

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION  
Slezská 7, 120 56 Praha 2, Czech Republic  
Fax: (00422) 25 70 90

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## MICROBES, ENVIRONMENT AND BIOTECHNOLOGY IN THE YEAR OF LOUIS PASTEUR – A REVIEW

Z. Filip

*Umweltbundesamt, Institut für Wasser-, Boden- und Lufthygiene, Langen, Germany*

The year 1995 has been designated "The Year of Louis Pasteur" to mark the hundredth anniversary of his death. This anniversary was commemorated by six International Scientific Symposia that should summarize the current progress in the major fields of Pasteur's scientific activities. Under a broad scale topic "Microbes, Environment, Biotechnology" one of the Louis Pasteur Symposia was held in Papeete, Tahiti, in May 1995. This article consists of a comprehensive report of the selected papers that were presented at the symposium. They included the following themes: (a) Old and New Microbes, (b) Carbon Recycling, (c) Nitrogen Fixation and Symbiosis, and (d) Protection of Environment. Because no symposium proceedings will be published, the review should reveal an actual information to everyone interested in microbial ecology and environmental biotechnology.

old and new microbes; carbon recycling; nitrogen fixation; protection of environment

### INTRODUCTION

Organized by UNESCO and the Institute Pasteur (Paris, France) 1995 celebrates the 100th Anniversary of the death of Louis Pasteur. Pasteur's astonishing scientific performances started in the field of crystallography. The discovery of molecular asymmetry as one of the fundamental characteristics of living matter led him, a few years later, to the germ theory of fermentation and of diseases, and to the view that different chemical alterations, as well as pathological processes, are caused by specific types of microbes. These scientific theories have a far-reaching impact on modern microbiology and biotechnology. Louis Pasteur became also aware of the fact that the metabolic activities of microbes are profoundly influenced by environmental factors. He contributed to scientific philosophy by perceiving that all forms of life are integrated components of a global ecological system.

The Year of Louis Pasteur included six international scientific symposia held in each of five continents of the world. One of them was the international symposium on "Microbes, Environment, Biotechnology" which was held on May 8-12, 1995 in Papeete, Tahiti, French Polynesia. In this review article selected presentations made at this important scientific event and belonging to its major topics will be summarized.

#### OLD AND NEW MICROBES

Despite the fact that prokaryotes evolved about 3.8 billion years ago, the two prokaryotic domains, *Archaea* and *Bacteria*, presently contain only about 3 250 validly described species (excluding *Cyanobacteria*), belonging to about 220 genera. Over the last 15 years, the yearly increase in number of new species has ranged between 70 and 150, and that of new genera between 15 and 30. According to Stackebrandt (Germany), the fraction of prokaryotic species is presently only about 0.2% of the total number of described biological species. A question arises, whether as yet uncultured prokaryotes (which may account for over one million) represent novel taxa or whether they belong to known, culturable species. It should be stressed, however, that unknown biodiversity of prokaryotes is not restricted to the uncultured organisms. As a result of the novel isolation techniques an increase in the number of novel isolates has been also demonstrated. As an example Stackebrandt pointed on the recently isolated members of the order *Planctomycetales*. Using 16S rDNA sequence analysis, nine and eight isolates could be assigned to the genera *Planctomyces* and *Pirellula*, respectively. Three of the novel isolates were of soil origin. One prerequisite for the assessment of microbial diversity in natural environments is the availability of an extensive molecular database of cultured organisms which can serve as a reference for the comparison of sequences from both isolates and uncultured strains. The comparison of sequences from environmental rDNA to each other and to cultured strains, will also allow the recognition of putative target sites for oligonucleotides, suitable for the specific detection of the respective strains directly in their natural habitats.

Existing examples of novel isolates represent hyperthermophilic *Bacteria* and *Archaea* described by Stetter (Germany). In contrast to moderate thermophiles, hyperthermophiles are unable to grow at ambient temperature. Some of them are adapted to superheated water, and temperatures of 80 °C are still too low to support growth of them. About 47 species of hyperthermophilic *Bacteria* and *Archaea* are known, which are grouped into 23 genera and 11 orders (Tab. I). Within the *Archaea*, members of the genera *Pyrobaculum*, *Pyrococcus*, *Pyrodictium* and *Methanopyrus*, e.g., exhibit the growth

I. Genera and orders of hyperthermophiles (from Stetter, 1995)

Order	Genus	T max	DNA (GC mol %)	
BACTERIA	<i>Thermotogales</i>	<i>Thermotoga</i>	90	46
		<i>Thermosipho</i>	77	30
		<i>Fervidobacterium</i>	80	41
	<i>Aquificales</i>	<i>Aquifex</i>	95	40
ARCHAEA	<i>Sulfolobales</i>	<i>Sulfolobus</i>	87	37
		<i>Methallosphaera</i>	80	45
		<i>Acidianus</i> (= <i>Desulfurolobus</i> )	95	31
		<i>Stygiolobus</i>	89	38
	<i>Thermoproteales</i>	<i>Thermoproteus</i>	97	56
		<i>Pyrobaculum</i>	104	46
		<i>Thermofilum</i>	95	57
	<i>Desulfurococcales</i>	<i>Desulfurococcus</i>	97	51
		<i>Staphylothermus</i>	98	35
	<i>Pyrodictiales</i>	<i>Pyrodictium</i>	110	62
		<i>Hyperthermus</i>	108	57
		<i>Thermodiscus</i>	98	49
	<i>Thermococcales</i>	<i>Thermococcus</i>	98	57
		<i>Pyrococcus</i>	103	38
	<i>Archaeoglobales</i>	<i>Archaeoglobus</i>	92	46
	<i>Methanobacteriales</i>	<i>Methanothermus</i>	97	33
<i>Methanococcales</i>	<i>Methanococcus</i>	91	31	
<i>Methanopyrales</i>	<i>Methanopyrus</i>	110	60	

temperatures between 103 °C and 110 °C. Hyperthermophiles had been isolated mainly from water-containing active volcanic areas like terrestrial sulfataric fields and hot springs, and submarine shallow and abyssal hot vent systems and seamounts. Man-made biotops are geothermal power plants and smoldering coal refuse piles. Recently communities of hyperthermophiles have been detected in deep geothermally heated oil reservoirs. In an ecological sense, hyperthermophiles are either primary producers or consumers of

II. Energy yielding reactions in chemolithoautotrophic hyperthermophiles (from Stetter, 1995)

Energy-yielding reaction	Genera (examples)
$4 \text{ H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{ H}_2\text{O}$	<i>Methanopyrus, Methanothermus, Methanococcus</i>
$\text{H}_2 + \text{S}^0 \rightarrow \text{H}_2\text{S}$	<i>Pyrodictium, Thermoproteus, Pyrobaculum, Acidianus, Stygiolobus</i>
$4 \text{ H}_2 + \text{H}_2\text{SO}_4 \rightarrow \text{H}_2\text{S} + 4 \text{ H}_2\text{O}$	<i>Archaeoglobus</i>
$\text{H}_2 + \text{HNO}_3 \rightarrow \text{HNO}_2 + \text{H}_2\text{O}$	<i>Pyrobaculum, Aquifex</i>
$\text{H}_2 + 1/2 \text{ O}_2 \rightarrow \text{H}_2\text{O}$	<i>Pyrobaculum, Sulfolobus, Acidianus, Metallosphaera, Aquifex</i>
$2 \text{ S}^0 + 3 \text{ O}_2 + 2 \text{ H}_2\text{O} \rightarrow 2 \text{ H}_2\text{SO}_4$	<i>Sulfolobus, Acidianus, Metallosphaera, Aquifex</i>

organic matter within their biotops. Primary producers gain energy by anaerobic and aerobic types of respiration, in which molecular hydrogen is used predominately as an electron donor (Tab. II). Consumers gain energy either by anaerobic or aerobic respiration or by fermentation. Recent *in situ* 16S rRNA analysis indicate the existence of a great amount of so far uncultured hyperthermophiles with unknown properties.

Due to the extensive studies carried out especially by Japanese scientists, a broad knowledge already exist on the alkaliphilic microbes. They grow optimally or very well at pH values above 9 but cannot grow or only grow slowly at neutral pH. According to Horikoshi (Japan), isolation of alkaliphilic microbes must be carried out in media containing between 0.5–2% of sodium carbonate, sodium bicarbonate, potassium carbonate or sodium borate. The number of alkaliphilic bacteria found in soil was about 1/10 to 1/100 that of neutrophilic bacteria. They have been also isolated from sea sediment collected at 6 500 m depth. Studies of alkaliphiles have led to the discovery of many types of enzymes which exhibit unique properties in many respects. Some of them are listed in Tab. III. A number of enzymes have been already produced in industrial scale plants. Alkaline proteases from *Bacillus* sp., e.g., have found an application in the detergent industry. These enzymes can be also used to decompose the gelatinous coating of films, from which silver was recovered. Alkaline cellulases produced by an other *Bacillus* sp. strain, and which are commercially available, are used in the laundry detergents to improve the washing effect. Xylanases with an optimum pH 9 and temperature 70 °C are proposed for use in biobleaching processes.

In tropical and semi-tropical saline lakes, halophilic bacteria represent the largest development of *Archaea* that occurs in nature. The genera *Natronobacterium*, *Natronococcus*, *Haloferax*, *Haloarcula*, *Halococcus* and *Halobacterium* probably represent relict organisms from the distant past. In samples

III. Different enzymes produced by alkaliphiles (from Horikoshi, 1995)

Enzyme	Optimum pH	Stability pH	Mol. weight x 10 <sup>4</sup>
Alkaline alginase	9	8–10	4
Alkaline amylase	10–11	6–11	6
Alkaline cellulase	6–11	5–11	4–8
Alkaline DNase	9	6–10	4
Alkaline pectinase	10	5–10	6–7
Alkaline protease	10.5–11	4–12	2–3
Alkaline RNase	9	6–10	1.2
Glukose dehydrogenase	9.8	6–10	5.1
Maltose dehydrogenase	10	6–10	3.9
Uricase	9	10	10
Xylanase	5.5–9	5–9	3.6
β-galactosidase	6.5	5.5–9	18.5
β-1,3-glucanase	8.5	5–9	3.6

of brine from different geological locations and of different ages (26–230 mill. years) halobacteria have been isolated together with a range of non-archaeal halophilic prokaryotes (Grant et al., UK). Some halobacteria become incorporated in a viable state within pockets of brine inclusions that are a permanent feature of the salt crystal structure. These bacteria, e.g. from solar salts, are responsible for the ready spoilage of proteinaceous materials such as salt treated hides and fish. Gene sequence studies using polymerase chain amplification and 16S rRNA are necessary to determine the evolutionary distances between halobacteria and different halotolerant prokaryotes.

CARBON RECYCLING

The degradation of cellulose contributes a major part to the carbon cycle in the biosphere. Cellulose-utilizing organisms belong almost exclusively to eubacteria and fungi. Cellulolytic bacteria occur in a wide variety of taxonomic groups, encompassing numerous Gram-positive and Gram-negative organisms. Cellulolytic fungi include various aerobic species belonging to *Ascomycetes*, *Deuteromycetes* and *Basidiomycetes*, and several anaerobic species, particularly *Chytridiomycetes*, which are present in the gastrointestinal tract of ruminants. All organisms that are able to utilize nature cellulose produce sets of extracellular cellulases with complementary specificities.

Classically, cellulases have been ordered into three major categories: (a) endoglucanases, which attack cellulose chains at random but preferentially within the amorphous regions of the fibrils; (b) cellobiohydrolases, which attack cellulose chains stepwise from the non-reducing end; (c)  $\beta$ -glucosidases, which hydrolyse cellobiose and low molecular weight cellodextrins into glucose.

Beguin (France) extensively studied the cellulase system of *Clostridium thermocellum*. This is a Gram-positive, sporogenic and strictly anaerobic bacterium with an optimum growth temperature of about 60 °C. Cellulose is rapidly degraded by *C. thermocellum* and fermented into ethanol, acetate, lactate, formate, hydrogen and carbon dioxide. The cellulolytic enzymes of this bacterium are assembled into specific complexes – cellulosomes. The cellulosome contains at least 14 different components; the majority of them possess endoglucanase activity. Different endoglucanase genes, two  $\beta$ -glucosidase genes, two xylanase genes and one  $\beta$ -1,3- $\beta$ -1,4-glucanase gene were cloned from *C. thermocellum*. The cellulosome also consists of a large, non-catalytic component, now termed CipA (for cellulosome integrating protein) that might act as a scaffolding element of the complex and as a cellulose-binding factor mediating attachment of the catalytic subunits to the substrate. Besides CipA, the OlpA protein located in the cell envelope of *C. thermocellum* seems to be involved in anchoring individual cellulolytic components to the cell surface. These components may promote cell-bound cellulolysis when the bacteria make a close contact with the substrate.

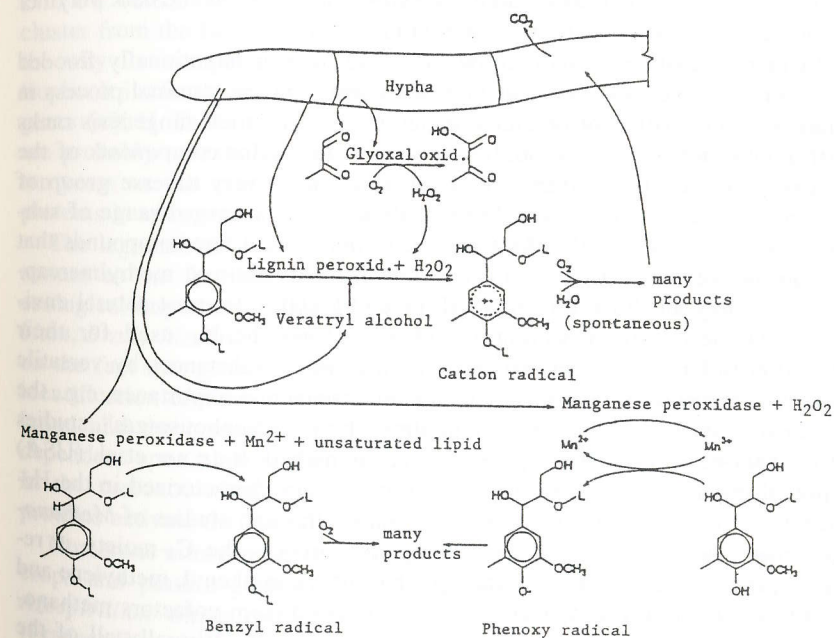
Mastromei et al. (Italy) isolated *Streptomyces rochei*, a cellulolytic actinomycete from the gut of termites. From this microbe two cellulase genes have been cloned: a  $\beta$ -glucosidase, and an endoglucanase gene. In these enzymes the catalytic domain and the cellulose binding domain have been identified. Southern hybridization analysis showed sequences similar to the *S. rochei* genes also in the majority of 156 *Streptomyces* strains isolated from various sources.

Lignin is second only to cellulose in abundance natural polymer in the terrestrial biosphere, and it physically protects the cellulose and hemicelluloses in woody tissues from attack by the enzymes of non-lignolytic microbes. Lignin degradation occurs not only in wood, but also in the litter of forest floors, in soils, and in other ecosystems. According to Kirk and Hamme (USA) white rot fungi are the most efficient decomposers of lignin. In fact, they decompose all three structural components of wood-cellulose, hemicelluloses and lignin, and under optimized conditions, the rates at which they mineralize lignin rival those of polysaccharide degradation.

Mubyana (New Guinea) isolated different fungi such as *Fusarium* sp., *Gliocladium* sp., *Helminthosporium* sp., *Chaetomium* sp., *Polyporus* sp.,

*Penicillium* sp., *Aspergillus niger*, and *Trichoderma viride* from decaying timber. The fungal colonization of timber, however, became significantly reduced after spraying the timber with inhibitory bacteria. Among the twelve bacteria tested, *Bacillus subtilis*, *Serratia marcescens*, *Micrococcus roseus* and *Micrococcus luteus* showed the highest fungal inhibition.

Recently, most research on the biochemistry of lignin degradation has been done with the experimentally advantageous fungus *Phanerochaete chrysosporium* (Burds). An extracellular lignolytic system of *P. chrysosporium* as currently understood is shown in Fig. 1. It consists of lignin peroxidases (LiP) manganese peroxidases (MnP), and glyoxal peroxidases (GLOX), the activities of which supplement each other. The LiPs are encoded by at least ten structurally related genes; the MnPs are known to be encoded by a minimum of four genes. GLOX is encoded by a single gene with two allelic variants unlinked to any LiP or MnP genes. LiPs and MnPs are involved also in the initial oxidation of certain polycyclic aromatic hydrocarbons and chlorinated phenols by *P. chrysosporium*. These activities make them attractive candi-



1. Schematic illustrating the extracellular lignolytic system of *P. chrysosporium* (from Kirk and Hamme, 1995)

dates for use in the bioremediation of chemically polluted sites. Contaminants can be either mineralized or "humified" with lignocellulose-derived polyphenols or with existing humic acids as a result of the activity of fungal enzymes. Humification results in irreversible covalent binding of the pollutants to humic materials, rendering them biologically unavailable and thus intoxic.

Humic substances are a major component of the refractory organic matter not only in soils but also in recent marine sediments. Their origin and characteristics, however, may differ from those of soils. Filip and Alberts (Germany/USA) studied two fungal species – *Phaeosphaeria spartnicola* and *Phaeosphaeria halima* – in order to determine if they contribute to the formation of humic substances. These fungi commonly colonize leaves and stalks of *Spartina alterniflora* (Loisel.), a smooth cordgrass dominant in the salt marshes of the Atlantic coasts of the USA. In incubations of up to one year in duration, the fungi did not form dark pigments when grown in an artificial, full nutrient medium. However, if the nutrient solution was enriched with a water extract of *S. alterniflora*, appreciable amounts of dark brown substances were produced. The results of elemental (C, H, N, O), spectral (UV, Vis, FTIR) and electrophoretic (PAGE) analyses show the dark polymer substances to be similar to salt marsh humic acids.

In marine sediments, natural swamps, and also in intentionally flooded soils, such as rice paddies, methanogenesis represent the terminal process in anaerobic degradation of organic compounds. In fact, methanogenesis ranks with photosynthesis and respiration to one of the major components of the global carbon cycle. Methanogens themselves are a very diverse group of microbes which seems to be able to catabolize only a limited range of substrates to CH<sub>4</sub>, specifically CO, CO<sub>2</sub> and formate and a few compounds that contain preformed methyl groups (methanol, methylamines, methylmercaptan, secondary alcohols, dimethyl sulfide and acetate). In most natural environments these carbon-containing substrates and the H<sub>2</sub> used for their reduction to CH<sub>4</sub> are generated from more complex substances by versatile microbes. Because of their economic and environmental importance, e.g., the increasing concern for methane as an atmospheric "greenhouse-gas", studies of methanogens and methanogenesis have intensified. Reeve et al. (USA) reported on seven biochemical steps that have been characterized in the H<sub>2</sub>-dependent reduction of CO<sub>2</sub> to CH<sub>4</sub>, primarily through studies of *Methanobacterium thermoautotrophicum*. During this process the C<sub>1</sub>-moiety is reduced progressively, from CO<sub>2</sub> through the formyl, methenyl, methylene and methyl reduction levels to CH<sub>4</sub>, and is transferred from cofactors methanofuran to tetrahydromethanopterin and to coenzyme M. Virtually all of the enzymes that participate directly in the methanogenesis pathway leading from CO<sub>2</sub> to CH<sub>4</sub> have now been purified and characterized and most of their

encoding genes have been cloned and sequenced. That the synthesis of different methanogenesis enzymes can be controlled in the laboratory by growth conditions can now also be included in the future design, and in current manipulations of anaerobic digestors that are used for waste treatment and biogas production.

## NITROGEN FIXATION AND SYMBIOSIS

The study of nitrogen cycle consists of several examples of how Pasteur's work was extended in the 19th century. The denitrifying bacteria that transform nitrate into molecular nitrogen were first isolated by Gayon and Dupetit in 1886. In 1890 Winogradski described first the bacteria *Nitrosomonas* that bring about nitrification, and in 1893 the same scientist isolated in France the free-living nitrogen-fixing bacterium, the anaerobe *Clostridium pasteurianum*. In the last years molecular genetic analysis has led to enormous strides in our knowledge of the environmental regulation of diazotrophy and the elucidation of signal transduction pathways controlling nitrogen fixation. According to Dixon (UK), the complete sequence of the twenty genes in the *nif* gene cluster from the facultative anaerobe *Klebsiella pneumoniae*, e.g., has paved the way for comparative sequence analysis of nitrogen fixation in diazotrophs. It is clearly apparent that each organism maintains a common core of genes (*nif*, H, D, K, T, Y, E, N, X, U, S, V, Z, W, M, B, Q) whose products are probably essential for efficient biosynthesis of nitrogenase. In the next ten years we can expect a far more detailed picture of the role of individual proteins in nitrogenase biosynthesis and metallocluster assembly.

The legume-rhizobia symbiosis is estimated to fix as much nitrogen per annum as the fertilizer industry produces and is of great agronomic and ecological importance. Rhizobia are now classified into three genera, *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium*. Denarie et al. (France) pointed on the fact that genetic manipulation of the rhizobial partner has led to the identification of a set of genes (the *nod* genes) required for infection, nodulation and the control of host specificity. The expression of these genes is dependent upon plant signals, usually flavonoids, excreted in root exudates. In the presence of appropriate plant inducers, regulatory rhizobial NodD proteins activate the transcription of the structural *nod* genes.

Numerous nitrogen-fixing bacterial species have been isolated from cereal crops and pasture grasses. Special attention has been given to the genes *Azospirillum* which colonizes the root system. According to Elmerich (France) five species have been described on the basis of phenotypic properties and DNA characteristics: *A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraefens* and *A. irakense*. These bacteria are Gram-negative chemoorga-

notrophs of vibroid shape. Their motility is ensured by a polar flagellum. Strains have been found in association with monocotyledons, including maize, rice, sugarcane, sorghum, forage grasses, and with dicotyledons. Bacteria induce an enhanced proliferation of the lateral roots and of root hairs. They are also known to produce siderophores and bacteriocins which may serve as biocontrol agents in the competition with other members of the soil microflora. The nitrogen fixation genes in *Azospirillum* seem to be expressed during association with the host plant. Experiments using the  $^{15}\text{N}$  isotope indicated that the  $\text{N}_2$  fixation by *Azospirillum* can account for several per cent of the total nitrogen in the plant.

Further research should also address the actinomycete genus *Frankia*. The family *Frankiaceae*, as now defined, contains the genera *Frankia*, *Geodermatophilus* and *Blastococcus*. According to Silvester (New Zealand) *Frankia* is a uniquely diverse organism, having variable structure both in symbiosis and in the free-living state. Strains are characterized by extensive hyphae and terminal or intercalary multilocular sporangia. A most important feature of the genes is the production of vesicles in culture, i.e., lipid encapsulate spheres 2–6  $\mu\text{m}$  in diameter, born on short stalks cut off by a cross wall. They are the sites of nitrogenase activity. The nitrogenase is a standard Mo-Fe system which is strongly  $\text{O}_2$  labile, requires Mg-ATP and a source of reductant and produces  $\text{NH}_4 + \text{H}_2$ . *Frankia* forms effective symbioses with a wide range of angiosperm hosts, however, there is a reason to believe that some *Frankia* strains are ubiquitous and persist in the absence of host plants.

Haselkorn et al. (USA) underlined the importance of the cyanobacterium *Anabaena* which occurs from polar to tropical regions, although abundance is probably greater in the latter. *Anabaena* grows in filaments containing up to several hundred vegetative cells that carry out photosynthesis, evolving oxygen. Under condition of nitrogen starvation, heterocysts specialized for  $\text{N}_2$  fixation differentiate at regular intervals along each filament. The conversion of an oxygen-evolving vegetative cell into a nitrogen-fixing heterocyst requires the differential expression of more than a thousand genes. Among them the *hetR* and *patA* belongs to the early acting genes that increase in abundance within an hour of transfer to nitrogen-free medium.

## PROTECTION OF ENVIRONMENT

During the past few decades large quantities of different chemicals have been released into the environment. Still, microorganisms have been exposed to these environmental pollutants for only a short period of evolutionary time. Thus, current pathways for the metabolism of xenobiotics could hardly reach an optimal state of development. Hence, the microbial activities that can serve

as the basis of biotechnological protection of environment should be improved. Pieper et al. (Germany) summarized some examples of how novel degradative pathways have been constructed in *Pseudomonas putida* by a combination of some unspecific degradative sequences with the *ortho*-cleavage pathway for chlorocatechol. However, when added to contaminated sites, these bacteria usually exhibit poor survival and their contribution to bioremediation is less than predicted from laboratory experiments. Furthermore, pollutants such as chloroaromatics can be only cooxidized, and in the process generated compounds can be more toxic than those initially present. A judicious combination of segments of different metabolic pathways in suitable bacteria should be developed in order to construct effective microbial tools for bioremediation. The utilization of broad-host range plasmids should gain more attention according to Mergeay et al. (Belgium). These plasmids are able to self-transfer and/or self-replicate in a wide range of taxonomically distant hosts. Genetic transfer of metal resistance, e.g., is intended to decrease the burden exerted by toxic heavy metals on the biodegradation of organic compounds.

Richard et al. (USA) were able to demonstrate the usefulness of bioremediation used as a supplemental cleanup tool in the Exxon Valdez oil spill, in Prince William Sound, Alaska. The oil degradation by indigenous microflora could be significantly accelerated especially by the application of a slow release formulation of organic nitrogen and phosphorus fertilizers.

Several bacteria, such as *Acinetobacter* sp., *Arthrobacter* sp., *Azotobacter* sp., *Bacillus* sp., *Citrobacter* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Thiobacillus* sp., but also yeasts, actinomycetes and filamentous fungi, have been screened and found capable of degrading a large scale of chemical pollutants according to Song and Yang (China). The successful collection of these microbes has constituted an infrastructure for the development of microbiological processes for waste treatment in China.

Chet et al. (Israel) stressed the importance of antagonistic microbes in biological control. Two major approaches include: (a) the introduction of specific microbial antagonists into the soil or plant material; (b) the enhancement of the antagonistic activity of indigenous microbes, e.g., by organic soil amendments. The mechanisms of antagonistic interactions involve parasitism or lysis, antibiosis and competitions. In many studies *Pseudomonas* sp. have been used for the biological control of soil-borne diseases. In most cases, the antagonistic activity of these bacteria was related to siderophore ( $\text{Fe}^{2+}$  chelators) and/or antibiotics production. Recently, a  $\beta$ -1,3-glucanase-producing *Pseudomonas cepacia* has been found to decrease the incidence of plant diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium ultimum* by up

to 85%. The ability to produce hydrolytic enzymes that degrade fungal cell walls is considered to be an important characteristic of biocontrol agents. Due to the production of extracellular lytic enzymes (e.g., chitinase,  $\beta$ -1,3-glucanase, lipase, proteinase), several species from the fungal genus *Trichoderma* represent strong biocontrol agents of some soil-borne plant pathogenic fungi.

Also the exploration of *Bacillus thuringiensis* as a biocontrol agent will certainly continue. The lack of its widespread adoption is due to several inherent biological characteristics which according to Klier (France) include: (i) host range specificity; (ii) inability to target pests that feed internally or on roots; (iii) degradation on foliage by sunlight or other environmental factors; (iv) lack of residual activity in water due to rapid settling of spores and crystals and adsorption to organic particles; (v) relative quick degradation by soil microbes. The application of genetic technology may help to overcome these limitations and to make the use of *B. thuringiensis* more successful. Other microbial hosts for the toxic crystal proteins could also present interesting alternatives and, eventually, the plant itself may become the vehicle for administering biopesticides.

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(All references represent the scientific contributions to "The Year of Louis Pasteur International Symposium – Microbes, Environment, Biotechnology", held on May 8–12, 1995 in Papeete, Tahiti, French Polynesia, and included in "Abstracts".)

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FILIP, Z. (Umweltbundesamt, Institut für Wasser-, Boden- und Lufthygiene, Langen, Německo):

## Mikroby, prostředí a biotechnologie v roce Louise Pasteura — přehledný referát.

Scientia Agric. Bohem., 27, 1996 (1): 67–80.

Rok 1995 byl v mezinárodním kalendáři vědeckých jubileí označen jako „Rok Louise Pasteura“ k uctění památky 100. výročí úmrtí tohoto významného badatele v oblasti přírodních věd. V rámci tohoto jubilea zorganizovaly UNESCO a pařížský Institut Pasteur šest mezinárodních vědeckých sympozií v pěti kontinentech světa. Jejich cílem byla souhrnná diskuse o aktuálních poznatcích v těch oblastech mikrobiologie, kterým Louis Pasteur a jeho vědecká škola položili základ. Jedno ze sympozií, které se zabývalo problematikou mikroorganismů, prostředí a biotechnologie, se konalo od 8. do 12. května 1995 v Papeete na Tahiti (Francouzská Polynésie). Protože materiály ze sympozia nebudou zveřejněny, i když mají značný informativní význam pro odbornou veřejnost, je v tomto článku podána souhrnná informace o obsahu hlavních příspěvků. Zahrnuta jsou tato témata: a) známé a nové mikroorganismy, b) koloběh uhlíku, c) fixace dusíku a symbióza, d) ochrana prostředí.

V prvním tématu se pojednává o některých mikroorganismech taxonomických řádů *Bacteria* a *Archaea*, z nichž řada vyniká neobvyklými vlastnostmi. Patří k nim např. hypertermofilové rostoucí při teplotách nad 100 °C a dále alkalofilní a halofilní mikroorganismy. V kapitole o koloběhu uhlíku se pojednává o rozkladu celulózy a ligninu, o tvorbě humusových látek a o metanogenezi. Další část článku je věnována otázkám fixace atmosférického dusíku za účasti symbiotických i nesymbiotických

mikroorganismů. Úsek věnovaný problematice ochrany prostředí přináší poznatky o mikrobiálním rozkladu antropogenních škodlivin a také o použití mikroorganismů v ochraně rostlin. Ve velkém počtu diskutovaných prací byl patrný vzrůstající podíl molekulárně-biologických metodických přístupů k řešení dané problematiky.

známé a nové mikroorganismy; koloběh uhlíku; fixace dusíku; ochrana prostředí

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*Contact Address:*

Prof. Ing. Dr. Dr.h.c. Zdenek Filip, Umweltbundesamt, Institut für Wasser-, Boden- und Lufthygiene, Paul-Ehrlich-Str. 29, D-63225 Langen, BR Deutschland, tel.: +49/61 03/70 41 60, fax: +49/61 03/70 41 47

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