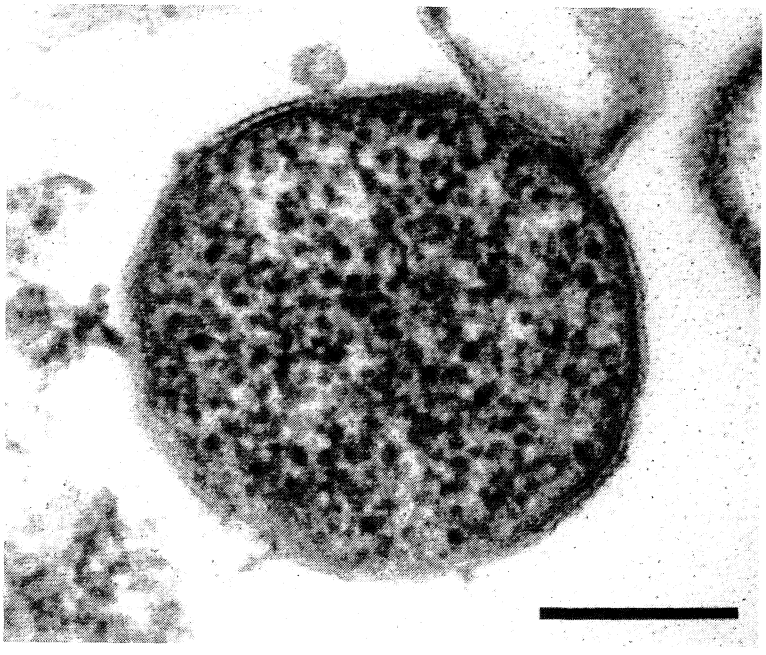
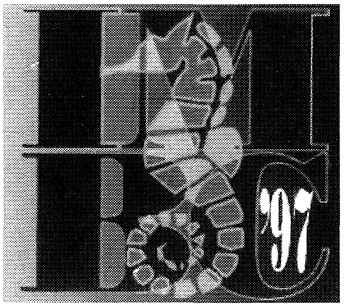


FIG. 1. Transmission electron micrograph of *"Dehalococcus ethenogenes"* strain 195, the first microorganism isolated which is capable of reductively dechlorinating tetrachlorethene (PCE) to ethene. Marker bar represents 0.2 μm.

degraded also by bacteria. Lower chlorinated compounds can be degraded (detoxified and, in some cases mineralized) in the presence of oxygen by aerobic bacteria, whereas higher chlorinated ones, which are persistent under oxic conditions, can be detoxified in the absence of oxygen by anaerobic bacteria. The latter occurs mainly via the process of reductive dechlorination.

The knowledge acquired in recent years by microbiologists predicts a promising future for the biodetoxification of chlorinated pollutants. For environmental engineers to apply this biological method of degradation, the bacteria involved, the nutrient requirements, the optimal biodegradation conditions, the physiology of the processes and the possible formation of toxic intermediates in the degradation of chlorinated pollutants have to be well understood. Here is where microbiologists come strongly into play. The research efforts of microbiologists and civil, environmental engineers have to be organized and shared among them. Only a combined effort will direct applied research towards a faster and improved application of bioremediation technologies.

An example of a successful cooperation has been that between Prof.S. H. Zinder (a microbiologist) and Prof. J. M. Gossett (a civil, environmental engineer) at Cornell University. Their combined efforts have led to the isolation of a novel microorganism, the first capable of dechlorinating PCE (a widespread recalcitrant carcinogenic compound) to ethene, therefore completely detoxifying it. There are several keys to a successful cooperation, but maybe the major one is the ability to understand each other's profession. Engineers need to understand the basics of microbial ecology, physiology and molecular biology, whereas microbiologists need to understand basic engineering concepts. With this in view, their different outlooks and methods will come together to complement in the accomplishment of a common goal: the bioremediation of our environment.



4th International Marine Biotechnology Conference IMBC '97

22–29 September 1997

Sorrento, Paestum, Capo Rizzuto, Oranto Pugnochiuso – Italy

IMBC '97 will consist of two phases: Phase I will take place in Sorrento from September 22 to 25 and will focus on research presentations and poster sessions. Phase II will consist of a four-day scientific working tour of southern continental Italy: In the evenings, Keynote lectures will be delivered by: B. S. Blumberg, Philadelphia, USA, A. Kornberg, Standford, USA, J. Lederberg, New York, USA, M. Rodbell, Research Triangle Park, USA, H. K. Schachman, Berkeley, USA, M. Smith, Vancouver Canada.
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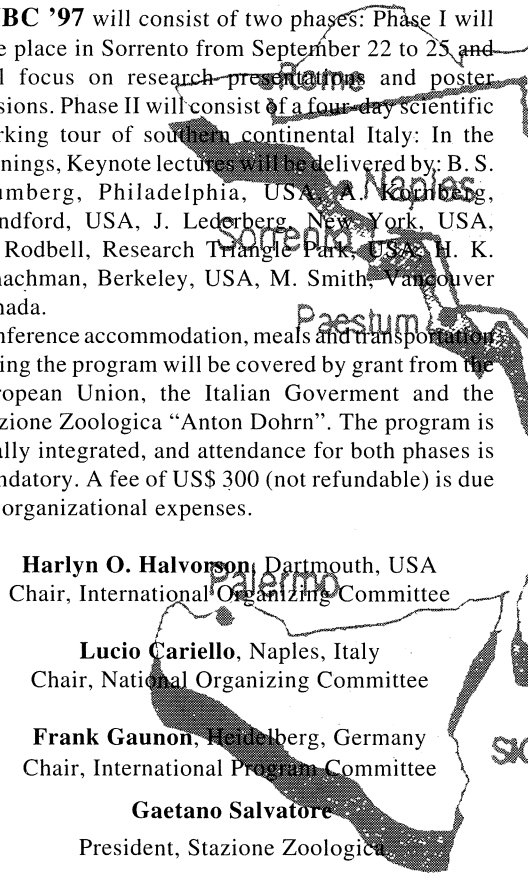

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MICROBIOLOGÍA SEM **13** (1997), 89–94 **OPINION**

Patarroyo and the strategies to develop a malaria vaccine*

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In the last few years, a series of alliances and calls for alliance creations have arisen from the groups that have traditionally fought against malaria and had competed with each other, as well as from the administrations responsible for health policies. Thus, a small number of big, well-organized teams which attempt to find a solution to the malaria problem have originated from a set of many small, less organized ones. This merging has coincided with the development of a vaccine against this disease by a Colombian-Spanish research group. The still limited efficacy of this vaccine leaves some hope for the various groups investigating a definitive end to malaria.

The disease

Forty per cent of the world population lives in regions affected by malaria, a disease which, according to the World Health Organization (WHO), kills 2.7 million people a year, and affects 500 million. As a matter of fact, these figures may be much higher, since WHO only relies on censuses from hospitals. The aforementioned figures become even more serious if one considers that about a million of these deaths correspond to small children. Most endemic countries belong to the third world, with scarce resources and infrastructure to fight the disease. Besides, the organizations that take the decisions on malaria research are located far away from the regions affected.

The history of Colombian vaccine SPF66

In March 1988 *Nature* published (10) a study by Manuel Elkin Patarroyo with the results of the inoculation of some soldiers with a malaria vaccine. The vaccine proved to be partially effective. The scientific community's

*This article constitutes a part of a larger journalistic study on the role of the different participants in the development of the SPF66 malaria vaccine. The different analyses considered are: that of the scientific community, that of the media, that of the institutions responsible for the scientific policies, that of the market and the national context in which the discovery took place. The aim is an identification of the key factors of success and failure in the Colombian-Spanish work. The study began in February 1994.

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first reaction to those results was a strong criticism of Patarroyo's risking volunteers' lives (6). Contemporary malaria researchers were surprised, as they not only were unaware of Patarroyo's previous trials on monkeys (9), but knew nothing about the Colombian physician.

This was clearly reflected in the article "La recrudescence mondiale du paludisme" (i.e., the intensifying universal fight against malaria) published in *Le Monde* on October 23, 1989. This article explained that, according to WHO, the fight against malaria was becoming more and more ineffective. The article presented Marc Gentilini as "one of the best specialists in tropical diseases"; he claimed that "no malaria vaccine will be developed before the year 2000. The situation is charaterized by the lack of a vaccine, the demobilization of the pharmaceutical companies and the development of a new and disturbing phenomenon: the diminishing efficiency of the available products against malaria."

Significantly enough, Gentilini did not mention Patarroyo's potentially definitive vaccine against malaria. The vaccine was not taken into account, partly because of the fact that Patarroyo's clinical trials were not carried out in accordance with the basic rules of epidemiological research. It was for this reason that, once more, the Colombian physician became the subject of criticism by the scientific community. In June 1990, experts from WHO made a public call for clinical trials in Africa to be carried out by independent groups.

Collaboration with Spanish groups

In 1990, Spanish epidemiologist Pedro Alonso (1), a reseacher at the Gambian center of the Medical Research Council (MRC), met with Patarroyo to establish an agreement about a trial in the MRC British center. The trial was subsequently suspended by the MRC head office in London. These refusals, the last occurring in December 1990, led both the Colombian and the Spaniard to consider the need to establish a close cooperation which would assure that the vaccine be tested according to WHO requirements. Thus, the development of a vaccine, which had been hindered to that moment, became a concern of the Spanish Consejo Superior de Investigaciones Científicas (CSIC) [Spanish Council for Scientific Research]. Pedro Alonso resigned from MRC, and began to work for CSIC. The Colombian-Spanish alliance sought the characterization of its product and the possibility of testing it in Africa with the established epidemiological procedure.

In January 1992, the first license for clinical trials was provided by the Dirección General de Farmacia (Spanish Pharmaceutical General Office); in March the same year an agreement was reached with Tanzanian authorities, the Swiss Tropical Institute and CSIC, with the purpose of carrying out trials of the SPF66 vaccine in Tanzania. In March 1993 *The Lancet* (12) published the results of the first Colombian-Spanish medical trial. This trial, in Phase III, had been randomised, double-blind, placebo-controlled, and had been carried out in La Tola (Colombia).

In June 1993, Patarroyo announced his decision to donate his vaccine to WHO. In October 1994, two months after the last trial of the SPF66 in Tanzania, the results were published: SPF66 efficiency had been 31% (2). This was considered to be too low a percentage by the scientific community (5, 8). Patarroyo received his third criticism. In February 1995, the Colombian government—together with the Spanish government—negotiated funding for the production and application of the vaccine in Tanzania. The general program of the development and implementation of the vaccine was the result of the collaboration among Colombian, Spanish, Swiss and

Tanzanian institutions, and it was granted by the Agencia Española de Cooperación Internacional (AECI) [Spanish Agency for International Cooperation] and the Swiss Development Cooperation (SDC). Pedro Alonso was technically responsible for the project. In May 1995, an agreement was signed by which Patarroyo sold his vaccine patent to WHO on the condition that the production would be made in Colombia, and that its price would be no more than one dollar per unit. Patarroyo stated that the reason and the conditions for the donation of the vaccine lay in his own interest in making it sure that those who need the vaccine would get it at a low price.

In 1996, the Colombian-Spanish team carried out trials in Mozambique, and Patarroyo announced that the Institute of Immunology which he directed had discovered a new vaccine, based on the previous one, but with a much higher efficacy. This vaccine has not yet been presented to the scientific community. Nevertheless, a study by Cochrane Collaboration (4) at the beginning of 1996, which was based on five trials of Patarroyo's vaccine, concluded that in the first clinical phase the vaccine's efficacy was 27% (99% CI 13–38%).

Research on malaria in the United States

In 1993 the world spent 84 million dollars on malaria research, according to a report published by Wellcome Trust in 1996 (*Malaria Research: an Audit of International Activity*). About half of this amount was supplied by the National Institutes of Health (NIH) of the United States. This budget appears conspicuously low if compared with the budget for other diseases. The American research budget for cancer was about 2.2 billion dollars a year, AIDS went from zero to 1.5 billion dollars in the last 15 years, and Alzheimer research received about 300 million dollars. In total, the USA spent up to 12 billion dollars in medical research, plus an extra billion dollars which comes from federal agencies.

As a reaction to these priorities, a report on malaria research, made public by the Institute of Medicine in July 1996, i.e. *Vaccines Against Malaria. Hopes in a Gathering Storm*, admitted that "the pace of vaccine development appears to be slowing because of diminishing public funds, fragmented public sector efforts and limited interest within the vaccine industry." This report made a call for the creation of a federal Malaria Vaccine Development Board "to finance and orchestrate research in universities, government laboratories and industry." The study was carried out by the MacArthur Foundation, the Rockefeller Foundation, the Burroughs Wellcome Fund, the NIH, and the Naval Medical Research Institute from the American Department of Defence (DOD). Since a few years ago, this latter institution has coordinated the whole research program on malaria vaccines in the USA.

Walter Reed Army Institute of Research (WRAIR) was placed immediately below the American DOD, as the most important center for medical research of the American Army. WRAIR led the Thai trial of the SP66 vaccine manufactured in the USA (7) and presented the results of the first phase of the trial of a recombinant vaccine against malaria in the *The New England Journal of Medicine* in January 1997 (11). The vaccine, named RTS'S, proved a protection in five out of seven volunteers.

Prior to that project, the New York Vaccine (NYVAC) had been developed. Two private companies, Virogenetics and Pasteur-Mérieux-Connaught, took part in this work, together with WRAIR. The vaccine was presented by colonel Ripley Ballou from WRAIR in November 1994, when he announced that a first dose had been administered to 30 soldiers. It never showed any efficacy against the disease.

The Australian group

Twenty years ago, Australia decided that tropical medicine was a priority, and a coordinated effort among several groups began. Today the Australian team is composed by the Saramane Consortium, Hoffmann la Roche, Biotech Australia and universities around the country. The Australian research groups are planning to carry out a medical trial in phase III in Papua New Guinea in 1997, which is to test a genetic engineered vaccine.

Besides, the Saramane Consortium has carried out trials of toxicity on another candidate, a recombinant vaccine as well, with which it intends to test security and immunogenicity on human beings in Australia. The Australian team is the one which has been investigating for the longest time in basic research, medical trial, and industry.

The European project

Wellcome Trust, with trials in Kenya and Australia, MRC (3), and the Institute Pasteur are all involved in the development of a malaria vaccine. None of them have by themselves the capacity enough to go from basic research to massive trials, which are necessary for the development of a malaria vaccine. This was the reason for creating in February 1995 the African Malaria Vaccine Testing Network, from which the Spanish malaria vaccine researchers were excluded. This fact is especially relevant if one considers that the Spanish team is the only team that had undertaken medical trials on malaria vaccines in Africa.

On December 3, 1995, the Joint EU-USA Action Plan was signed by the President of the European Union (Felipe González), the President of the European Commission (Jacques Santer), and the President of the United States (Bill Clinton). That document stated that the European Union and the U.S. administration intend to work towards a common selection of projects and an exchange of information. This would include the development of a malaria vaccine.

The Colombian-Spanish team

Bearing in mind the aforementioned initiatives, the Colombian-Spanish team is characterized and differentiated from the other research groups in that: (i) Its malaria research work is currently the most developed, as it has carried out trials in Phase III, in Africa and in South-America, with the SPF66 vaccine. (ii) It lacks active participation from any Anglosaxon research group. (iii) It is not linked with any multinational pharmaceutical company. The whole project is funded by only Colombian and Spanish administrations. (iv) It pursues only one goal: basic investigation on tropical diseases like malaria, leprosy, leishmania, as well as tuberculosis and hepatitis. These diseases affect the poor, and must be fought by means of chemical vaccines. To this end, Patarroyo's institute is made up of an interdisciplinary group of around 130 researchers (physicians, chemists, pharmacologists, bacteriologists, general microbiologists, as well as system engineers). (v) The Institute of Immunology is directed personally by Manuel Patarroyo, who determines the relevant lines of research.

The role of WHO

Although WHO has recognized the fight against malaria as one of its main priorities since its creation in 1955, it has not succeeded in

winning the battle. In the 1993 Amsterdam Conference, it was admitted that this failure was due to problems in the biological and socio-administrative sector.

The organization has a Tropical Disease Research (TDR) program, in which malaria research is included. A report on TDR progress 1975–1994 and the 1993–1994 highlights was published in 1995 by WHO. According to that report, of the global TDR budget, 49% goes for malaria. The program is funded—through WHO—by the World Bank and the United Nations Development Organization, as well as by some member states. USA, Canada, Norway, the Netherlands, Denmark and Sweden are the countries which have contributed most to the program. Thus, from 1974 to 1989, the U.S. contributed 33,003,912 U.S. dollars and 1,500,000 U.S. dollars in 1994. The country which has contributed to this project with the highest financial support, however, has been Denmark, with up to 36,923,321 U.S. dollars between 1974 and 1989, and 2,668,823 in 1994.

Two years before the United States called for the creation of the Malaria Vaccine Development Board, a press release from WHO, dated February 13, 1994, informed that a malaria vaccine might be about to appear and called for a global effort to achieve that goal. In that article, TDR director Tore Godal claimed that the Tanzania SPF66 results were a proof that we were halfway through the development of the first effective vaccine against malaria. That same press release informed that at least five other promising vaccines were being tested in endemic countries, and another 20, or even more, were being developed in laboratories all over the world. "To realize all the accumulated potential, —in Dr. Godal's words—we need a global collaboration among institutions working on malaria vaccines, so each can make its own special contribution."

The press release added that "the U.S. National Institutes of Health, the Walter Reed Army Institute of Research and the U.S. Navy are major participants in malaria research." American scientists account for about half the research effort towards malaria vaccines and contribute significantly towards its molecular biological "leading edge". "Other major laboratories and centres are supported by Australia, France, Spain, Switzerland, the United Kingdom and several other countries, including malaria endemic countries themselves." It also noted that "several major private pharmaceutical companies are also involved, and their contribution is essential."

Conclusions

The fact that for the time being the Colombian-Spanish team is the closest to a definite solution to the disease is basically due to the fact that:

(i) It has not based its interest on financial reasons.

(ii) The group does not suffer from the fragmentation of research efforts which is so common in western countries (with the exception of Australia).

(iii) The financial support from the last four presidents of Colombia has provided independence from administrative ups and downs and a little burocratization of the project.

Acknowledgements

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References

1. Alonso, P. L., Lindsay, S. W., Armstrong, J. R. M., Conteh, M., Hill, A. G., David, P. H., Fegan, G., de Francisco, A., Hall, A. J., Shenton, F. C., Cham, K., Greenvood, B. M. (1991). The effect of insecticide-treated bed nets on mortality of Gambian children. Lancet **337**, 1499–1502.
2. Alonso, P. L., Smith, T., Amstrong Schellenberg, J. R. M., Masanja, H., Mwankusye, S., Urassa, H., Bastos de Azevedo, I., Chongela, J., Kobero, S., Menéndez, C., Hurt, N., Thomas, M. C., Lyimo, E., Weiss, N. A., Hayes, R., Kitua, A. Y., López, M. C., Kilama, W. L., Teuscher, T., Tanner, M. (1994). Randomised trial of efficacy of SPF66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. Lancet **344**, 1175–1181.
3. D'Alessandro, U., Leach, A., Drakeley, Ch. J., Bennet, S., Olaleye, B. O., Fegan, G. W., Jawara, M., Langerock, P., George, M. O., Targett, G. A. T., Greenvood, B. M. (1995). Efficacy trial of malaria vaccine SPF66 in Gambian infants. Lancet, **346**, 462–467.
4. Lancet (1996). Malaria, where now? Lancet **348**, 695.
5. Maurice, J. (1995). Malaria Vaccine Raises a Dilemma. Science **267**, 320–323.
6. Miller, L. H. (1988). Malaria. Effective vaccine for humans? Nature **332**, 109–110.
7. Nosten, F., Luxemburguer, Ch., Kyle, D. E., Ballow, W. R., Wittes, J., Wah, Eh., Chongsuphajaisiddhi, T., Gordon, D. M., White, N. J., Sadoff, J. C., Heppner, D. G., the Shaklo SPF66 Malaria Vaccine Trial Group. (1996). Randomised double-blind placebo-controlled trial of SPF66 malaria vaccine in children in northwestern Thailand. Lancet **348**, 701–707.
8. Nussenzweig, R. S., Long, C. A. (1994). Vaccines. Frontiers in medicine. Malaria Vaccines: Multiple Targets. Science **265**, 1381–1383.
9. Patarroyo, M. E., Romero, P., Torres, M. L., Clavijo, M. L., Moreno, A., Martínez, A., Rodríguez, R., Guzman, F., Cabezas, E., (1987). Induction of protective immunity against experimental infection with malaria sing synthetic peptides. Nature **32**, 629–632.
10. Patarroyo, M. E., Amador R., Clavijo, P., Moreno, A., Guzman, F., Romero, P., Tascón, R., Franco, A., Murillo, L. A., Ponton, G., Trujillo, G. (1988). A synthetic vaccine protects humans against challenge with asexual blood stages of *Plasmodium falciparum* malaria. Nature **332**, 158–161.
11. Stoute, J. A., Slaoui, M., Heppner, D. G., Momin, P., Kester, K. E., Desmonds, P., Wellde, B. T., Garcon, N., Krzych, U., Marchand, M., Ballow, W. R., Cohen, J. D. (1997). A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. The New England J. Medicine **336**, 86–91.
12. Valero, M. V., Amador, L. R., Galindo, C., Figueroa, J., Bello, M. S., Murillo, L. A., Mora, A. L., Patarroyo, G., Rocha, C. L., Rojas, M., Aponte, J. J., Sarmiento, L. E., Lozada, D. M., Coronell, C. G., Ortega, N. M., Rosas, J. E., Alonso, P. A., Patarroyo, M. E. (1993). Vaccination with SPF66, a chemically synthesised vaccine, against *Plasmodium falciparum* malaria in Colombia. Lancet **341**, 705–710.

MICROBIOLOGÍA SEM **13** (1997), 95–100 **OPINIÓN**

Consideraciones en torno a la ciencia como producto cultural

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La comprensión de la ciencia

Científicos y filósofos de la ciencia han reflexionado sobre las características de la ciencia moderna, la manera de trabajar del científico en la elaboración y progreso de la misma, y la evolución del conocimiento científico a través de los tiempos. Como en muchos otros aspectos del quehacer humano, no existen unanimidades absolutas, pero sí se aprecia algún acuerdo en considerar la ciencia como un producto cultural con características lo suficientemente peculiares para permitir distinguirlo de otros productos culturales, como la filosofía o el arte.

Karl Popper (1902–1994) ha sido, sin ninguna duda, uno de los pensadores que ha tenido más influencia en la comprensión de la ciencia moderna y en la búsqueda de sus raíces epistemológicas e ideológicas, así como de las motivaciones que rigen el quehacer científico. Este autor considera que la ciencia moderna, o ciencia empírica, actúa a partir de una hipótesis que, si al ser contrastada a través de resultados se demuestra falsa, debe ser abandonada. El análisis de falsación se hace por medios empíricos, lo que supone, en muchos casos, la planificación y realización de experimentos adecuados. Cuando, o mientras, los resultados de los experimentos cumplen las condiciones previstas por la hipótesis que les sirve de base, esta hipótesis se mantiene vigente y accede al rango de teoría científica válida (1).

La postura de Popper frente a la ciencia, sea por sus afirmaciones, sea por la interpretación de su pensamiento en algunas de sus obras, se ha tomado en un sentido reduccionista como, por ejemplo, en la frase del biólogo celular Grinell citada por Schumm (2): "Investigators know that reproducibility of experiments is a requirement of scientific research. While one can raise questions about unique events, only recurring events can be subjected to scientific investigation." Si esto fuera cierto quedarían al margen de la ciencia moderna numerosas ra-

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mas de las ciencias o de parte importante de las materias que competen a muchas de esas ramas científicas. El mismo Schumm, después de la frase entrecomillada, afirma que, si esto es verdad, "there is no Earth science".

La postura reduccionista atribuye una uniformidad a la ciencia, que está lejos de existir (3). Sin embargo, sí se dan algunos elementos comunes en todas y cada una de las ciencias consideradas como tales actualmente, como por ejemplo la asimilación de la ciencia a un producto cultural autocorrectivo por definición

En primer lugar, el experimento o la observación realizados con el rigor requerido, cuyos resultados contradicen una teoría, deben ser sometidos a examen y, si después de las pruebas necesarias se mantiene la incompatibilidad, se impone modificar o incluso abandonar la teoría. En segundo lugar, la ciencia moderna es **empírica** y plantea interrogantes, suscita hipótesis y las corrobora o invalida a través de la observación y el experimento. En tercer lugar, la ciencia es **sistemática,** y por ello requiere que sea consistente en sí misma como discurso racional. Finalmente, a causa y en apoyo de estos elementos, la ciencia agradece el uso de métodos **cuantitativos** para comprender y fijar los resultados, y para manejarlos más fácilmente. El cálculo y las matemáticas son instrumentos de gran interés y utilidad en la elaboración de la ciencia moderna, y con frecuencia preceden o permiten la creación de hipótesis y teorías.

El recientemente fallecido Thomas S. Kuhn (1922–1996) ha aportado ideas que contribuyen a corregir el excesivo simplismo que derivaría de una interpretación literal de lo que acabamos de decir. En efecto, con los elementos citados podría pensarse en un progreso lineal de la ciencia, y eso es algo que no ocurre así y que muchos autores modernos, además de Kuhn, han subrayado. Kuhn utilizó la palabra paradigma, que tiene bastante en común con el concepto de "estilo de pensamiento", desarrollado por Ludwick Fleck (1896–1961).

Según la opinión de Kuhn (4), la ciencia progresa por medio de revoluciones que se concretan en cambios de paradigmas. Estas revoluciones suceden cuando la ciencia establecida empieza a entrar en crisis por la incapacidad de asumir nuevas aportaciones en el ámbito de una determinada ciencia en la que se consideraba válido un determinado paradigma. Sin embargo, y haciendo hincapié en las ideas de Kuhn y de Fleck, y en la experiencia moderna del imparable avance tecnológico, es preciso incluir la posibilidad de que una experimentación no cumpla las predicciones de la teoría, no por falta de consistencia, sino por falta del instrumento adecuado. En este caso, pasado el tiempo y con nuevas posibilidades técnicas, la teoría podría cumplirse.

El enfoque de Kuhn plantea, más que la manera del proceder consciente de los investigadores, la existencia de unos "sistemas" científicos que se suceden en el tiempo. Representa un viraje en la reflexión sobre la ciencia que, desde muy diversas líneas y con diferentes argumentos, está siendo considerado por muchos pensadores en la actualidad. Esta nueva reflexión está más ligada a la ciencia como producto cultural, que como doctrina racional. Representa una diversidad de enfoque cuyo resultado proclamaría que son más bien los sociólogos que los filósofos de la ciencia los que crean teorías al respecto. Dentro de este mismo contexto, Paul R. Gross y Norman Lewitt han dedicado el libro *Higher Superstition* (5) a criticar duramente a destacados grupos de sociólogos de la ciencia que han elaborado teorías que parecen ser incompatibles con lo que la ciencia es realmente. Estaríamos ante posiciones ideológicas que manipulan, de una manera particularmente radical, los aspectos culturales de la ciencia para negar su validez universal y la de sus resultados.

La ciencia como producto cultural

No es nuestro interés entrar en la mencionada polémica, ni pretendemos ocuparnos explícitamente de los temas tratados en el libro que acabamos de citar, aunque indirectamente hagamos alguna referencia a los mismos. Más bien intentamos apuntar algunas ideas que nos permitan progresar en el camino de los "sistemas" científicos, y no sólo en el tiempo, sino también en el espacio. Por ello, no trataremos del contenido de las ciencias, sino sobre todo del "feedback" constante que se produce entre la subjetividad social e individual y la objetividad de los conocimientos científicos.

Hemos usado el término "producto cultural" aplicándolo a la ciencia, y no pensamos que nadie dude de la validez de esta atribución. Los seres humanos producen también materiales estrictamente "naturales", es decir, productos en los que la racionalidad y el sentimiento apenas interfieren. Es el caso de los productos de la acción mecánica, espontánea o controlada del cuerpo, como el sudor, la orina, secreciones, etc. En otro sentido, hemos acuñado el término de "productos artificiales" para todo aquello que hacen los hombres y las mujeres y que entronca con los procesos psicológicos conscientes que afectan al pensamiento y a la sensibilidad. Todos ellos pueden ser denominados productos culturales, que van desde un objeto concreto a cualquier creación humana, por compleja que sea. Considerado desde esta perspectiva, un receptor de radio y la ciencia moderna son igualmente productos culturales contemporáneos.

Ahora bien, un **producto cultural** es algo que se crea dentro de una determinada cultura, ya que todos los productos culturales nacen del pensamiento, del sentimiento y de la acción humanos en el interior de lo que podría llamarse una **etnia cultural**. Dicho en otras palabras, pensamos, sentimos y obramos siempre en el interior de un imaginario que nos viene dado por nuestra pertenencia a un ámbito cultural determinado, situado en un espacio y un tiempo particulares e intransferibles. Este imaginario puede ser llamado con propiedad **paisaje cultural** porque participa de las propiedades de los paisajes geográficos y se solapa con ellos. Las etnias culturales se sienten ligadas a un territorio concreto, con unos elementos tan materiales como sus condiciones climáticas, su tipo de relieve y de vegetación, sus animales y sus tipos de hombres y mujeres presentes en él. Por otra parte, las tradiciones y creencias, la historia y la situación actual configuran una manera de pensar y de sentir que pueden estereotiparse. De este modo, simplificando, se puede decir que los latinos son y actúan de una determinada manera distinta a la de los germánicos, o que los católicos reaccionan de forma diferente en muchos aspectos de la vida a como lo hacen los protestantes.

Desde esta perspectiva, los productos culturales llevan la impronta de la etnia cultural que los crea y están insertos en el paisaje cultural de esta misma etnia. Dicho en palabras más simples, todo producto cultural es dependiente de la cultura que lo crea, de modo que sin ella no se habría dado tal cual es. Aunque otra cultura produzca algo que tenga la misma función, y aunque use materiales iguales o parecidos, el diseño será diferente. Sin embargo, a modo de paréntesis, no estaría de más reflexionar sobre el efecto que la intercomunicación está ejerciendo y seguirá haciéndolo en el futuro, y considerar hasta qué punto los marchamos culturales pueden ver modificadas sus características distintivas.

Pero resulta claro que la ciencia moderna es un producto cultural de determinadas clases sociales de los países occidentales, que responde a unas necesidades y expectativas. Por consiguiente, las motivaciones que condujeron a su creación y desarrollo, las formas de expresión y la presentación de sus resultados, corresponden

a la cultura que la originó. Desde este punto de vista cabe considerar que la ciencia no es un producto universal ni, por tanto, comprensible de la misma manera para todos los pueblos.

De este hecho algunos pensadores deducen que la ciencia es un producto sólo válido en la cultura que lo creó, de manera que los paisajes culturales serían tan absolutamente impermeables que no podrían integrar elementos derivados de otras culturas. Nada más falso. El producto cultural, una vez creado, es relativamente independiente de la cultura que lo creó, aunque lleve eternamente el marchamo o diseño original. Un reloj japonés marca la hora en Europa y en África, aunque su diseño continúe siendo el original. Las teorías que propugnan que las culturas son realidades absolutamente cerradas no pueden ser aceptadas, aunque no se pueden despreciar las dificultades de comprensión y aceptación de los productos culturales de una determinada etnia cultural por parte de las otras. Cabe retomar aquí la reflexión sobre el efecto de la propagación de la información y la facilidad de comunicación propia de nuestra época para dudar de la posibilidad de cerrazón permanente de algunas culturas. Experiencias sociopolíticas muy recientes demuestran todo lo contrario.

De lo que acabamos de decir se infiere claramente la universalidad de la ciencia, de manera que la causa de la caída de una manzana de un árbol viene definida precisamente por la ley de Newton, tanto en Europa como en China. Así mismo, la Tierra gira alrededor del Sol siguiendo las pautas descubiertas hace siglos por la ciencia europea. No se pueden poner adjetivos a la ciencia, ni en relación a los pueblos o grupos sociales donde se desarrollan, ni a las ideologías que la integran. Este hecho no invalida la existencia de un "regionalismo" en la mentalidad de los científicos, según su pertenencia a una etnia cultural o, más claramente, según su propio paisaje cultural. Esta dependencia de la comprensión de la ciencia derivada de cada uno de los paisajes culturales nos hace ver que la ciencia es a un tiempo universal y "regional". La aceptación de esta peculiaridad permitirá abordar con rigor el diálogo entre científicos para tratar de vencer los obstáculos que dificultan enormemente la expansión y difusión de una cultura científica ("scientific literacy") (6). Y ello irá también en beneficio de un público que no se dedica a la ciencia profesionalmente, pero que tiene derecho a exigir saber y comprender sobre ella.

Formas de "regionalismo" científico

Trataré de discutir brevemente algunos tipos especialmente destacados de regionalismo. A mi entender, se originan tanto a partir de la influencia del paisaje cultural de determinadas etnias culturales, como por la presencia de ciertos factores en la formación personal de los propios científicos. Con ello desearía invitar a la reflexión sobre unos temas que me parecen de particular interés en un momento histórico como el actual, cuando los logros de la ciencia y de la técnica tienen en todo el mundo una influencia tan grande en la vida de las personas.

Muchos científicos comprenden la ciencia como un valor casi absoluto en el orden cognoscitivo, de modo que reducen drásticamente el ámbito del conocimiento a la ciencia. Este reduccionismo deriva de una actitud cultural excesivamente simplista, presente en determinados paisajes culturales que han aceptado ciertos principios que son propios de algunas tendencias de la filosofía moderna y contemporánea. Es una actitud que se corresponde con lo que ha sido llamado a veces "cientismo", consistente en creer que las respuestas a todas las preguntas solamente son posibles con la aplicación universal del razonamiento científico. Es una fe ciega en el progreso y en las posibilidades

de la ciencia. Se ha acusado, quizá con alguna razón, a los científicos anglosajones de pertenecer mayoritariamente a este grupo, pero también se da en amplias capas de la población en los países en los que la ciencia moderna tiene un cierto desarrollo.

Entre los científicos que no han sucumbido al puro cientismo podemos distinguir dos tipos:

(i) El primero corresponde a aquéllos en los que su particular percepción del mundo y de los acontecimientos, que denominaremos cosmovisión, deriva de la ciencia y forma parte de su paisaje cultural. No han excluido la cosmovisión que adquirieron a través de la educación y la tradición y que nutrió las propias reflexiones; pero apenas se produce conexión entre ambas cosmovisiones, que discurren paralelas sin ninguna relación reflexiva.

(ii) Un segundo tipo está formado por los científicos cuya cosmovisión científica interactúa constantemente con la que obtienen de otros campos del conocimiento, produciéndose una especie de fecundación mutua. En este segundo tipo se suelen encontrar quienes poseen una cosmovisión que no ha sido adquirida por la ciencia, dotada de un vigor y una vitalidad especialmente notables.

En la captación de los fenómenos científicos por parte del gran público, se encuentran también diversos tipos de integración de la ciencia en el propio paisaje cultural. Por una parte, existe el tipo constituido por los que aceptan el valor de la ciencia, pero mantienen un cierto sentido crítico frente a la avalancha sensacionalista de noticias, real o supuestamente científicas, frecuentes en los medios de comunicación de masas.

Por otra parte, podemos distinguir también otras dos clases, opuestas, de comportamiento. Por un lado, el de los que "sacralizan" la ciencia, de manera que la convierten y consideran un instrumento de salvación, ejerciendo un papel similar al que las religiones se han ido atribuyendo frecuentemente a través de las distintas épocas. Este grupo tendría muchos puntos en común con la postura cientista a la que hemos aludido en párrafos anteriores.

En el otro extremo, en los países que cuentan con un mayor y mejor desarrollo de la ciencia moderna, están proliferando determinados grupos sociales que han expulsado de su paisaje cultural la ciencia ordinaria, para sustituirla por formas y fórmulas diversas de paraciencia o pseudociencia (7).

A modo de conclusión

Las líneas que anteceden pretenden describir de una manera sucinta algunos de los temas que pueden ser estudiados desde la perspectiva de la ciencia como producto cultural. Contienen, al mismo tiempo, algunos juicios de valor sobre determinados aspectos que, en nuestra opinión, interfieren tanto en el desarrollo armónico de la ciencia, como en la comprensión eficaz y provechosa de los avances de este producto cultural. Un producto que, nacido en el occidente europeo, se expande con resultados enormemente desiguales por todo el mundo.

Comprender la ciencia y aceptar que las distintas culturas, al conferirle sus propias características, le van dando esa conformación universal es a la vez una garantía de seriedad y un ejercicio de modestia por parte de los científicos. Es también la base para el diálogo intercultural, cada vez más urgente, dado que, si bien la información llega a todas partes, la captación precisa de la misma no se produce con igual facilidad. Este hecho, en ciencia, es particularmente dramático, como demuestran, entre otros ejemplos, las estadísticas sobre la esperanza de vida e incidencia de enfermedades en los diversos países del mundo.

Notas

1. Diversos libros ofrecen una explicación detallada de nuestra comprensión de la ciencia moderna. Yo he tomado datos de Popper y Valentine&Ayala, entre otros, del libro: Boladeras, Margarita (ed.) (1982). *Metodologia de la ciència. Materials per a una metateoria de la Geologia.* Publicacions i Edicions de la Universitat de Barcelona.
2. Schumm, Stanley A. (1991). *To Interpret the Earth. Ten Ways to Be Wrong.* Cambridge University Press, Cambridge.
3. Dupré, John (1993). *The disorder of things. Metaphysical Foundations of the Disunity of Science.* Harvard University Press, Cambridge, Mass.
4. Chica, Carmen (1996). Los paradigmas perdidos, Thomas S. Kuhn. *Microbiología SEM* **12**, 641–646. Presenta una visión resumida muy interesante de la vida y pensamiento de Kuhn.
5. Gróss, Paul R. & Lewitt, Norman (1994). *Higher Superstition: Left and Its Quarrels with Science.* The Johns Hopkins University Press, Baltimore. Los autores consideran que muchas de las posturas anticientíficas manifestadas por los sociólogos y pensadores a los que se refiere el libro, son más fruto de posturas ideológicas que de un debate serio y racional. Bajo estas premisas, esas posturas resultan particularmente inconsistentes.
6. La preocupación por la expansión de la cultura científica entre el público no especialista ha dado lugar a muchas iniciativas. Una de las más importantes es el "Project 2061: Science Literacy for a Changing Future", patrocinado por la American Association for the Advancement of Science. El tema de la posibilidad, conveniencia y utilidad de extender a amplias zonas de la humanidad un mínimo de cultura científica ha dado lugar a discusiones apasionadas y a libros expresamente dedicados a ese fin. Las reseñas y críticas de esos libros y comentarios de las discusiones han aparecido en revistas tan importantes como, entre otras, *Nature, Science* y *The Sciences.*
7. Véase, por ejemplo, el libro: Zimmerman, Michael (1995). *Science, Nonscience and Nonsense.* The Johns Hopkins University Press, Baltimore. Algunas organizaciones se ocupan en España de estos temas, por ejemplo, "Alternativa racional a las pseudociencias", que publica la revista *La Alternativa Racional*, o, en Estados Unidos, el "Committee for the Scientific Investigation of Claims of the Paranormal" (CSICOP), que publica la revista *The Skeptical Inquirer.* Para ver la importancia que eminentes científicos dan a este fenómeno reciente, cada vez más extendido y, en muchos aspectos, peligroso para la sociedad, basta citar los nombres de algunos de los miembros de este comité: Carl Sagan, Mario Bunge y Stephen J. Gould. En su último libro, *The Demon-Haunted World. Science as a Candle in the Dark* (Random House, New York, 1995), el recientemente fallecido Carl Sagan (1934–1996) trata de este problema, con respecto al cual el título es especialmente significativo.

MICROBIOLOGÍA SEM **13** (1997), 101–111

Revisión de libros
Book reviews

Science and the Quiet Art. The Role of Medical Research in Health Care

David Weatherall

W. W. Norton & Co., New York, 1995. 375 pp.
Price: $ 25. ISBN 0-393-03744-4

In this wide ranging, general interest book, David Weatherall, Regius Professor of Medicine at the University of Oxford, explores the tension that exists between doctoring as a bedside empirical practice (the quiet art) and as the application of laboratory-based scientific research. He argues strongly that scientific medicine is the superior orientation for doctors and that basic medical research, rather than environmental mediation, should continue to be society's priority. The book is a product of the New York City-based Commonwealth Fund Book Program, a collection started (in 1985) and directed until his death by Lewis Thomas (1913–1993). The book is therefore the work of an experienced scientist, reflecting on his specialty and placing it in a wider social context. The references are informally placed at the end of the book rather than being cited in the text.

Professor Weatherall, a specialist in human genetics, begins with a brief, eurocentric account of the history of medicine, emphasizing the increasing historical influence of experimental scientific research on medical practices. He explores heart disease, pernicious anemia and infectious diseases in general to show how scientific research has contributed to solve medical problems, but also to show how the practical application of research results may not arise until years or decades later. In part III, "How much do we really know?", the author has a fascinating account of the development of cardiology and a review of the current state of morbidity and mortality in the Western industrialized societies and the developing world.

The remainder of the book is a tour through a number of interesting topics including the genetic vs. environmental causes of diseases, the evolutionary origin of heritable diseases, with an emphasis on diseases related to the structure of hemoglobin, and the development and application of molecular biology techniques to medicine. The concluding chapters discuss possible future developments in genetic markers, gene therapy and other modern diagnostic and therapeutic techniques. The author examines a wide diversity of medical problems including diabetes, heart disease, psychiatric disorders, and cancer.

Because the book is an attempt to include several different topics (the training of doctors, a history of medicine, the biological basis of diseases and infections, and the development of health care policy) the author succeeds at covering some topics more than others. It seems he produced an amalgam of topics to reach a wider audience. The way doctors are trained

and orient their practices, although ostensibly the subject of the book as indicated by the title, is probably of limited interest to the general public. Similarly, in-depth accounts of the limits in our knowledge of the biology of diseases, such as the difficulties in treating cancer with chemotherapy or AIDS with AZT without damaging the patients immune system, although of interest to readers of this journal, may not carry the interest of a nonspecialist audience. The book is actually very limited on microbiology and immunology.

The biggest shortcoming, however, is in the wider social context of health care policy. Although the author repeatedly refers to the high costs of medicine, to economies being crippled by the expense, or to governments not taking care of the poor, the book contains no discussion of the political economy of health care. Why does medical care cost so much? What are the costs to society from for-profit pharmaceutical companies and (in the USA) for-profit hospitals? What is the wider social context for the poverty of the developing countries, where seven million children, or perhaps even more, die each year from preventable infections and other diseases? The book, in fact, is an uncritical acceptance of the existing social relations of health care, which really seem to be at the heart of public criticism of the medical establishment.

Despite these criticisms, *Science and the Quiet Art* is a useful and absorbing account of the history and current state of medical research. It provides a good introduction to the scientific and ethical problems associated with medicine and gives the nonspecialist public the concepts it needs to understand the genetic component of health problems.

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Informe mundial sobre la ciencia 1996

Ediciones UNESCO, Madrid, 1996. 372 pp.
Precio orientativo: 4600 PTA.
ISBN 84-294-5128-5 (Santillana)
ISBN 92-3-303220-5 (UNESCO)

Coincidiendo con el 50 aniversario de su fundación, UNESCO ha publicado su segundo *Informe mundial sobre la ciencia*. Como indica en el prefacio Federico Mayor, su director general, "es un momento oportuno para reflexionar y hacer un balance [...] rendir un homenaje [...] a los fundadores de la UNESCO cuando decidieron que la ciencia debía desempeñar un papel importante en el programa de la Organización para propiciar la consecución de sus objetivos de fomento de la paz, las capacidades endógenas y la democracia". El *Informe* cumple esta finalidad; es a un tiempo reflexión y balance muy completo de la situación de la ciencia en el mundo. El libro se estructura en tres bloques, dedicados a otras tantas áreas temáticas, precedidos del prefacio de Federico Mayor y de un preámbulo sobre conocimientos científicos básicos a cargo de Francisco J. Ayala, ex-presidente de la AAAS (American Association for the Advancement of Science).

Ayala incide en la necesidad universal de que la población general posea un mínimo de conocimientos funcionales de la ciencia, que equipara a la necesidad de alfabetización. Por una parte, destaca la necesidad que tienen los países modernos, para su crecimiento económico, de contar con mano de obra capacitada técnicamente; en la mayoría de países existe una estrecha correlación entre el porcentaje del PIB invertido en I + D y las diferentes medidas de su bienestar económico (vease el Editorial de *Microbiología SEM* de junio de 1996 [**12**, 163–

165}, de F. J. Ayala). Por otra parte, las decisiones políticas requieren cada vez más de unos conocimientos científicos y tecnológicos; los gobiernos deberán contar con asesores, pero los propios gobernantes tendrán que ser capaces de comprender y evaluar los consejos de los expertos. Además, la democracia a que deberían aspirar todos los países no será completa si sólo una parte de la población puede comprender la base científica y tecnológica en que se apoyan las decisiones de sus gobernantes. Una población ignorante es más vulnerable a la explotación por parte de individuos o grupos desaprensivos que puedan utilizar la demagogia o la falsedad para sus propios intereses. Insiste Ayala en que la instrucción científica debe arrancar de la escuela primaria, proseguir a lo largo de todo el período de escolarización, y consolidarse a través de los medios de comunicación, especialmente la televisión, que, además de información, deben transmitir conocimientos.

El primer bloque del informe, "La situación de la ciencia en el mundo", se inicia con una panorámica mundial, con estadísticas que permiten comparar la dedicación de las diferentes zonas geográficas a la ciencia y la tecnología. Aunque en algunos casos las estadísticas son demasiado generales y proporcionan una visión distorsionada de la situación, los capítulos siguientes aportan datos más concretos. Así vemos que aunque la Unión Europea destina un 1,9% del PIB a los gastos de I + D, se dan variaciones significativas entre los distintos países que la integran. Por ejemplo, Alemania y el Reino Unido superan con creces ese porcentaje, mientras que España, Portugal y Grecia ocupan los últimos lugares, con menos del 1%.

Resulta curioso que, junto a la mayoría de capítulos que consideran grandes zonas geográficas del planeta (América del Norte, America Latina, Europa Occidental, Europa Occidental, Comunidad de Estados Independientes [ex-Unión Soviética], etc.), se incluya uno que trata exclusivamente de la ciencia en Irán. En cambio, resulta infructuosa la búsqueda en el índice de un capítulo dedicado a Oceanía. Un examen detallado permite localizar los datos referentes a Australia y a Nueva Zelanda en el capítulo sobre Asia Oriental y Sudoriental. Una nota advierte que aquellos dos países se incluyen allí "para establecer comparaciones, ya que en el último decenio han comenzado a establecer relaciones cada vez más estrechas con el resto de Asia".

El segundo bloque, "Los debates contemporáneos", expone algunos de los temas que más controversia despiertan actualmente en los medios de comunicación y en la población mundial. Se inicia con el capítulo "La ética de la ciencia: entre humanismo y modernidad", escrito por Noëlle Lenoir, presidenta del Comité de Bioética de la Unesco. Es una reflexión sobre los principios éticos que deben regir toda práctica científica, y que resume en cuatro ideas clave: respeto a la dignidad de la persona y a su libertad; prevención de los riesgos tecnológicos de los que depende el futuro de la humanidad; preservación de la libertad de creación científica; y solidaridad intelectual y moral, que permita que las ventajas del progreso beneficien a toda la humanidad. Los otros temas considerados son: "La macrociencia", que se refiere a proyectos o programas muy ambiciosos, tendencia imperante en algunos sectores científicos como la investigación espacial, el estudio del cambio mundial, o el proyecto sobre el genoma humano; "Geociencia y medio ambiente: comprender los efectos de las actividades humanas en los procesos naturales", que preconiza el establecimiento de una nueva disciplina para evaluar los efectos de las actividades humanas en el ambiente, predecir sus consecuencias y establecer planes para evitar los posibles impactos negativos; "La degradación de la tie-

rra", que es un ejemplo de un caso aplicado de geociencia ambiental; "Diversidad biológica", cuyo autor, Francesco Di Castri, define como la totalidad de genes, especies y ecosistemas de una región determinada, y trata de las extinciones y las acciones emprendidas a escala internacional para evitarlas; "La biotecnología y el desarrollo", amplio capítulo sobre un campo de la ciencia casi tan antiguo como la propia humanidad, pero que ha experimentado un cambio vertiginoso con los avances de la genética y la tecnología; "Las tecnologías de la información", que analiza principalmente la red de comunicación global Internet, resultado de la convergencia de la electrónica, la informática y las comunicaciones; y "Ciencia e ingeniería de los materiales", que trata de una revolución más callada que la producida por las tecnologías de la información y las biotecnologías, pero que puede aportar soluciones a problemas relacionados con el ambiente, la energía, el transporte y la medicina.

El último bloque, " El lugar de las mujeres en la ciencia y la tecnología", intenta explicar la interacción de las relaciones de género con las instituciones y los proyectos científicos y tecnológicos. La presencia de la mujer en el mundo de la ciencia dista aún mucho de alcanzar la del hombre. Aunque no se posee una amplia información a escala mundial, los datos disponibles indican que la incorporación de la mujer a este campo del conocimiento no va ligado al menor o mayor desarrollo económico y cultural de su propio país. Este apartado se complementa con una referencia a los servicios disponibles en Internet en relación al lugar de la mujer en la ciencia y la tecnología. Sandra Harding , profesora de filosofía y estudios sobre la mujer de la University of Delaware y de la University of California, Los Angeles, y Elizabeth McGregor, directora de estudios del Grupo de Estudio de la Comisión de Ciencia y Tecnología para el Desarrollo, de Naciones Unidas, han elaborado la mayor parte de este bloque, que, como ellas mismas indican, es una muestra de "los resultados de dos décadas de estudios de las relaciones entre los sexos y, por lo menos, de tres de historia y sociología de las ciencias y la tecnología en el Norte y en el Sur". El bloque se completa con varias contribuciones de otras autoras. Merece la pena destacar la reseña histórica dedicada a las mujeres en la investigación científica moderna de Pnina G. Abir-Am, actualemente becaria del Dibner Institute for History of Science and Technology (Cambridge, MA, EE.UU.).

Los datos objetivos que contiene el *Informe* hacen de él una útil obra de referencia. Sería conveniente que, en próximas ediciones, los capítulos sobre la situación de la ciencia en el mundo presentasen mayor coherencia en cuanto al espacio dedicado a los diferentes países o regiones geográficas. Sería también aconsejable que las estadísticas sobre los indicadores del desarrollo científico se refiriesen a datos equivalentes, para permitir establecer comparaciones. El interés de esta obra va más allá del que podría despertar una mera recopilación de datos que intentasen expresar, de manera objetiva, la situación de la ciencia en el mundo. Aporta también la visión de especialistas en cada uno de los temas tratados. En este punto, por su relación con la microbiología, queremos mencionar la presencia, entre los autores, de la Prof. Rita R. Colwell, que hace poco comentaba en *Microbiología SEM* la reciente epidemia de cólera que empezó en Perú (véase el Editorial en el número de diciembre de 1996 [**12**, 519–522]). Sin duda estamos ante una obra de referencia muy recomendable, y esperamos vayan apareciendo nuevas ediciones en años sucesivos.

Mercè Piqueras
Redacción de Microbiología SEM

Manual para la redacción, traducción y publicación de textos médicos

José Luis Puerta López-Cózar, Assumpta Mauri Mas

MASSON, S. A., Barcelona, 1995. 445 pp. Precio: 6.400 PTA. ISBN 84-458-0255-0

Cada día se insiste más en la necesidad de utilizar un lenguaje correcto. Aunque desanime comprobar el escaso interés de los medios que más deberían cuidarlo, lo cierto es que van apareciendo obras que vienen en ayuda de quienes se preocupan por los aspectos lingüísticos y el uso de la nomenclatura y terminología apropiada en sus escritos. Tal vez sean muchos los autores veteranos que se pregunten ¿por qué no las había en mis tiempos? La utilidad de estos instrumentos ha llevado a estos dos autores a recopilar y adaptar en un *Manual* el material que generalmente se tiene que consultar en varias obras separadas.

La redacción de un artículo, habida cuenta que el contenido reúne los requisitos de interés, novedad e importancia necesarios para desear comunicarlo, necesita (i) **lógica**, **estructura** y **continuidad** a través de los diferentes apartados, (ii) **equilibrio** entre los elementos (texto, tablas, figuras, bibliografía) que lo componen y (iii) **consistencia** en la utilización de la terminología. Escribir un artículo, como saben muy bien los autores, no es un acto reflejo, ni obedece a leyes intuitivas, antes obliga a ejercer el sentido de la organización para encajar la información en la estructura del artículo científico o ensayo clínico.

Aunque es distinto el caso de la traducción, donde, además, es requisito indispensable el conocimiento de las dos lenguas para verter con fidelidad (que no quiere decir literalidad) al idioma al que se traduce las explicaciones del autor, rigen, sin embargo, idénticos criterios de claridad, concisión y precisión.

El *Manual* aparece prologado por el Prof. Pedro Laín Entralgo, quien destaca el valor de la precisión en la palabra para decir con exactitud y corrección lo que se tiene que decir. Para ello, insiste, es preciso conocer, para bien emplear, la palabra más adecuada y respetar las reglas sintácticas del idioma en que se escribe.

El libro consta de ocho capítulos: 1. Las normas de publicación de Vancouver (que todos los autores de textos científicos y médicos deberían conocer). 2. Consideraciones gramaticales, ortográficas y tipográficas, con términos y explicaciones que suelen plantear dudas, como en el caso de abreviaturas y siglas. 3. Abreviaturas y símbolos científicos. Sistemas de nomenclatura, donde se recogen términos médicos y de otras áreas muy relacionadas. 4. Unidades del SI y otros sistemas de medida utilizados en medicina. 5. Agentes infecciosos, de utilidad para localizar de forma rápida la escritura correcta de los agentes infecciosos más frecuentes; ofrece una clasificación alfabética de los géneros, con indicación de los nombres populares o sinonimias. 6. Glosarios. Es la parte más extensa de toda la obra e incluye abreviaturas y acrónimos médicos en inglés y en español, así como términos de especialidades terapéuticas y de investigación clínica. Los dos capítulos finales (7. Abreviaturas de las revistas médicas y 8. Bibliografía) tienen una indudable utilidad práctica a la hora de redactar trabajos y de citar las referencias con propiedad, algo que no siempre se consigue, y a lo que las editoriales conceden la misma importancia que al resto de apartados que componen el artículo o ensayo.

Carmen Chica
Redacción de Microbiología SEM

Maintaining Cultures for Biotechnology and Industry

Jennie C. Hunter-Cevera,
Angela Belt (ed.)

Academic Press, London, 1996. 263 pp.
ISBN 0-12-361946-7

La reposición y mantenimiento de los cultivos, además del interés para la investigación científica básica, resulta una necesidad en la actividad industrial basada en la biotecnología. Por este motivo siempre son deseables nuevos métodos, técnicas y conocimientos que mejoren el proceso, permitiendo eliminar los problemas de contaminación y mantener la viabilidad y estabilidad genética de los organismos, algo que reviste particular importancia por su relación con la obtención y explotación de patentes. Tanto en el ámbito privado como en el público, la pérdida de cepas provoca pérdidas costosas en tiempo y dinero y acarrea fustraciones, algo que cuidadores de colecciones, estudiantes e investigadores conocen sobradamente.

Maintaining Cultures for Biotechnology and Industry es un libro que quiere responder a las necesidades de mantenimiento y conservación de la biodiversidad tanto en las industrias como en las instituciones. Un conjunto de autores aportan la experiencia de muchos años dedicados a los cultivos de laboratorio y presentan técnicas que no habían aparecido en la literatura especializada. Constituyen procedimientos que permiten una elección y adaptación a los recursos disponibles para obtener los resultados deseados. A la vez, no deja de ser ventajoso saber que existen otros métodos entre los que elegir para mantener y conservar con todas sus cualidades las colecciones de cultivos.

El libro se distribuye en doce capítulos. Tras la información sobre aspectos legales, patentes y requisitos de los depósitos de colecciones de cultivos (cap. 1), ofrece una perspectiva sobre los principios, desarrollo técnico y continuos avances en la materia (cap. 2). Los siguientes capítulos se ocupan del mantenimiento y cultivo de diferentes tipos de células y organismos —algas, eubacterias, actinomicetes, hongos, protozoos, células animales, virus de animales y de humanos, plantas, virus de plantas y viroides. Cada capítulo se presenta con la misma estructura; una primera parte donde introduce los aspectos básicos que comprenden clasificación, diversidad, importancia industrial y caracterización y una segunda, más extensa y detallada, sobre los métodos de conservación, agrupados según las diferentes técnicas y objetivos. Puede así utilizarse a modo de guía para localizar la información necesaria. En el último capítulo se proponen vías para evaluar y caracterizar cultivos utilizados en biotecnología y en las industrias relacionadas. Contiene tablas y metodología para la conservación de células, cultivos y virus y se exponen formas de conservación por frío, liofilización, crioprotección, propagación continua y subcultivo (que a veces resulta imprescindible), entre otros muchos procedimientos.

Especialmente útil es la inclusión de los depósitos de colecciones de cultivo de cada clase de organismo en todo el mundo, con las direcciones completas para acceder a ellos. En conjunto, toda la información que se ha introducido en el texto tiene interés práctico. Permite la consulta rápida y la obtención de los datos necesarios para aclarar dudas y resolver problemas de quien, como responsable específico, personal en formación o usuario de colecciones, debe atender a su cuidado.

Antoni Navarrete
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Concepts in Biotechnology

D. Balasubramanian, C. F. A. Bryce, K. Dharmalingam, J. Green, K. Jayaraman (ed.)

Universities Press, India, 1996. 425 pp.
Precio: £ 19,95. ISBN 0-86311-667-1

La publicación de este libro de texto corresponde a una iniciativa conjunta del Committee on Science and Technology in Developing Countries asociado con la International Biosciences Networks (COSTED–IBN) y ha sido financiado en su totalidad por la International Council of Scientific Unions (ICSU), por la UNESCO y por la International Union of Microbiological Societies (IUMS). El objetivo genérico del COSTED–IBN es diseñar mecanismos para fomentar el progreso de la ciencia y tecnología en los países en vías de desarrollo.

En concreto, este libro de texto es un proyecto del COSTED–IBN para el establecimiento de un currículum en biotecnología —tanto de licenciatura como para postgraduados— de países en vías de desarrollo. Existe en el mercado un buen número de libros de texto en esta materia publicados por expertos e instituciones de países desarrollados, en los cuales los métodos de enseñanza, el entorno para la investigación, las infraestructuras científicas son muy diferentes de las que existen en los países en vías de desarrollo. Como hecho adicional, los precios de dichos libros de texto superan las posibilidades de cualquier estudiante de nivel económico medio de estos últimos países.

El libro está dividido en 17 capítulos, que cubren suficientemente los principios y conceptos fundamentales que forman la base de la materia (macromoléculas, célula, estructura y expresión génicas, proteínas, enzimas), así como ilustran sus aplicaciones en áreas seleccionadas, como son la salud, la agricultura, la zootecnia, las tecnologías de bioproceso y el ambiente. Un último capítulo —olvidado en la mayoría de libros de texto de los países desarrollados— trata sobre las implicaciones económicas, sociales y éticas de la biotecnología.

Los capítulos del libro han sido escritos por expertos en la materia de diferentes partes del mundo, y guardan un cierto equilibrio de sus procedencias entre países desarrollados y en vías de desarrollo. Destaca la autoría del capítulo 15 sobre biotecnología marina, a cargo de Rita R. Colwell. Cada capítulo comienza por un índice y acaba con una bibliografía recomendada para el estudiante que quiera ampliar sus conocimientos en ese tema. Este último aspecto es muy apropiado, dadas las generalidades que deben acometerse en una publicación que quiera abarcar toda la biotecnología. Los capítulos tienen, además, unos ejercicios que incitan al estudiante a trabajar el libro más que limitarse a leerlo. Estos ejercicios refuerzan notablemente los conceptos explicados con anterioridad, además de estimular la capacidad de interrelación. Las soluciones a los ejercicios se encuentran al final de cada capítulo.

En definitiva, esta obra es recomendable tanto desde el punto de vista pedagógico como científico. Su estructura, extensión y contenido son muy adecuados para impartir esta materia. El esfuerzo de los promotores de esta publicación en editar un texto dirigido especialmente a estudiantes de países en vías de desarrollo, ha redundado en una gran eficiencia pedagógica. También hay que decir que el libro —por cuestiones obvias de coste— se aparta mucho, en cuanto al formato y calidad de los materiales empleados, de las ediciones de países desarrollados, sobre todo americanas.

Jordi Mas-Castellà
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Un regulador genético

El fago λ y los organismos superiores

Mark Ptashne

Traducción de la 2ª edición en inglés:
Teresa Alonso, Universidad de La Laguna

Consejo Superior de Investigaciones Científicas, Madrid, 1996. 188 pp. ISBN 84-00-07590-0

En el prefacio a la primera edición, de 1986, Mark Ptashne, profesor de bioquímica y biología molecular en Harvard, expresaba su intención de describir "los resultados de más de veinticinco años de investigación sobre cómo el virus llamado λ utiliza sus genes, su DNA, para dirigir su crecimiento". Cuando al cabo de seis años (en 1992) apareció la segunda edición, el autor incorporaba dos nuevos capítulos que tratan los mecanismos que regulan la expresión génica en los organismos superiores. Asevera el autor que, hasta cierto punto, el modelo de λ, sin duda el regulador de la expresión génica del que más sabemos, ofrece similitudes con los mecanismos de regulación de los eucariotas.

El libro ha sido saludado por la crítica como un ejemplo de la mejor ciencia y excelente método para enseñarla. Estamos de acuerdo. La sencillez y claridad de las explicaciones, junto a unos excelentes dibujos, consiguen una obra de una elevada calidad científica y didáctica.

En seis capítulos el autor desarrolla los elementos capitales del control, las interacciones proteína-DNA y el control génico, los circuitos de control, la descripción de los experimentos clave, el panorama general de la regulación génica en eucariotas, experimentos y ejemplos, donde incorpora los resultados de la experimentación reciente con el represor de λ. Añade un apéndice sobre cómo diseñar una proteína que se fije al DNA y otro sobre interacciones débiles y fuertes. Desde la introducción, ideada para proporcionar los elementos necesarios de la biología molecular (aunque es de suponer que quien afronta su lectura domina ya mínimamente la materia) y a través de los sucesivos capítulos, se describen los aspectos básicos de los genes y del funcionamiento de los mismos y el desarrollo de λ desde diferentes perspectivas. Una, distante, muestra el patrón generalizado de la interacción entre el virus y la célula hospedadora. Otra, cercana, permite observar las interacciones moleculares que dan lugar a dicho proceso. Esta segunda edición permite una más amplia comprensión de la regulación génica de λ y a ello ha contribuido el que los modelos presentados procedan de experimentos relacionados entre sí, y no de observaciones aisladas.

El libro recoge treinta años de investigación en biología molecular, en general, y sobre el fago λ, en particular, en cuya labor han intervenido científicos de todo el mundo. Algunos ya renombrados en los momentos iniciales y otros que, con el tiempo, alcanzaron ese renombre. André Lwoff y François Jacob, en el Instituto Pasteur, fueron algunos de ellos. Hace casi 50 años describieron que las células de *Escherichia coli* irradiadas con luz ultravioleta a dosis bajas detenían su crecimiento y, al cabo de 90 minutos, se lisaban liberando una cantidad de bacteriófagos que serían llamados λ. A pesar del tiempo y de la acumulación de conocimientos, todavía no se ha podido generalizar un modelo que explique la forma en que las células seleccionan los genes que han de ser expresados. Más que un lamento del autor, es el reconocimiento de los límites que hay que superar.

Creo que debemos aplaudir la iniciativa del CSIC de publicar esta magnífica obra en español, con una edición muy cuidada.

Antoni Navarrete
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Microbiología General (traducción de la 7ª ed. alemana, 1992)

Hans G. Schlegel

Traducción: Jordi Lalucat, Universitat de les Illes Balears

Ediciones Omega, Barcelona, 1997. 654 pp. Precio: 5750 PTA. ISBN 84-282-1030-6

De la misma manera que la potencia de una cultura no se mide por el número de personas que la integran, sino por las obras que produce, la importancia de un libro no depende de su tamaño ni de su vistosa presentación, sino del contenido que alberga en sus páginas. Son varios los libros que merecen figurar en toda biblioteca microbiológica, obras de autores que han sido investigadores esenciales para el desarrollo de la microbiología del siglo XX. Algunas, como "el Stanier" o "el Brock" (véase la revisión de este último en *Microbiología SEM*, **12**, 671–672, dic. 1996), tratan la materia extensamente y ocupan un lugar destacado de nuestra librería; y no sólo figurada, sino también físicamente, por su volumen.

Otros libros, aparentemente más modestos, son pequeños tesoros que el tiempo deteriora únicamente en su aspecto físico. Mencionaremos, entre otros pocos, *The Microbe's Contribution to Biology*, de A. J. Kluyver y C. B van Niel, publicado en 1956. En sus 182 páginas explica los principales mecanismos que rigen los procesos biológicos. Los ejemplos microbianos que incluye demuestran una unidad de comportamiento en los seres vivos.

Un libro breve en su contenido, pero extenso en sus implicaciones, es "el Schlegel". El autor, también es un destacado microbiólogo, en el campo del metabolismo bacteriano. La obra, *Microbiología General*, es un texto ya clásico de la microbiología (se publicó por primera vez, en alemán, en 1969), y ocupa un nicho específico en el sector del libro de texto. Tal como el propio autor reconocía en el prólogo a la primera edición, esta obra no pretende competir con los grandes manuales de microbiología. Intenta más bien ofrecer —y lo consigue con creces— una visión general de la materia a estudiantes de microbiología general, al tiempo que proporciona los conocimientos básicos de esta asignatura a estudiantes de botánica, zoología, farmacia, medicina, agricultura, bromatología, química, etc. Aunque el número de páginas ha aumentado considerablemente desde la primera edición, su formato reducido y compacto lo hacen muy manejable. (Y para dejar también constancia de sus propiedades físicas, diremos que el libro —el más pequeño de los de texto de microbiología actuales que conocemos— tiene 654 pp., de un tamaño de 19 × 12 cm, y un peso de 714 g; las cuales pueden compararse con las del último Brock [8ª ed., en rústica], con 1038 pp. [con apéndices], 27,5 × 21,5 cm, y 2155 g.)

El libro, centrado en los aspectos esenciales de la microbiología, es un magnífico complemento de obras imprescindibles como el "Stanier" o el "Brock", y facilita la comprensión elemental de la materia. Es recomendable no sólo para alumnos, sino también para profesores. Los avances de las últimas décadas en muchos sectores de la microbiología se reflejan en el cambio que este libro ha experimentado en sus diferentes ediciones. Sin embargo, su autor reconoce no haber tenido en cuenta las tendencias impuestas por la moda, que también se da en la ciencia. La estructura actual del libro es muy similar a la de la primera edición, con igual número de capítulos, que tratan en general los mismos temas, pero incorporando a cada uno de ellos nuevos conocimientos y mejorando su aspecto didáctico, con inclusión de figuras nuevas y reestructuración de algunas ya presentes

en anteriores ediciones. No existe una división en secciones, pero los capítulos siguen una secuencia lógica. Los cinco primeros tratan de circunscribir a los microorganismos en el conjunto de los seres vivos, describiendo sus características generales y localizándolos en el árbol filogenético universal. Ahí es donde encontramos un punto flaco a esta obra, que mantiene la división haeckeliana en tres reinos (Animales, Vegetales y Protistas), sin tener en cuenta, no ya la reciente separación en tres dominios, sino incluso la división en cinco reinos, casi tan clásica como este libro. Tras el sexto capítulo, que trata principalmente de los medios de cultivo y la fisiología del crecimiento, sigue un detallado estudio del metabolismo bacteriano. El conjunto de estos capítulos, que ha sido muy ampliado desde la primera edición, constituye el núcleo de la obra y ofrece un excelente estudio de la variabilidad fisiológica de los microorganismos, con sus múltiples capacidades metabólicas. A continuación se tratan los principales aspectos de la genética bacteriana y los distintos mecanismos raguladores del metabolismo. El último capítulo difiere notablemente del final de las primeras ediciones, que era muy breve y dedicado a evolución; esta edición acaba con unas consideraciones que constituyen una introducción a la ecología microbiana.

En cuanto a la presentación del libro, la edición española ha sido muy cuidada, lo que no ocurre en otras versiones. La traducción inglesa (General Microbiology [7th ed.], Cambridge University Press, 1992), por ejemplo, no se ha puesto al día en este aspecto, y parece haber mantenido los mismos gráficos y figuras de las primeras ediciones, en blanco y negro, y con fórmulas químicas y esquemas de ciclos metabólicos que se diría están hechos a máquina o con las plantillas que se utilizaban cuando no se disponía de programas de ordenador. La edición española, en cambio, destaca por la pulcritud y nitidez de gráficos y figuras, que son de gran calidad y en muchos casos a dos tintas. Incluye una reproducción del clásico árbol filogenético de Haeckel, que no aparece en la versión inglesa. Además, la composición tipográfica es excelente y resulta un texto fácil de leer. En cuanto a la traducción, excelente en general, presenta algunos fallos concretos, que, por otra parte, se observan con frecuencia en muchos textos de biología. Como son el añadido (calco del inglés) de la preposición "para" entre el verbo codificar y su complemento directo, el mantenimiento (igual que en el original) del término "flora" aplicado a bacterias, o la asignación del género masculino a la palabra enzima, que aunque etimológicamente debería ser así (y así lo cree también el autor de esta revisión), no se ajusta a las indicaciones del *Vocabulario Científico y Técnico* de la Real Academia de Ciencias, que ha escogido la forma femenina. Muchas veces hay que ceder en la rigurosidad —aunque no estemos de acuerdo— en beneficio de la homogeneidad.

En resumen, se trata de una obra muy apreciable (en contenido, traducción y presentación), y de la que sólo sentimos que la editorial haya tardado tanto en publicar. Se podría sugerir a las editoriales españolas que sigan el ejemplo de algunas extranjeras, que colocan el nombre del traductor en lugares destacados (en el caso de la traducción de Cambridge University Press, antes mencionada, pone el nombre del traductor en la portada). Es un modo de reconocer algo la importancia de esa ingrata tarea (poco apreciada, generalmente, y mal retribuida, siempre), que resulta esencial para la difusión de obras publicadas en otras lenguas.Confiemos en que las futuras traducciones de esta obra no se hagan esperar tanto.

Ricard Guerrero
Universidad de Barcelona

El llibre digital

Una exploració sobre la informació electrònica i el futur de l'edició

Lluís Codina
Centre d'Investigació de la Comunicació, Generalitat de Catalunya, Barcelona, 1996.
191 pp. ISBN 84-393-3995-X

Este novedoso libro, escrito en catalán, realiza un análisis conceptual sobre la publicación electrónica y plantea elementos de discusión sobre aspectos como las características y propiedades de la información digital, la estructura del libro electrónico y las innovaciones que aporta en relación a la accesibilidad y distribución de la información. Discute el futuro del libro digital y de la cultura de la letra impresa, y el papel de autores y editores en el contexto de la publicación electrónica.

La obra es el resultado de un trabajo de investigación que el autor ha realizado consultando diversas bases de datos mediante búsquedas a través de Internet. La metodología y las fuentes se especifican con detalle en el libro, donde a lo largo de cinco capítulos se tratan los siguiente temas : (1) los microordenadores como máquinas literarias; (2) las características y propiedades de la información digital; (3) la anatomía de un libro electrónico; (4) los aspectos económicos y sociales de la publicación electrónica; y (5) resumen y conclusiones. Una extensa bibliografía comprende, además de obras recientes sobre el desarrollo de los nuevos sistemas de comunicación, otras que profundizan en sus fundamentos, posibilidades y efectos. Concluye el libro con tres anexos que contienen el álgebra de Boole, direcciones de robots, buscadores y "navegadores" de la "web", y de servidores dedicados al periodismo electrónico. Se incluye un breve glosario de los términos clave utilizados. Merece la pena destacar las tablas y figuras por la concisa explicación que proporcionan. Un buen ejemplo es la presentación de las características de los documentos digitales, en contraposición con los analógicos, y los tipos de información que se adaptan mejor a uno u otro tipo, con la tecnología disponible.

Las autopistas de la información y la red Internet son tratados analíticamente. Aun reconociendo las ventajas del sistema, el problema que se nos plantea a muchos usuarios es la dificultad de acceder a la información deseada a causa de la gran cantidad entre la que hay que seleccionar. El autor lo atribuye a que se han creado ideas irracionales sobre la nueva sociedad de la información y la exposición ilimitada a un gran número de mensajes produce mayor desinformación. Pero no duda que a la resolución del problema contribuirán las redes telemáticas, los microordenadores y los libros electrónicos, que permitirán seleccionar con mayor grado de probabilidad la información que realmente necesitamos.

La sustitución del libro en su forma tradicional por el soporte digital, aunque técnicamente posible, no se considera que vaya a suceder en un futuro cercano. No por falta de viabilidad técnica, sino porque median hábitos culturales y tradicionales muy establecidos que no tendría sentido modificar por el momento. Recuerda el autor que parecido temor se produjo cuando, al inicio de la expansión de las técnicas audiovisuales se auguró el fin de los medios que utilizan la letra impresa. Y, sin embargo, el libro clásico no han perdido vitalidad, antes al contrario. Pero, sería ingenuo ignorar los cambios que en las industrias editoriales, grandes y pequeñas, lo mismo que en los propios autores, están introduciendo los nuevos sistemas.

Carmen Chica
Redacción de Microbiología SEM

MARINE MICROORGANISMS FOR INDUSTRY

17–19 September 1997

ADEBIO, MUSEUM ET COLLEGE DE FRANCE, IFREMER, ESMB

Aims of the conference

Biotechnology is spreading widely in as diverse fields as bioactive substances and environment. This meeting between researchers and industry people is an opportunity to assess the potentialities of marine microorganims (viruses, bacteria, microalgae, fungi, protozoa, etc.) and associated processes. It is held in the heart of European countries of the Atlantic Bow, whose marine aptitude promote the development of this subject in both scientific and industrial ways.

Programme

The conference will last three days, and will include lecture sessions, poster presentations, and exhibition of equipment and products. Three major topics are selected:

- **products** (lipids, enzymes, polymers, bioactive substances)
- **processes** (production, separation, analysis)
- **prospects**

Call for papers

Prospective participants are invited to offer a short **oral presentation** (20 minutes) or a **poster**. In either case, an abstract (maxium one side of A4 paper) should be submitted in duplicate to the Secretariat of the Conference before **31 March 1997**. Special reduced registration fees will be allowed to PhD students.

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BP 225
F-29182 Concarneau, France
Fax: +33–2–98 97 81 24
E-mail: ylegal@sb-roscoff.fr

Instructions to authors

Microbiología SEM (the official journal of the Spanish Society for Microbiology, SEM) publishes original research articles, research notes and reviews covering all aspects of microbiology. All submissions should be written in English (preferably) or Spanish. The decision to accept manuscripts is made by the Editorial Board. Submission of an article to this journal is understood to imply that it has not previously been published and that it is not being considered for publication elsewhere. Consent will be given for reproduction of papers published in this journal if the source is credited.

ORGANIZATION AND FORMAT OF THE MANUSCRIPTS. Type every portion of the manuscript double-space with wide margin at the left on UNE A-4 format sheets. Only one side of the sheet should be used and the pages should be numbered sequentially. Articles must be restricted to a maximum of 16 printed pages, including figures and tables (this corresponds to approximately 25 typewritten pages).

The front page should include title, name(s) of the author(s), institution affiliation(s) and complete address(es). Three to five "key words" should also be included. Articles should be divided into: Abstracts in English and in Spanish (not exceeding 250 words each), Introduction, Materials and methods, Results, Discussion, Acknowledgments, and References. Results and Discussion can be combined.

Abbreviations and symbols should follow the recommendations of the IUPAC-IUB Commission. The *Système International d'Unités* (SI) is to be used throughout.

Cite each listed reference by number in the text. References should be numbered and arranged in alphabetical order as indicated in the following examples:

Miller, J. H. (1972). Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Seeberg, E., Nissez-Meyer, J., Strike, P. (1976). *den*V gene of bacteriophage T4 determines a DNA glycosilate specific for pyrimidine dimers in DNA. J. Virol. **35**, 790–797.

Tomasz, A. (1984). Building and breaking in the cell wall of bacteria. The role for autolysins. *In* Nombela, C. (ed.), Microbial Cell Wall Synthesis and Autolysis, pp. 3–12. Elsevier Science Pub., Amsterdam.

References to thesis, manuscripts not yet accepted for publication or meetings should be indicated in the text as follows: (García, P. et al. 1985, in preparation), (Smith, T. 1985. Ph. D. thesis, University of Massachusetts, Amherst) or (Suárez, A., González, F. 1975. V Congr. Nac. Microbiol., p. 1845).

Only those photographs which are strictly necessary for the understanding of the article should be submitted. Photoprints must be of sufficient quality to ensure good reproduction. They should be numbered on the back and identified with the first author's name written in pencil. Legends for line-drawings and photoprints must be typed double-space on a separate sheet. The size of the photographs should not exceed the printing area (13 × 20 cm). All elements in the drawing should be prepared to withstand reductions. Drawings and line figures should be drawn in black ink on tracing paper and should be prepared as indicated for the photographs. Colored illustrations are not accepted.

Tables should be compiled on separate sheets with a descriptive title and numbered independently of the figures using Arabic numerals. Please indicate with a soft pencil the approximate location of tables and figures in the left margin of the pages of the manuscript.

NOTES. Notes should be restricted to 6 typewritten pages and are intended to present experimental observations and descriptions of techniques or methodological changes of interest. They should be written according to the instructions given for articles, but without the heading divisions, and their abstracts should not exceed 50 words. Figures and tables should be restricted to a maximum of 2 figures and 1 table or vice versa.

MINIREVIEWS. Minireview articles should deal with microbiological subjects of broad interest. They will be written in English. Specialists will be called upon to write them. However, if some authors are interested in publishing minireviews, these can be submitted for publication. They should be between 12 and 20 double-spaced typewritten pages, including the space needed for figures and tables.

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Normas para los autores

Microbiología SEM (la revista científica de la Sociedad Española de Microbiología, SEM) acepta artículos y notas de investigación originales dentro del campo de la microbiología y, ocasionalmente, artículos de revisión. Textos en inglés (preferentemente) o español. La aceptación corresponde al Consejo Editorial. Sólo se admitirán trabajos inéditos que no estén pendientes de publicación en cualquier otra revista. Los originales publicados en *Microbiología SEM* podrán ser reproducidos siempre que se indique su origen.

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Las abreviaturas, símbolos y siglas deberán seguir las recomendaciones de la Comisión IUPAC-IUB sobre nomenclatura bioquímica. Deberá emplearse siempre el Sistema Internacional de Unidades (SI).

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Las referencias a tesis doctorales, originales no aceptados todavía o comunicaciones presentadas a congresos, deben incluirse en el texto del artículo de acuerdo con los siguientes ejemplos: (García, P. et al. 1985, en preparación), (Smith, T. 1985. Tesis doctoral University of Massachusetts, Amherst) o (Suárez, A., González, F. 1975. Res. V Congr. Nac. Microbiol., p. 1845).

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Continued from p. 4

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