Biodegradation of Organic Pollutants by Halophilic Bacteria and Archaea

Sylvie Le Borgne[a]   Dayanira Paniagua[b]   Rafael Vazquez-Duhalt[b]

[a]UAM-Cuajimalpa, México, D.F., and [b]Instituto de Biotecnología, Universidad Nacional Autónoma de México UNAM, Cuernavaca, Morelos, Mexico

Abstract

Hypersaline environments are important for both surface extension and ecological significance. As all other ecosystems, they are impacted by pollution. However, little information is available on the biodegradation of organic pollutants by halophilic microorganisms in such environments. In addition, it is estimated that 5% of industrial effluents are saline and hypersaline. Conventional nonextremophilic microorganisms are unable to efficiently perform the removal of organic pollutants at high salt concentrations. Halophilic microorganisms are metabolically different and are adapted to extreme salinity; these microorganisms are good candidates for the bioremediation of hypersaline environments and treatment of saline effluents. This literature survey indicates that both the moderately halophilic bacteria and the extremely halophilic archaea have a broader catabolic versatility and capability than previously thought. A diversity of contaminating compounds is susceptible to be degraded by halotolerant and halophile bacteria. Nevertheless, significant research efforts are still necessary in order to estimate the true potential of these microorganisms to be applied in environmental processes and in the remediation of contaminated hypersaline ecosystems. This effort should be also fo-

cused on basic research to understand the overall degradation mechanism, to identify the enzymes involved in the degradation process and the metabolism regulation.

Copyright © 2008 S. Karger AG, Basel

Introduction

Environmental pollution due to anthropogenic activity has spread to all types of ecosystems. Marine and fresh water, soils and air have been impacted by the dispersion of contaminants. Contamination and biodegradation in extreme environments has received little attention although many contaminated ecosystems present high or low temperatures, extreme acidic or alkaline pH, high pressures or high salinity [Margesin and Schinner, 2001a]. Extremophilic microorganisms (extremophiles) are adapted to thrive in such hostile environments. Extremophiles include psychrophiles: optimal growth temperature <20°C; thermophiles and hyperthermophiles: optimal growth temperature >50 and 75°C, respectively; barophiles: optimal growth at greater than 1 atm; acidophiles and alkalophiles: optimal growth at pH <3 and >10, respectively, and high salt concentrations; halophiles: optimal growth in 2.0–5.2 M sodium chloride [Rotschild and Mancinelli, 2001]. Not only can these microorganisms survive and grow under extreme conditions but usually require these conditions for survival

Sylvie Le Borgne
UAM-Cuajimalpa
Av. Pedro A. de los Santos 84
11850 México, D.F. (Mexico)
Tel. +52 55 5804 64 08, Fax +52 55 5804 64 07, E-Mail sylvielb@correo.cua.mx

and growth. Tolerant microorganisms can grow under extreme conditions but these conditions are not their optimal growth conditions.

Halophilic and halotolerant microorganisms are able to thrive and grow in saline and hypersaline environments. These microorganisms are being the object of basic studies in relation to the origin of life in our planet and the molecular mechanisms of adaptation to saline and hypersaline conditions [DasSarma and Aora, 2002]. The oldest prokaryote fossils found in 3,500 million-year-old stromatolites resemble the contemporary microbial mats found in hypersaline environments. Halobacteria and methanogenic bacteria are both placed on a very ancient phylogenetic branch of archaea. Apart from their evolutionary and ecological significance, halophiles have promising biotechnological applications including food industry pigments, organic osmotic stabilizers, surfactants, enzymes able to function at low water activites, bacteriorhodopsin applications including holography, optical computers and optical memory, production of renewable energy and biodegradation of organic pollutants [Margesin and Schinner, 2001b; Oren, 2002a, b].

The degradation or transformation of organic pollutants by halophilic and halotolerant microorganisms has received little attention. However, a survey of the literature indicates that halophilic microorganisms have more catabolic versatility than previously thought. The specific aim of this work is to provide a critical review of organic pollutants degradation by halophilic and halotolerant microorganisms with emphasis on aerobic and facultative anaerobic halophilic bacteria and archaea. The range of compounds known to be degraded by anaerobic halophilic bacteria is very limited, so the potential of this group of microorganisms for the degradation of organic pollutants has been stated to be doubtful [Oren, 1992]. A short overview on halophilic bacteria and archaea is presented focused on the problematic of saline and hypersaline pollution.

Pollution of Hypersaline Ecosystems

A variety of saline and hypersaline ecosystems are present on Earth. The salt concentration in these environments can vary from 3.5% (w/v) of total dissolved salts, as in seawater, to concentrations close to saturation (35%). Hypersaline environments are those containing salt concentrations in excess of seawater. These systems have considerable economic, ecological and scientific value and can be both of natural and man made origin,

including natural saline and hypersaline lakes and ponds, salt marshes, solar salt production facilities, brine inclusions in salt mine crystals and petroleum deposits. Saline and hypersaline aquatic environments can be classified as athalassohaline or thalassohaline depending on their origin. Athalassohaline waters are mainly formed from salt deposits due to evaporative events in inland water bodies. They are not of marine origin. Natural thalassohaline environments are originated from the evaporation of seawater due to the inland isolation of sea water bodies. Their composition resembles those of seawater and it is mainly dominated by sodium chloride and sulfate ions. Solar salterns are artificial thalassohaline environments where seawater is evaporated to obtain salt for commercial purposes. The Great Salt Lake in Utah, the Dead Sea in Israel, the Red Sea and lakes of the Atacama Desert in Chile are typical examples of hypersaline environments. Another type of hypersaline environment is the saline alkaline soda lake where the high salt concentration is combined with a high pH due to the presence of carbonate. All these environments are ecological niches of halophilic microorganisms [Oren, 2002a].

As all natural ecosystems on our planet, hypersaline environments are subjected to environmental contamination (fig. 1). Industrial and municipal effluents are often discharged into saline and hypersaline depression and intertidal zones, especially in developing countries [Lefebvre, 2004]. Several industrial processes, such as pesticide, chemical and pharmaceutical production as well as gas and oil extraction, generate thousands of millions of liters of saline to highly saline wastewaters [Lefebvre and Moletta, 2006]. It has been estimated that 5% of the total world effluents are highly saline [Lefebvre, 2004]. The petroleum industry generates a huge amount of oily and saline residual waters (oily brines, production waters) with salinities up to 10% or more after separation of crude oil from reservoir water. The most abundant extraneous material in the crude oil extraction process is water. Most wells, especially during their declining years, produce vast quantities of highly saline waters which have to be disposed [Speight, 1998]. The main contaminants in these production waters are aromatic and polycyclic hydrocarbons (PAHs). Due to decreasing freshwater availability, water re-use as well as seawater use and re-use strategies are being implemented in industries, increasing the volume of saline and hypersaline effluents to be treated [Alva and Peyton, 2003].

The biological treatment of industrial hypersaline wastewaters and the bioremediation of polluted hypersaline environments is not possible with conventional mi-

Fig. 1. Waste pond with salt built up.

croorganism [Oren, 2002b; Pieper and Reineke, 2000]. Conventional microorganisms are unable to operate efficiently at salinities above that of seawater and their capacity of adaptation to salinity is easily lost after exposition to low salinity conditions. High and fluctuating salinity promotes the loss of cell wall integrity, protein denaturalization and changes in osmotic pressure [Pernetti and Di Palma, 2005]. Such inhibition effects due to high salinity have been reported in several conventional wastewater treatment plants [Kargi and Dincer, 1997; Woolard and Irving, 1994]. Thus, halophilic microorganisms are potential candidates for the degradation of pollutants at high salt concentrations.

Halophilic Bacteria and Archaea

Halophiles grow and carry out their metabolic functions under hypersaline conditions [Litchfield, 1998]. Halophilic microorganisms can be classified according their salt requirements (table 1) [DasSarma and Aora, 2002]. Nonhalophilic microorganisms show an optimal growth below 0.2 M NaCl while halotolerant microorganisms are able to grow both under high and low salinity conditions. As the salt concentration increases, the microbial diversity is reduced and halotolerant and halophilic species tend to dominate [Oren, 2002b].

Table 1. Classification of halophilic microorganisms according to their NaCl requirements

Classification	NaCl concentration	
	mol/l	% (w/v)
Slight halophiles	0.2–0.85	2–5
Moderate halophiles	0.85–3.4	5–20
Extreme halophiles	3.4–5.1	20–30

Halophilic microorganisms include moderately halophilic and halotolerant bacteria found in many subgroups within the kingdom bacteria. Extreme halophilic bacteria are found in the archaea kingdom. Halophiles and halotolerant organisms are also present in the eukarya kingdom; they are not the subject of this review. The diversity of halophilic bacteria and archaea has been extensively reviewed and several characteristic groups have been distinguished [Grant et al., 1998; Kamekura, 1998; Ventosa et al., 1998a, b]. The phototrophic bacteria are generally found under the cyanobacteria in anaerobic zones of microbial mats, typical genera are *Chlorobium*, *Chromatium, Chloroflex, Thiocapsa, Rhodospirillum* and *Ectothiorhodospira*. Aerobic heterotrophic Gram-negative bacteria are typically found in moderately saline

brines with the following representative genera: *Halomonas*, *Acinetobacter*, *Alteromonas*, *Marinomonas* and *Pseudomonas*, among others. Aerobic heterotrophic Gram-positive bacteria are also present but less abundantly, they include *Marinococcus*, *Sporosarcina*, *Salinococcus* and *Bacillus*. Hypersaline waters with neutral pH are generally inhabited by *Halobacteriaceae* which are extreme halophilic archaea mainly from the *Haloarcula*, *Halobacterium*, *Haloferax*, *Halorubrum*, *Halococcus* and *Halorubrum* genera. Methanogenic archaea (*Methanohalophilus*) are also present. The microbial diversity of soda lakes has also been reviewed showing the presence of the same microbial groups as those mentioned above as well as alkaliphilic and haloalkaliphilic microorganisms and sulfur-oxidizing bacteria [Jones et al., 1998].

Most halophilic archaea are red pigmented and, until recently, were generally thought to require high salt concentrations of at least 15–20% for growth, limiting the range of habitats where they could be found. However, new specimens of halophilic archaea were recently isolated from environments having salinities close to these of seawater. This archaea is able to grow at salinities from 2.5% NaCl, extending the range of habitats of this type of microorganisms [Purdy et al., 2004]. Halophilic archaea preferentially use amino acids as carbon and energy sources and they also can obtain energy from light through the bacteriorhodopsin proton pump. Halophilic bacteria have attracted less attention than the red halophilic archaea even though they inhabit a wider range of habitats; they have a wider range of metabolic capabilities and considerable diversity with respect to the carbon and energy sources they can use [Ventosa et al., 1998a, b].

Halophilic bacteria and archaea have the ability to adapt to fluctuations in external osmotic pressure and maintain an osmotic balance between their cytoplasm and the hypersaline extracellular environment. Two main specific mechanisms of osmoadaptation exist: the 'salt-in' mechanism in which the intracellular salt concentration is maintained high, and the accumulation of organic osmotic solutes mechanism in which the intracellular salt concentration is maintained low. Halophilic archaea use the 'salt-in' mechanism which consists in accumulating high intracellular concentrations of salts, mainly K^+ and Cl^-. The cellular structural components, the intracellular machinery and both the intra- and the extracellular enzymes have evolved to the presence of high salt concentrations and are adapted to operate under very high salinities [Oren, 2002b]. On the other hand, halophilic bacteria mainly use the 'compatible solutes' adaptation strategy that consists of the biosynthesis and intracellular accumulation of uncharged and highly water-soluble organic solutes which reduce the thermodynamic activity of water in order to compensate the external osmotic pressure [Oren, 1999]. An exception is the family of anaerobic halophilic bacteria (*Haloanaerobiaceae*) that have a high intracellular salt concentration [Oren, 1986].

Biodegradation of Organic Pollutant by Halophilic Bacteria and Archaea

Petroleum Hydrocarbons

Petroleum hydrocarbons are the origin of important pollution in almost all types of ecosystems. Atmosphere, soils, superficial and underground waters, and marine environment have been continuously affected by pollution produced during the extraction, refining, transport and use of petroleum. There is a significant amount of literature concerning hydrocarbon biodegradation by marine microorganisms, starting by the classical reviews, such as Atlas and Bartha [1972] and Colwell [1977], or more recent reviews [Harayama, 1999, 2004; Head and Swannell, 1999; Head et al., 2006]. However, information on hydrocarbon degradation in the presence of high salt concentrations is scarce. As mentioned above, hydrocarbon biodegradation in the presence of high salt concentrations is important because the bioremediation of oil-polluted salt marshes and treatment of industrial wastewaters.

It has often been reported that the biodegradation potential of extreme halophilic, acidophilic, alkaliphilic, or barophilic microorganisms is limited [Margesin and Schinner, 2001b]. In most cases, the metabolic activity and diversity decreases as the salt concentration increases, reducing the hydrocarbon metabolization [Kleinsteuber et al., 2006; Kuznetsov et al., 1992; Riis et al., 2003; Ward and Brock, 1978]. However, after long exposition times, degradation can be observed and the amount of metabolized hydrocarbons increases in saline environments [Kleinsteuber et al., 2006; Riis et al., 2003], suggesting an adaptation of the microbial consortia to high salt concentrations [Kleinsteuber et al., 2006]. Nevertheless, there are reports in which the hydrocarbon biodegradation was not affected in a wide salinity range [Kerr and Capone, 1988], while other reports claim that a salinity increase enhanced the hydrocarbon degradation [Diaz et al., 2000; Yang et al., 2000]. This diversity of results shows the complexity of microbial communities and the specificity of the polluted sites.

Several studies were performed using microbial consortia because they generally show higher degradation performance than axenic cultures. Unfortunately, most microbial consortia are not characterized in terms of their biodegradation capacity, as in the case of an oil-bearing stratum that showed oxidation of petroleum hydrocarbons at salinities higher than 15% NaCl and in which the presence of extreme halophilic archaea was detected [Kulichevskaya et al., 1992]. Bacterial consortia isolated from the North Sea and able to metabolize petroleum hydrocarbons at a salinity range from 0 to 22% of NaCl mainly included *Marinobacter* ssp., *Erwinia ananas* and *Bacillus* spp. [Díaz et al., 2000, 2002]. The degradation of aliphatic hydrocarbons was independent of the salinity level, while a salinity increase enhanced the degradation of pristane and phytane, but the degradation of total hydrocarbon was reduced. On the other hand, a consortium containing *Cellulomonas* spp., *Bacillus marisflavi*, *Dietzia maris* and *Halomonas eurihalina* was able to metabolize diesel oil from 0 to 17.5% of NaCl, showing an optimal hydrocarbon degradation at 10% NaCl [Riis et al., 2003]. The dynamic behavior and complexity of hydrocarbon-degrading microbial consortia at high salt concentrations was clearly demonstrated by Kleinsteuber et al. [2006]. This microbial consortium showed a preferential growth from 7.5 to 15% of NaCl with diesel fuel as solo source of carbon and energy. *Halomonas*, *Ralstonia* and *Ditzia* showed a wide salinity tolerance (from 0 to 20% of NaCl), *Idiomarina* was a dominant species at 7.5% and *Alcanivorax* and *Marinobacter* were dominant at 15% of NaCl in 42-day cultures, but these species disappeared in older cultures. An α-proteobacterium closely related to *Caulobacterse* was found between salinities of 15 and 20% in 84-day cultures. In older cultures and at different salinities, other species appeared, such as *Sphingomonas* (a well-known PAH degrader), *Janibacter* (a xenobiotic-degrading actinomycete) as well as bacteria from the *Ectothiorhodospiraceae* and *Methylophilaceae* families, *Desulfobacula*, *Desulfosporosius*, *Halomonas*, *Ralstonia* and *Dietzia* (the last two species are known as hydrocarbon degraders). As expected, several viable and noncultivable bacterial cells were found during the experiments.

Cyanobacteria-rich microbial mats showed efficient degradation of crude oil at salinities up to 21% [Abed et al., 2006; Cohen, 2002]. Cyanobacterial strains isolated from this microbial mat, however, did not degrade crude oil in axenic cultures. The complexity of this microbial consortium shows a high complementarity in terms of metabolic functions. Hydrocarbon degradation of oil was done primarily by aerobic heterotrophic bacteria. The oxygenic photosynthesis of oil-insensitive cyanobacteria supplied the molecular oxygen for the efficient aerobic metabolism. Environmental conditions at the mat surface switch from highly oxic conditions in the light to anaerobic sulfide-rich habitat in the dark, allowing the combined aerobic and anaerobic degradation of crude oil [Cohen, 2002]. Another microbial mat showed higher capacity to metabolize aromatic hydrocarbons than aliphatic compounds at 7.5% of NaCl [Grötzschel et al., 2002].

Few studies of hydrocarbon degradation at high salt concentration have been carried out using axenic cultures. Bacteria as *Rhodococcus, Micrococcus* and *Arthrobacter* were able to grow in a wide salinity range from 0.5 to 25% NaCl but hydrocarbon metabolization was observed only up to 15% of NaCl [Kulichevskaya et al., 1992; Zvyagintseva et al., 2001].

Extreme halophilic archaea have been reported to be able to metabolize hydrocarbons. *Halobacterium* sp. showed a high capacity to degrade C_{10}–C_{30} n-alkanes in a medium containing 30% NaCl. Hydrocarbon co-metabolization has been reported for *H. salinarium, H. volcanii and H. distributum* [Kulichevskaya et al., 1992]. Another extreme halophilic archaea strain, EH4, having oxidase and catalase activities was able to metabolize saturated hydrocarbons (tetradecane, eicosane, hexadecane, heneicosane, pristane) and PAHs (acenaphtene, phenanthrene, anthracene, 9-metylanthracene) growing in a medium with 310 g/l of salts [Bertrand et al., 1990]. In this strain, the decrease of salinity had a strong effect on both the bacterial growth and hydrocarbon degradation. Maximal growth was obtained at 20% NaCl, while no hydrocarbon degradation was found below 10% NaCl. As several nonhalophilic bacteria, the PAH-degrader *Halomonas eurihalina* produces an emulsifier to improve the mass transfer during hydrocarbon metabolization [Martínez-Checa et al., 2002]. This emulsifying compound is an exopolysaccharide that emulsifies crude oil more efficiently than synthetic surfactants.

On the other hand, *Dietzia maris*, an environmental actinomycete, is able to degrade paraffin and other petroleum derivatives at NaCl concentrations up to 10% [Zvyagintseva et al., 2001]. The halotolerant actinomycete *Streptomyces albiaxialis* is able to grow at salinities up to 30% using crude oil as unique source of carbon and energy in a salinity range of 3–10% NaCl [Kuznetsov et al., 1992]. Immobilization of hypersaline microbial mats and bacterial consortium improved both salinity tolerance and hydrocarbon degradation [Diaz et al., 2002; Grötzschel et al., 2002].

This review of the scarce information on hydrocarbon degradation at high salt concentration shows that most of the studies are phenomenological and there are still many unanswered questions. Are metabolic pathways similar than for mesophilic microorganisms? Are enzymes more stable to salt concentration or temperature? Enzymes from extremophiles could be very interesting for industrial applications. Thus, research efforts focused on the basic characterization of the microorganisms able to transform hydrocarbons in hypersaline ecosystems and the metabolic mechanisms involved in the petroleum degradation under extreme conditions are necessary.

Aromatic Compounds

Aromatic Acids. This category includes compounds that contain both an aromatic ring and a COOH group. Aromatic acids are abundant in the biosphere originated by natural and anthropogenic activities, and some of them are pollutants. The 4-hydroxybenzoic, ferulic, *p*-coumaric, vanillic, cinnamic, and syringic acids are naturally present in plants and plant root exudates. Aromatic acids may also enter the environment due to their use as raw materials for the production of agrochemicals, pharmaceuticals, varnishes and other specialty materials. In addition, they can also indirectly enter the environment as intermediates during the biodegradation of natural polymers (lignins and tannins), from aromatic amino acid precursors and as intermediates during the biodegradation of pollutants such as pesticides or PAHs. Because of their anionic character at the pH of most soil and sediment environments, they are expected to move rapidly through the soil and therefore pose a great risk of groundwater contamination.

Benzoate has been used as a test substrate for the taxonomic characterization of some halophilic and halotolerant bacteria (not isolated for biodegradation purposes) , and only very few isolates were found to grow on this aromatic substrate [Del Moral et al., 1988; García et al., 1987; Ventosa et al., 1982]. This is not surprising considering that the reported bacteria were not isolated with benzoate as the sole source of carbon and energy.

Early studies indicated that the novel species *Pseudomonas halodurans*, a halotolerant bacterium able to grow at NaCl concentrations up to 2.65 M, was capable of *ortho* cleavage of aromatic compounds including benzoic acid [Rosenberg, 1983]. This bacterium was later renamed *Halomonas halodurans* [Hebert and Vreeland, 1987]. A diffusion gradient chamber was used to enrich microbial populations able to degrade toluate at high NaCl concentrations from oil brine contaminated soils [Emerson and Breznak, 1997]. Two dominant strains of halophilic and toluate-utilizing strains, identified as *Pseudomonas nautical,* could be isolated. Decreased levels of toluate degradation at increasing NaCl concentrations were observed. Poor growth was obtained at toluate concentrations above 3 mM in the presence of 5% NaCl and no growth was observed at any toluate concentration in the presence of NaCl concentrations above 10%. Recently, halophilic bacteria able to degrade a variety of aromatic acids including benzoic, *p*-hydroxybenzoic, cinnamic, salicylic, phenylacetic, phenylpropionic, *p*-coumaric, ferulic and *p*-aminosalicylic acids were isolated from water and sediment of salterns and hypersaline soils located near to oil refineries and food-processing industries [García et al., 2005a]. The bacteria were enriched in minimal medium containing individual aromatic acids at final concentrations of 1–5 mM as the sole carbon and energy source in the presence of 10% NaCl. The influence of NaCl concentration on growth and biodegradation was not studied, and the biodegradation products were not analyzed neither. Although bacteria could be isolated with all aromatic acids tested, the highest number of isolates was obtained using benzoic acid for the enrichment. All the isolates were moderately halophilic bacteria mainly from the genus *Halomonas*. A new species, *Halomonas organivorans*, able to degrade a wide range of aromatic acids (benzoic, *p*-hydroxybenzoic, cinnamic, salicylic, phenylacetic, phenylpropionic, *p*-coumaric, ferulic and *p*-aminosalicylic acids), was detected in this study [García et al., 2004]. These authors propose that *Halomonas* could be used as a model genus for elucidating the metabolism of aromatic compounds at high salt concentrations. The genus *Marinobacter*, as mentioned above, has been frequently associated to hydrocarbon degradation at high salinities [Gu et al., 2007; Marquez and Ventosa, 2005]; however, only one *Marinobacter* species was detected in this study. Some isolates belonging to the genera *Chromohalobacter*, *Salinococcus* and *Halobacillus* were also found. This is the first report in which members of these genera are able to degrade organic pollutants. The *Halomonas elongata* strain Mar isolated from table-olive fermentation brines is the first halophilic bacterium shown to be able to degrade ferulic acid into vanillic acid in the presence of 8% NaCl [Abdelkafi et al., 2006]. Vanillic acid is the starting material in the chemical synthesis of the flavor molecule vanillin.

Few isolates of halophilic archaea with aromatic acids biodegradative capabilities have been described. *Haloferax* sp. strain D1227 is the first reported archeon capable of aerobic metabolism of aromatic acids as benzoic, cin-
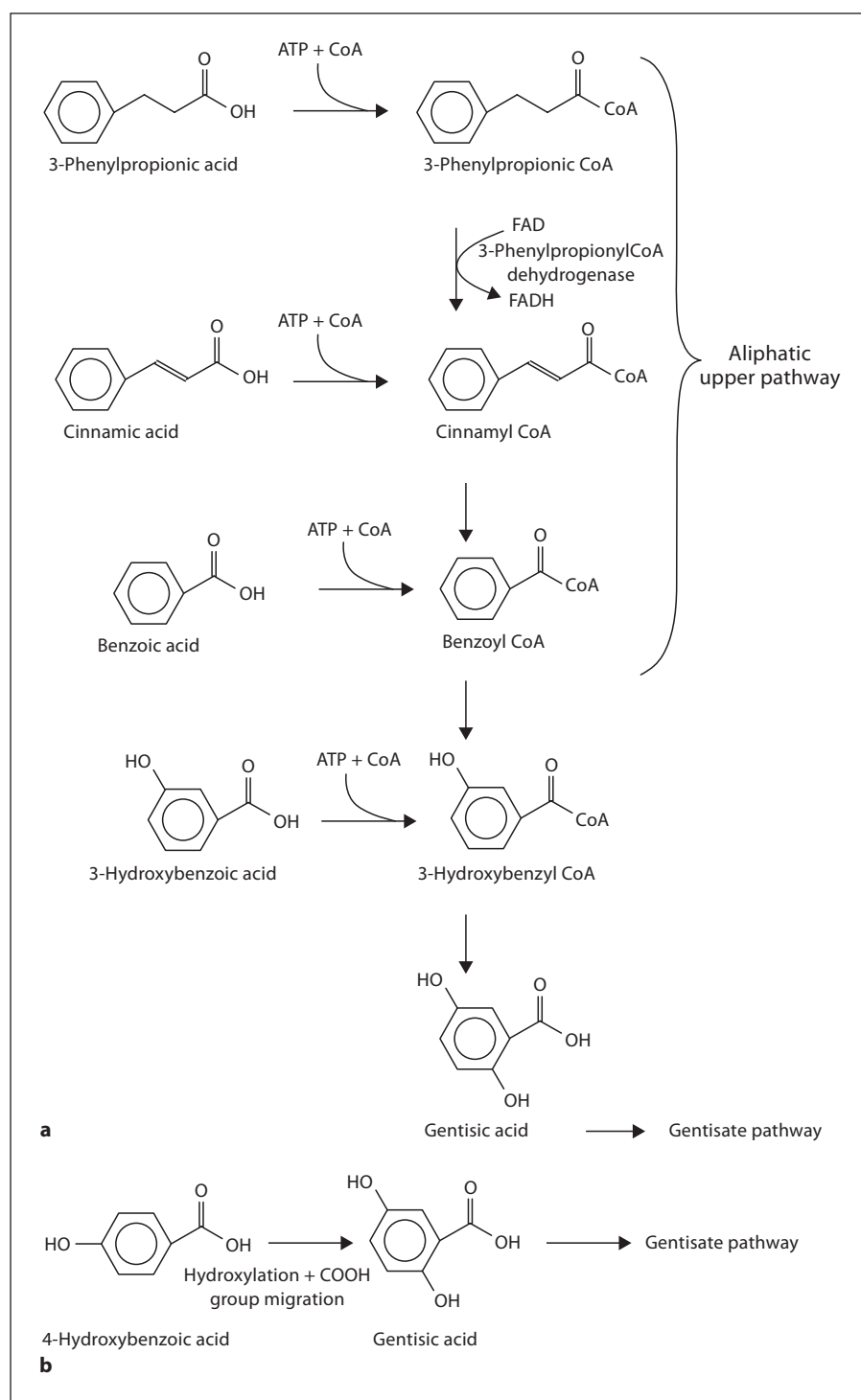
Fig. 2. Pathways for the degradation of aromatic acids in extremely halophilic archaea. **a** Degradation of 3-phenylpropionic acid by *Haloferax* sp. D1227. **b** Degradation of 4-hydroxybenzoic acid by *Haloharcula* sp. D1.

amic, and 3-phenylpropionic acid [Emerson et al., 1994]. This microorganism was isolated from petroleum-contaminated soil near Gran Rapids, Mich., USA. It belongs to the *Halobacteriaceae* family and requires 2 M NaCl for

optimal growth. The degradation of 3-phenylpropionic acid by the halophilic archeon *Haloferax* sp. D1227 was studied in more detail since this compound has an interesting structure with both an aromatic ring and an ali-
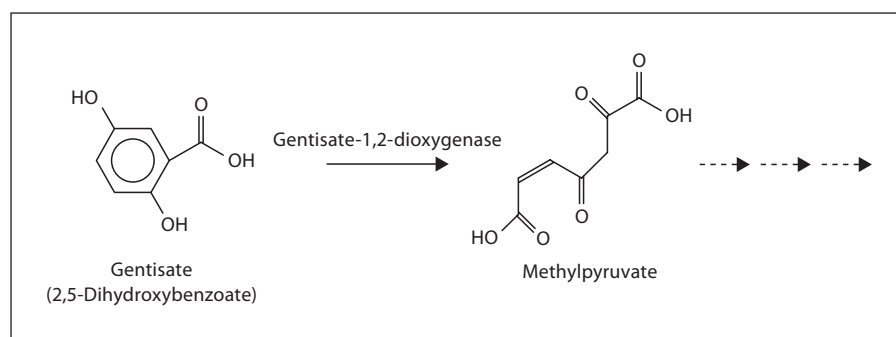
Fig. 3. Gentisate degradation pathway.

phatic side chain [Fu and Oriel, 1999]. It was shown that the catabolism of this compound proceeds by initial shortening of the aliphatic side chain to form benzoyl-CoA via a mechanism similar to fatty acids β-oxidation, followed by aromatic degradation through the gentisate pathway (fig. 2a, 3). A novel halophilic archaea strain (*Haloarcula* sp. D1), able to grow aerobically on 4-hydroxybenzoic acid (4HBA) as the sole source of carbon and energy, was isolated [Fairley et al., 2002]. This is the first halophilic archaon able to metabolize 4HBA, while the formerly isolated *Haloferax* sp. D1227 had been found to be unable to degrade this compound. 4HBA was metabolized through the gentisate pathway involving a hydroxylation that induces an intramolecular migration. More recently, the isolation of forty four new haloarchaeal strains able to grow with low concentrations of *p*-hydroxybenzoic acid (0.4 mM) as the sole carbon and energy source was reported [Cuadros-Orellana et al., 2006]. These authors claim that the ability to degrade *p*-hydroxybenzoic acid could be a widespread feature among the Halobacteriaceae.

Phenols and Phenolic Compounds. Phenols are major pollutants of industrial wastewaters since they are commonly used in many industries such as oil refining, coke conversion, pharmaceutical and resin manufacturing plants.

Biodegradation of phenol in hypersaline wastewaters was reported by Woolard and Irving [1994, 1995]. These authors used a halophilic bacteria biofilm isolated from a saltern at the Great Salt Lake. More than 99% of the phenol was removed from a synthetic wastewater containing from 0.1 to 0.13 g/l of phenol and 15% (w/v) NaCl in a batch-sequenced reactor. The bacteria present in the biofilm and responsible for biodegradation were not identified. Hinteregger and Schreiber [1997] studied the biodegradative capacity of a new phenol-degrading *Halomonas* sp. strain isolated from the Great Salt Lake. This strain degraded phenol as the sole source of carbon and energy in a model industrial saline wastewater. Optimum growth of this strain on phenol occurred at NaCl concentrations from 3 to 5% (w/v). At these salinities, 0.1 g/l of phenol was degraded in 13 h. The disappearance of phenol was accompanied by the accumulation of *cis,cis*-muconic acid, an intermediate of phenol degradation through the *ortho*-cleavage pathway (fig. 4).

Alva and Peyton [2003] reported phenol and catechol biodegradation by the haloalkaliphile bacterium *Halomonas campisalis*. This bacterium is able to grow with phenol as the sole source of carbon and energy at pH 8–11 and 0–150 g/l NaCl, and phenol was mineralized to CO_2. Catechol and *cis,cis*-muconic acid were identified as intermediate products indicating that phenol biodegradation proceeded through the *ortho*-cleavage pathway. *Cis,cis*-muconate accumulation was favored with increasing pH. These authors mention that *cis,cis*-muconate is normally effluxed from the cells. At high pH, this compound is present as an anion which may hinder permeability through cell membranes and re-incorporation into the cells for further degradation. When *cis,cis*-muconate was used as substrate, efficient degradation was found at low salinities but no degradation at medium and high salinity conditions could be detected. According to the authors, the cell membranes of *Halomonas campisalis* may be less compact and internally coherent at low lower salinities allowing *cis,cis*-muconate to re-enter the cell. Maskow and Kleinsteuber [2004] reported the isolation of the haloalkaliphile *Halomonas* sp. EF11 able to grow on phenol as the sole source for carbon and energy. This strain assimilated phenol through the *meta* pathway at a high C/N ratio and both through the *ortho* and *meta* pathways at a low C/N ratio (fig. 4).

During an extensive screening of aromatic compound-degrading halophilic bacteria, García et al. [2005a] isolated the new species *Halomonas organivorans* [García et
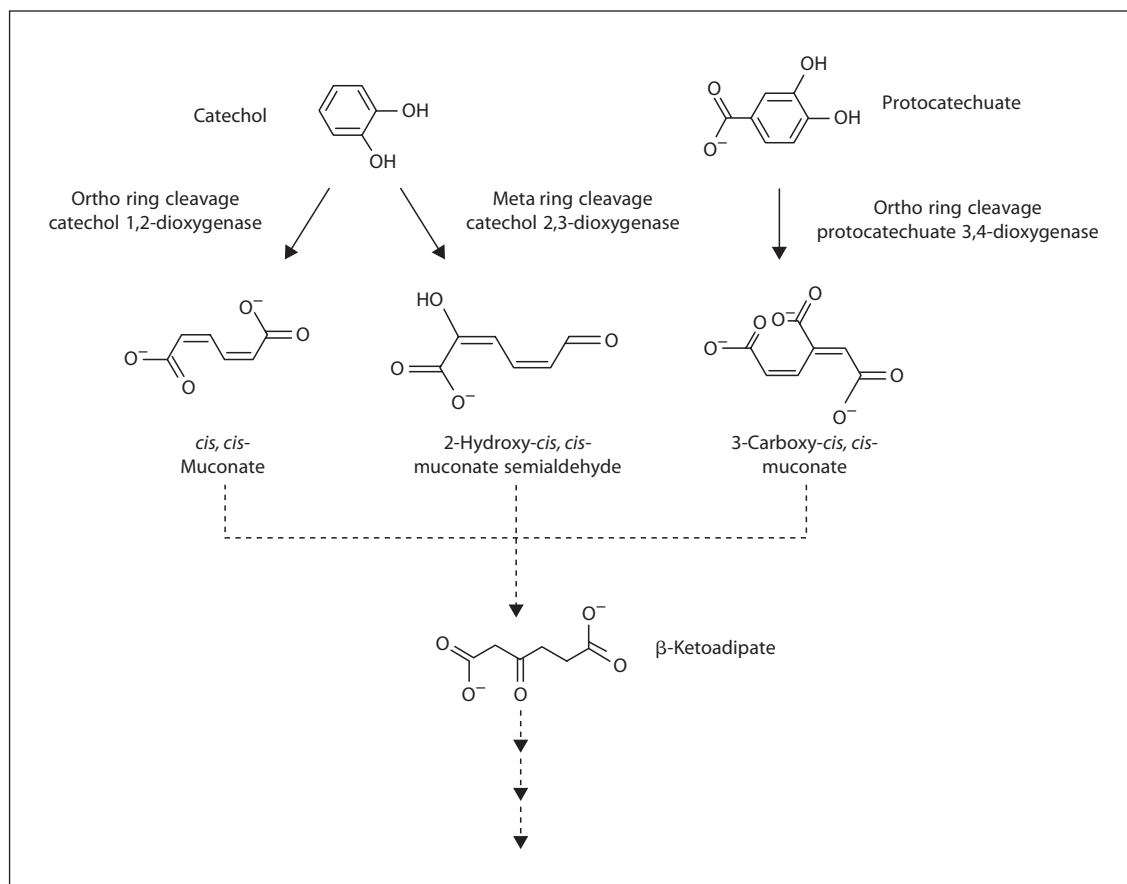
Fig. 4. Pathways for the degradation of phenol and protocatechuate.

al., 2004] and *Thalassobacillus devorans*, a moderately halophilic, phenol-degrading, Gram-positive bacterium [García et al., 2005b] able to use phenol as the sole source of carbon and energy. Both bacteria produced optimal growth in media containing 7.5–10% (w/v) NaCl. No bacteria could be isolated when *p*-cresol was the sole source of carbon and energy.

Tyrosol (*p*-hydroxyphenylethanol) is a phenolic compound that naturally occurs in olive mill wastewaters. A novel *Bacillus* sp., strain YAS1, could transform tyrosol into *p*-hydroxyphenylacetic acid under hypersaline conditions (50 g/l NaCl) in the presence of 1 g/l of yeast extract [Abdelkafi et al., 2005]. The strain was reported to be moderately halotolerant with an optimal growth at 3–6% NaCl. Biodegradation of nitro- and chloro-substituted phenols will be described below in the halogenated hydrocarbons section.

Benzene, Toluene, Ethylbenzene, Xylenes (BTEX). BTEX compounds are partially soluble in water and can contaminate soils and aquifers. These compounds can be found in the environment after oil and oil derivatives (gasoline, solvents) spills or released from oilfield wastewaters. Benzene is a well-known carcinogenic compound which makes it a priority pollutant. Little is known concerning the degradation of BTEX by halophilic and halotolerant bacteria. The biodegradation of benzene by an enrichment culture developed from an oil-brine soil sample in the presence of 145 g/l of NaCl was recently reported [Nicholson and Fathepure, 2004]. The obtained enriched culture was also able to degrade toluene, ethylbenzene and xylenes. Toluene was rapidly biodegraded (20 μmol in a week) while degradation of benzene, ethylbenzene and xylene required 2–3 weeks. Denaturing gradient gel electrophoresis (DGGE) analysis of the bacterial populations present in this enriched culture showed that it was dominated by *Marinobacter* species.

The genus *Marinobacter* was first described by Gauthier et al. [1992]. *Marinobacter* species are moderate halo-

philic bacteria affiliated to the gamma-proteobacteria. These bacteria have been mainly isolated from marine environments [Gorshkova et al., 2003; Green et al., 2006; Kim et al., 2006; Romanenko et al., 2005; Rontani et al., 1997, 2003; Yoon et al., 2003, 2004]. They also have been detected in oil reservoirs and oil-producing wells [Huu et al., 1999, Sette et al., 2006], as well as oil-impacted ecosystems [Brito et al., 2006; Gu et al., 2007; Piedad Diaz et al., 2000; Yakimov et al., 2005]. *Marinobacter* species tolerate a wide range of NaCl concentrations making them potentially useful for field applications in fluctuating conditions of salt concentration. *Marinobacter hydrocarbonoclasticus,* isolated from Mediterranean seawater near a petroleum refinery, grows at NaCl concentrations from 0.1 to 3.5 M with an optimum at 0.6 M. Additionally, these microbes have other extremophilic characteristics such as psychrophily, alkaliphily, thermotolerance and tolerance to heavy metals which make them versatile and interesting for eventual environmental applications [Deppe et al., 2005; Ivanova et al., 2002; Shieh et al., 2003; Shivaji et al., 2005; Takai et al., 2005]. In fact, the hydrocarbon-degrading bacterium *Marinobacter aquoeli* (heterotypic synonym of *M. hydrocarbonoclasticus*) is being currently sequenced at the DOE Joint Genome Institute.

Nicholson and Fathepure [2005] recently reported the degradation of benzene and toluene by enriched cultures initially developed from samples of uncontaminated hypersaline soils taken at the Great Salt Plains, Oklahoma and in the presence of benzene and 2.5 M NaCl. They demonstrate that microorganisms from hypersaline environments with no contamination history are able to degrade aromatic compounds as benzene and toluene. In this case, no degradation of ethylbenzene and xylenes occurred even after long incubation times. The DGGE analysis of the bacterial populations in this culture revealed that different types of bacteria were dominant depending on the NaCl concentration (*Acidovorax delafieldii* and *Pseudomonas* sp. in the absence of NaCl; *Halobacillus salinus* between 1 and 2.5 M NaCl; *Bacillus simplex* between 1 and 4 M NaCl as well as nonidentified bacteria above 2.5 M NaCl).

Halogenated Hydrocarbons

Halogenated hydrocarbons cover a wide group of aliphatic and aromatic compounds in which one (or more) of the hydrogen atoms is substituted by a halogen group (chlorine, fluorine, bromine or iodine). These compounds are used for industrial, petrochemical, food industry, and agricultural applications. Halogenated hydrocarbons are of environmental concern because of their persistence

and toxicity, some of them are thought to cause damage to the ozone layer and have been forbidden by most countries. In particular, chlorinated hydrocarbons are widely used because of their chemical and thermal stability as well as their fungicidal, herbicidal, and insecticidal properties.

2,4-Dichlorophenoxyacetic acid (2,4-D) is a widely used herbicide. A variety of nonextremophilic environmental microorganisms can degrade this compound [Fulthorpe et al., 1995]. Maltseva et al. [1996] reported the isolation of haloalkaliphilic bacteria related to the family *Halomonadaceae* (91% identity after analysis of 303 nucleotides of the 16S rRNA gene). These isolates were able to degrade 2,4-D as the sole source of carbon and energy, growing optimally at pH 8.4–9.4 and NaCl concentrations of 0.6–1.0 M. One of the isolates was further characterized revealing that it utilizes the same 2,4-D degradation pathway as most of the nonextremophilic 2,4-D-degrading bacteria. Additionally, this strain was also able to degrade benzoic acid, 3-chlorobenzoic acid and 4-chlorophenol [Oriel et al., 1997].

Chlorinated phenols have been widely used as wood preservatives and agricultural biocides. Maltseva and Oriel [1997] reported the isolation of the alkaliphilic and slightly halophilic *Nocardioides* sp. strain M6, able to grow on 2,4-dichlorophenol (2,4-DCP) as well as on 2,4,5- and 2,4,6-trichlorophenol (2,4,5-TCP and 2,4,6-TCP) as the sole source of carbon and energy. Strain M6 degraded 0.7 g/l of 2,4,6-TCP in 120 h and could grow at concentrations up to 1.6 g/l of 2,4,6-TCP; however, the length of the lag phase increased on increasing the concentration of this substrate.

Halophilic archaea from the genus *Halobacterium*, *Haloharcula* and *Haloferax* were submitted to an adaptation/selection process to high concentrations (up to 1 mM) of various halogenated compounds including TCP, lindane and DDT [Oesterhelt, 1998]. GC-MS analysis has shown that these bacteria could, at least, partially decompose these compounds.

Organic Solvents

Trichloroethylene (TCE) is a chlorinated hydrocarbon commonly used as an industrial solvent. Fuse [1998] reported the use of a halophilic methane-oxidizing bacterium *Methylomicrobium* sp. strain NI to oxidize TCE in a medium with a salt concentration of 2–6 % (w/v). Such bacteria could be useful for the bioremediation of TCE contamination in seawater or ecosystems containing salt concentration close to seawater.

A halotolerant, alkaliphilic and methane-oxidizing bacterium (methanotroph), isolated from highly alkaline and saline soda lakes, that can grow on methanol as carbon and energy source at pH 9–10 has been described [Khemelina et al., 1997; Sorokin et al., 2000]. *Methylobacterium* species and *Methylobacter alcaliphilus* growing with Na$^+$ concentrations up to 1.1 and 1.5 M, respectively, were able to metabolize methanol. *Methylobacterium* species growing with low methanol concentrations (2–5 mM) were inactivated after three passages [Sorokin et al., 2000]. This growth inhibition was attributed to formaldehyde accumulation due to low formaldehyde-oxidizing activity in these bacteria. Formaldehyde is an intermediate from methane and methanol degradation, and is further metabolized through assimilatory or dissimilatory pathways. On the contrary, *Methylobacter alcaliphilus* was grown in the presence of higher concentrations of methanol (50 mM) [Khemina et al., 1997].

Formaldehyde is used in the production of polymers and other chemicals and as a disinfectant. Oren et al. [1992] have described the isolation and partial characterization of a highly halotolerant bacterium (isolate MA-C) able to degrade formaldehyde in a medium containing between 0 and 20% (w/v) NaCl. This unidentified isolate was considered to be resistant to formaldehyde and grew well in media supplemented with 100 ppm formaldehyde. In the case of *Halomonas* species, the formaldehyde tolerance seems to be dependent on the composition and structure of outer membranes [Azachi et al., 1996].

N, N'-dimethylformamide (DMF) is a versatile solvent employed in the textile and pharmaceutical industries due to its solubility in both aqueous and organic solvents. Aerobic bacterial consortia, able to degrade 200 mg/l of DMF through a wide range of NaCl concentrations (up to 7%) [Bromley-Challenor et al., 2000], could be useful for the treatment DMF-contaminated effluents with fluctuating salinities.

Nitriles, such as acetonitrile, are important compounds for industry. Sorokin et al. [2007a] isolated a moderately salt-tolerant alkaliphilic bacteria (new genus and species) *Natronocella acetinitrilica* from a soda lake sediment sample. This bacterium was able to use acetonitrile and propionitrile as a carbon, energy and nitrogen source at extremely high pH (10) and moderate salinity (0.6 M NaCl).

Organophosphorus Compounds
Organophosphorus compounds are ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acids. They are widely used as pesticides, plasticizers and chemical warfare agents (nerve agents). Their continuous and extensive use as pesticides has led to contamination of several ecosystems in the world. These compounds are highly toxic to insects and mammalians. The microbial degradation of organophosphorus compounds by nonextremophilic microorganisms has been recently reviewed [Singh and Walker, 2006].

Few halophilic microorganisms, able to degrade organophosphorus compounds, have been described. De-Franck and Chen [1991] reported the isolation of a moderately halophilic bacterium from a hypersaline spring (24% w/v of salt) near the Great Salt Lake that showed high enzymatic activity for organophosphorus compounds transformation. The isolate, designated JD6.5, was reported to be an obligate halophile and tentatively identified as an *Alteromonas* species. An organophosphorus acid anhydrase, responsible for the hydrolysis of ester bond, was purified from this isolate, and the purified enzyme exhibited activity against diisopropylfluorophosphate, *p*-nitrophenylmethyl(phenyl)phosphinate, *p*-nitrophenylethyl(phenyl)phosphinate and diethyl *p*-nitrophenylphosphate (paroxon). The optimum pH and temperature reported for this enzyme were 8.5 and 50°C, respectively. This research group carried out a screening program to determine if organophosphorus anhydrases were commonly found in other species of *Alteromonas* [DeFranck et al., 1993]. A wide spectrum of hydrolytic activities against several organophosphorus compounds were detected in cell extracts from *Alteromonas* species and other nonidentified halophilic bacterial isolates. These results suggest that functionally similar enzymes might be present in the tested bacteria; these enzymes have potential for the decontamination of chemical warfare agents.

The archaeon *Halobacterium salinarum* was shown to contain an alkaline *p*-nitrophenyl phosphatase able to hydrolyze *p*-nitrophenylphosphate in reversed micelles in organic solvent [Marhuenda-Egea et al., 2002]. The hydrolysis of other organophosphorus compounds with this enzyme was not reported.

A Gram-negative halophilic bacterium, identified as *Chromohalobacter marimortui* or *Pseudomonas beijerinckii* after 16S rRNA gene sequencing, could utilize phosphonoacetate, 2-aminoethyl-, 3-aminopropyl-, 4-aminobutyl-, methyl- and ethyl-phosphonates as phosphorus sources for growth [Hayes et al., 2000]. This strain grew optimally at 10% (w/v) NaCl. The growth rate on different phosphonates and the range of utilized phosphonates decreased at elevated NaCl concentrations.

Nitrogen Compounds

Organic nitrogen compounds are found in foods, organic materials, fertilizers, poisons, and explosives. Information concerning the degradation of this type of compound by halophilic or halotolerant microorganisms is scarce.

The phenylurea herbicides are an important group of pesticides used worldwide. The microbial degradation of the phenyl ring in these compounds has been reported to be slow [Sørensen et al., 2003]. A moderate halophilic *Marinobacter* sp. isolate able to degrade 1,3-diphenylurea (DPU) was isolated from a contaminated ephemeral desert stream bed in the Negev Desert, Israel [Sørensen et al., 2002]. This isolated apparently could completely mineralize DPU, and aniline was detected as an intermediate metabolite suggesting that a metabolic pathway involving cleavage of the urea bridge between the phenyl structures. The optimum specific growth rate occurred in the presence of 0.51 M NaCl and at 35°C. The influence of the NaCl concentration on DPU degradation was not assayed.

A strictly halophilic denitrifying bacterium, able to degrade trimethylamine (TMA), was isolated from coastal sediments and wastewater contaminated by marine water [Kim et al., 2003]. TMA is a malodorous pollutant found in fish-meal manufacturing processes effluents which has teratogenic effects on animal embryos. Sequence analysis of 16S rRNA genes revealed that these isolates were clustered on a branch remote from other genera in the alpha-Proteobacteria and the nearest homologs were *Roseobacter* species. Maximum growth rate was found at 0.25–0.50 M NaCl. The versatility of these facultative anaerobic bacterial isolates was demonstrated because they could degrade TMA under both aerobic and denitrifying conditions. TMA degradation occurred via dimethylamine to ammonia and was coupled to respiratory nitrate reduction. These isolates possessed both a TMA monooxygenase and a TMA dehydrogenase for the oxidation of TMA under aerobic conditions.

Azo Dyes

Azo dyes are chemical compounds having the following general structure R–N=N–R′, in which R and R′ can be either aryl or alkyl. The N=N group is called an azo- or di-imide, and the more stable azo dyes derivatives contain two aryl groups. Azo dyes are released in wastewaters generated both by dye producing and dye consuming industries. Biodegradation of azo dyes can occur in both aerobic and anaerobic environments. In both cases, the initial step is the reductive cleavage of the azo bond.

Under aerobic conditions, this initial step is typically followed by hydroxylation and ring opening of the aromatic intermediates. The recalcitrant nature of such compounds could therefore be overcome by utilizing anaerobic-aerobic co-cultures [Field et al., 1995]. However, due to the electron-withdrawal character of their N=N groups, these compounds are not very susceptible to oxidative metabolism and, as a consequence of this recalcitrance in aerobic environments, these compounds preferentially end up in anaerobic sediments, aquifers and groundwaters [Razo-Flores et al., 1997]. Rupture of the N=N double bond leads to the decolorization. Guo et al. [2007] have described the decolorization of several azo dyes under anaerobic conditions by a new member of the *Halomonas* genus, strain GTW, isolated from coastal sediments in Dalian Bay (China). Optimal decolorization occurred at 30°C, pH 6.5–8.5 and NaCl 10–20% with yeast extract as the carbon source. Nevertheless, neither the precise anaerobic conditions nor the degradation products were described. Asad et al. [2007] reported the isolation of three new *Halomonas* strains from textile effluents that were able to use a wide range of azo dyes as the sole source of carbon. These strains could decolorize the azo dyes in a wide range of NaCl concentrations (up to 20%) and pH (5–11), and decolorization occurred only under anaerobic conditions and in static cultures but not under aerobic conditions (cultures submitted to agitation). HPLC analysis of the decolorized media indicated that decolorization proceeded through reduction of the azo bond followed by cleavage of the reduced bond to produce aromatic amines. The wide range of salinities for dye decolorization is an interesting characteristic for an eventual application to the decolorization of textile effluents that are generally saline and slightly alkaline.

Sulfur Compounds

Reduced organic sulfur compounds include mercaptanes and carbonyl- and methylsulfides. These volatile compounds play an important role in the processes of global warming, acid precipitation and global sulfur cycle. They have been identified as predominant odorants in several industrial gaseous emissions. The use of halo-alkaliphilic sulfur-oxidizing bacteria in a biotechnological process designed to remove hydrogene sulfide (H_2S) from gaseous emissions of petroleum industry has been recently described [van den Bosch et al., 2007]. The advantages of operating at alkaline pH and under saline conditions are the increased solubility of H_2S and the possibility to use and re-use seawater, respectively. No similar bioprocess under saline conditions has been de-

scribed for the abatement of volatile organic sulfur compounds odorous emissions yet. However, halophilic bacteria able to degrade dimethyl sulfide (DMS) have been described. Alkaliphilic halophilic and methylotrophic methanogens from the *Methanohalophilus* genus are able to catabolize DMS and grow in medium with a methyl group-containing substrate (methanogenic substrate, such as methanol or trimethylamine) as organic substrate [Mathrani et al., 1988]. Another *Methanohalophilus* strain able to grow over a range of external NaCl concentrations from 1.2 to 2.9 M and with methanol, trimethylamine and DMS as substrates for methanogenesis has been described [Robertson et al., 1992]. This strain produced different organic osmotic solutes to regulate the increase of external NaCl concentration. Interestingly, the relative ratio of these osmotic solutes seems depend more on the methanogenic substrate than on the external osmolarity.

Thiocyanate [SCN]$^-$ is a C_1 organic sulfur compound naturally produced during biological cyanide detoxification processes and also found in wastewater from coke and metal plants. Some microorganisms can utilize thiocyanate as an energy, carbon, nitrogen or sulfur source after it is hydrolyzed to sulfide, ammonia, and CO_2. Two thiocyanate degradation pathways have been described. In the first one, the C–S bond is broken to form H_2S and cyanate (N≡C–O$^-$) by a cyanase enzyme. The released H_2S is used as an electron donor and energy source. In the second one, the N≡C bond is broken to NH_3 and carbonyl sulfide (O=C=S) by a thiocyante hydrolase. The produced H_2S is eventually oxidized to sulfate. Three types of alkaliphilic halophilic bacteria able to utilize thiocyanate have been described by Sorokin et al. [2001]. The first group included obligate heterotrophes that utilized thiocyanate as a nitrogen source, but not as carbon or energy source. These bacteria were related to *Halomonas*. The second group included obligately autotrophic sulfur-oxidizing bacteria which utilized thiocyanate nitrogen during growth with thiosulfate as the energy source. These bacteria were related to *Thioalkalivibrio*. The third group included obligately autotrophic sulfur-oxidizing bacteria able to utilize thiocyanate as the sole source of nitrogen and energy (no thiosulfate required). These bacteria were also related to *Thioalkalivibrio*. The first two groups of bacteria showed high levels of cyanase activity contrary to the third group of bacteria. Recently described new genera and species of the third type of thiocyanate degrading bacteria included *Thioalkalivibrio paradoxus* and *Thioalkalivibrio thiocyanoxidans* [Sorokin et al., 2002]; *Thialkalivibrio thiocyanodenitrificans*,

able to grow with thiocyanate as electron donor, either aerobically or anaerobically, and with nitrate or nitrite as electron acceptor [Sorokin et al., 2004] and *Thiohalophilus thiocyanoxidans* capable of anaerobic growth with nitrite as electron acceptor and thiocyanate as electron donor under hypersaline conditions (2–4 M NaCl) [Sorokin et al., 2007b].

Metabolic Pathways, Enzymes and Genes

Most of the information on pollutant degradation in saline environments is phenomenological, and scarce information is available in literature on the metabolic mechanisms and enzymatic system involved in the pollutant degradation. Here, a review of the available information on metabolic pathways, enzymes and genes involved in the pollutant degradation by halophile microorganism is presented.

PAH Degradation by Marinobacter. The *Marinobacter* strain NCE312, isolated from a naphthalene-degrading culture inoculated from creosote-contaminated marine sediment, was shown to have a *Pseudomonas*-like naphthalene 1,2-dioxygenase [Hedlund et al., 2001]. This strain could grow on naphthalene as the sole source of carbon and energy producing bright yellow diffusible products characteristics from the *meta* ring cleavage of catechol through the action of a catechol 2,3-dioxygenase. This strain also degraded 2-methylnaphthalene but not 1-methylnaphthalene, 2,6-dimethylnaphthalene, acenaphthene, biphenyl, phenanthrene or fluorene. Figure 5 shows the naphthalene degradation via the catechol pathway of *Pseudomonas* based on Eaton et al. [1992]. The naphthalene 1,2-dioxygenase and the catechol 2,3-dioxygenase are enzymes involved in the naphathalene metabolism. Degenerate PCR primers directed to a central portion of the naphthalene 1,2-dioxygenase large subunit (NahAc) could successfully amplify a DNA fragment encoding a naphthalene dioxygenase in strain NCE312. According to a phylogenetic analysis, this dioxygenase was related to well-studied naphthalene 1,2-dioxygenase from *Pseudomonas*, *Burkholderia* and *Neptunomonas* and may have been acquired through horizontal gene transfer.

Aromatic Acids Degradation in Moderate Halophilic Bacteria and Halophilic Archaea. García et al. [2005a] amplified genes encoding the three ring-cleaving enzymes catechol 1,2-dioxygenase, catechol 2,3-dioxygenase and protocatechuate 3,4-dioxygenase of the β-ketoadipate pathway for the metabolism of aromatic com-

pounds in moderate halophilic bacteria. They have used degenerate PCR primers designed from conserved regions of nonhalophilic bacteria: *Acinetobacter radioresistens* for catechol 1,2-dioxygenase, *Pseudomonas putida* for catechol 2,3-dioxygenase and *Pseudomonas aeruginosa* for protocatechuate 3,4-dioxygenase. Figure 4 shows the reactions catalyzed by these enzymes. Some isolates seemed to be catabolically versatile since they contained the genes encoding for both the catechol 1,2- and the protocatechuate 3,4-dioxygenases. The degenerate primers designed for the catechol 2,3-dioxygenase gene gave slightly positive signals only for three of the tested strains; the majority of the strains yielded no signal or a smaller PCR product than expected. Catechol 1,2- and protocatechuate 3,4-dioxygenase activities were detected in *Halomonas organivorans* but not catechol 2,3-dioxygenase activity. According to the measured enzymatic activities, *Halomonas organivorans* preferentially used the catechol *ortho* pathway for degrading the benzoic, cinnamic, salicylic, phenylpropionic and *p*-aminosalicylic acid and, the protocatechuate pathway for degrading the *p*-hydroxybenzoic, *p*-coumaric and ferulic acid. The phenylacetic acid was apparently degraded through a different pathway since none of the ring-cleavage enzymatic activities assayed could be detected in this bacterium.

A gentisate 1,2-dioxygenase enzyme was purified and sequenced from the halophilic archaeon *Haloferax* sp. D1227 [Fu and Oriel, 1998]. As mentioned before, this microorganism is capable of aerobic metabolism of benzoic, cinnamic, and 3-phenylpropionic acid. Figure 3 shows the reaction catalyzed by the gentisate 1,2-dioxygenase. Ring fission occurs between the carbonyl and the hydroxyl groups and therefore it cannot be considered nor as an *ortho* or a *para* ring-fission type. The gentisate 1,2-dioxygenase from *Haloferax* D1227 has similar molecular weight and subunit structure as its eubacterial counterparts. It possess a four histidine cluster, His-X-His, and the amino acids His, Tyr and Glu in the extradiol dioxygenase fingerprint region. These sequences are related to iron ligand binding and catalytic function in bacterial extradiol dioxygenases (*meta* ring-cleavage enzymes). The optimal salt concentration for this enzyme was 2 M KCl or NaCl. This enzyme had a 9.2% excess of acidic over basic amino acids, this is a characteristic feature of halophilic enzymes.

A pathway was proposed for the degradation of the 3-phenylpropionic, cinnamic, benzoic and 3-hydroxybenzoic acids in *Haloferax* sp. D1227 [Fu and Oriel, 1999]. The first part of this pathway involves a pathway similar
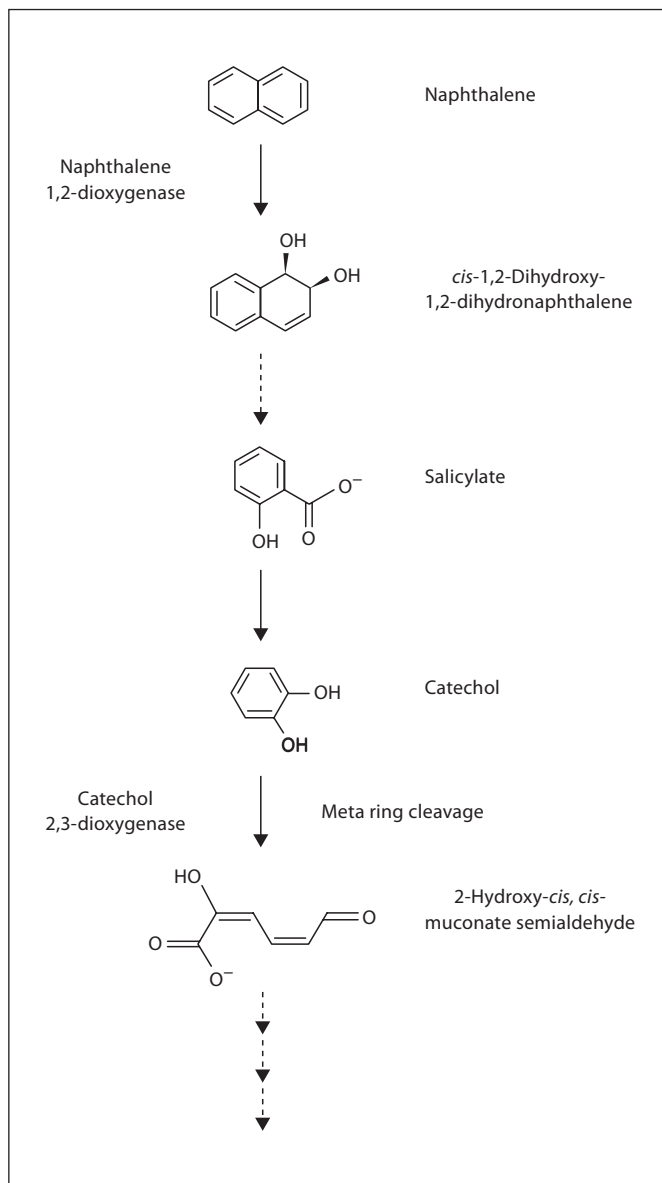
to fatty acids β oxidation in which CoA thioesters are formed (fig. 2a). The 3-phenylpropionic acid is metabolized by a 2-carbon consecutive cleavage to produce benzoylCoA. Each carbon is removed by coenzyme A as acetyl CoA. BenzoylCoA is further degraded through the gentisate pathway. The upper aliphatic pathway is induced by 3-phenylpropionic and cinnamic acid but not by benzoic acid. The lower gentisate pathway from benzoic to gentisic acid is induced by benzoate. Recently, Fairley et al. [2006] found that a gentisate 1,2-dioxygen-

Fig. 5. Pathway for the degradation of naphthalene.
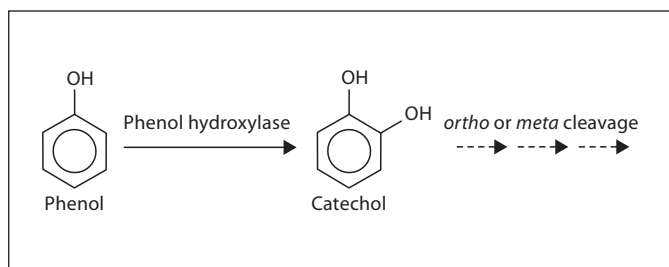
Fig. 6. Transformation of phenol into catechol by phenol hydroxylase.

ase gene (*gdoA*) was expressed during growth of *Haloferax* sp. D1227 on benzoate, 3-hydroxybenzoate, cinnamate, and 3-phenylpropionate. Both genes, encoding a putative CoA-synthetase subunit (*acdB*) and a CoA-thioesterase (*tieA*), were expressed during growth on benzoate, cinnamate, and 3-phenylpropionate but not on 3-hydroxybenzoate. These findings are consistent with the above proposed pathway. A *gdoA* gene was also detected in *Haloarcula* sp. strain D1, a halophilic archaeal isolate able to degrade 4-hydroxybenzoic acid (4HBA) as mentioned before [Fairley et al., 2002, 2006]. In this strain, the *gdoA* gene was expressed during growth on 4HBA but not on benzoate. However, gentisate 1,2-dioxygenase activity was detected on this substrate, suggesting that a second and independently regulated *gdoA* gene, with low homology with the *gdoA* gene expressed on 4HBA, might be present in this strain for the degradation of benzoate. No growth was detected on 3-phenylpropionate, cinnamate or 3-hydroxybenzoate. The *acdB* and *tieA* genes were not expressed during growth of this strain on benzoate, suggesting different pathways for the catabolism of aromatic acids in the two archaea *Haloarcula* and *Haloferax*. The *Haloarcula* sp. strain D1 was also shown to metabolize 4HBA via gentisate and not protocatechuate or catechol. The first step of 4HBA degradation did not involve the formation of CoA thioesters but a hydroxylation-induced intramolecular migration of the carboxyl group to form gentisic acid (fig. 2b) [Fairley et al., 2002]. This unusual pathway had been previously reported in *Bacillus stearothermophilus* [Keenan and Chapman, 1978].

Phenol Degradation by Moderate Halophilic Bacteria. Aerobic halophilic bacteria degrade phenol mainly by the *meta* and *ortho* cleavage pathways (fig. 4). Some bacteria degrade phenol use one of these two pathways, while others may degrade phenol using both pathways [Jiang et al., 2006]. The first step in the pathway, the conversion of phenol to catechol, is catalyzed by a multicomponent phenol hydroxylase (fig. 6) [Nordlund et al., 1990]. The accumulation of catechol and *cis,cis*-muconic acid, intermediates of phenol degradation through the *ortho*-cleavage pathway, has been observed during the growth of several *Halomonas* strains on phenol, indicating that these bacteria used the *ortho* pathway [Alva and Peyton, 2003; Hinteregger and Schreiber, 1997; Maskow and Kleinsteuber, 2004]. This is also the case for *Halomonas organivorans*, a moderate halophile able to degrade phenol and several aromatic acids that showed high catechol 1,2-dioxygenase activity, also indicating the *ortho* pathway [García et al., 2005a]. Interestingly, the haloalkaliphilic *Halomonas* sp. strain EF11 assimilated phenol through both the *ortho* and the *meta* pathways depending on the C/N ratio in the culture medium [Maskow and Kleinsteuber, 2004].

2,4-Dichlorophenoxyacetic Acid Degradation by Moderate Halophilic Bacteria. In nonhalophilic bacteria, catabolism of the herbicide 2,4-D involves enzymes encoded in the broad host range conjugative pJP4 plasmid. The chlorocatechol oxidative operon consisting of the *tfdCDEF* genes is encoded by this plasmid; it converts 3,5-dichlorocatechol to chloromaleylacetate; this compound is then dechlorinated and incorporated into the oxoadipate pathway (β-oxidation degradation) (fig. 7) [Perkins et al., 1990]. The moderately halophilic *Halomonas* sp. strain I-18 degraded the herbicide 2,4-D by using the same pathway as most nonextremophilic 2,4-D-degrading bacteria. Comparison of partial sequences of the *tfdA* gene (encoding a 2,4-D-α-ketoglutaric acid oxygenase) from this isolate with those of nonextremophilic bacteria suggested a common genetic origin of the 2,4-D degradation pathway [Maltseva et al., 1996]. The plasmid pJP4 was successfully used in conjugation experiments with mixed cultures enriched from water and sediment samples from an alkaline pond. One transconjugant, the alkaliphilic moderately halophilic *Halomonas* strain EF43, stably maintained the plasmid and degraded 2,4-D, but it could not use this compound as the sole source of carbon and energy [Kleinsteuber et al., 2001]. So, the nonextremophilic genes encoding 2,4-D degradation carried by the pJP4 plasmid were expressed in *Halomonas* and the plasmid-encoded 2,4-D degradative enzymes were active in this bacteria. This is not surprising since moderately halophilic organisms maintain a non-extreme low salt concentration in their cytoplasm in which nonextremophilic enzymes can be active; these bacteria could eventually be the hosts for degradative pathways from nonextremophilic organisms.

Final Remarks

Doubtless, saline and hypersaline ecosystems are very important because of both surface extension and natural equilibria. Over the last decades, fundamentally important contributions to our understanding of the ecology of complex microbial in saline and hypersaline communities have been made. Among these contributions are detailed studies of carbon, oxygen, nitrogen, and sulfur biogeochemical cycling, measurements of trace gas production and consumption, measurements of light penetration and photosynthesis, and surveys of the overwhelming diversity of microorganisms, as determined using molecular biological methods.

As all ecosystems on Earth, saline and hypersaline environments are submitted to pollution. Due to their reduced microbial diversity when compared with nonextreme environments, pollutant degradation becomes critical in these environments. Available information in the literature on the microbial degradation of xenobiotics and contaminants in these ecosystems is scarce. This work demonstrates that halotolerant and halophilic microorganisms are able to metabolize some pollutants. Nevertheless, significant research efforts are still necessary in order to estimate the potential of these natural systems to recover from pollution. These efforts should also be focused on basic research to understand the overall degradation mechanisms and to identify the enzymes involved in the degradation process and metabolism regulation.

From the application point of view, scientists have faith in their search for extremophiles, the bacteria that live in harsh environments. Some bacteria from extreme environments probably have enzymes that can direct novel chemical reactions. In addition, enzymes from halophilic bacteria could have several potential advantages. The first is obvious: a number of chemical processes require enzymes to act, or retain their activity, at high salt concentration.

The main challenge to use enzymes in industry has been lack of stability. Enzymes are nontoxic, biodegradable, and great catalysts, but many of them are too unstable under industrial process conditions. Halophilic enzymes could be more stable in some process conditions. Even if they are to be used at a low salt concentration, they may last far longer than regular enzymes. They may also resist the destructive effects of organic chemicals used in other steps of the industrial processes.
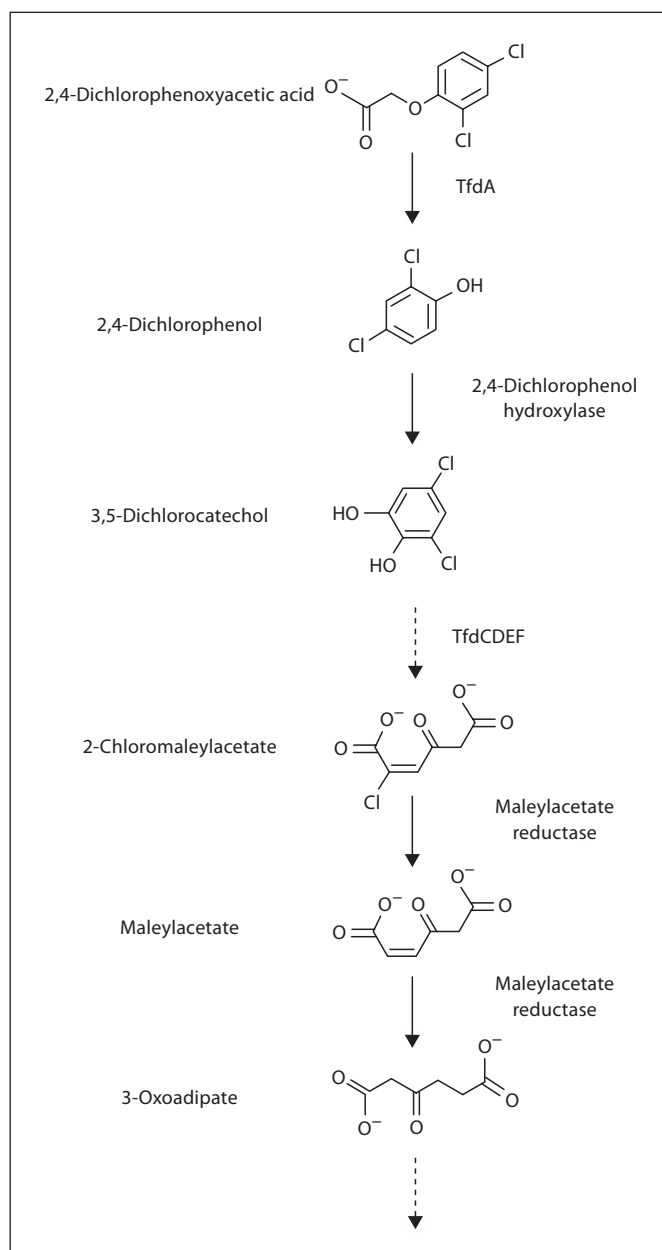
Fig. 7. Pathway for the degradation of 2,4-dichlorophenoxyacetic acid. TfdA is the 2,4-D:alpha-ketoglutaric acid dioxygenase. TfdCDEF are enzymes of the chlorocatechol degradation pathway.

Finally, microbes and enzymatic systems from mesophilic environments are unable to efficiently perform pollutant degradation at high salt concentration, as in the case of the treatment of saline effluents on conventional wastewater treatment plants. Thus, new bioremediation and biotreatment processes should be designed for resto-

ration of contaminated saline and hypersaline ecosystems and saline industrial effluents. To reach this goal, the understanding of the metabolic mechanisms involved in the transformation of pollutants by halotolerant and halophilic bacteria is imperative.

References

Abdelkafi S, Chambkha M, Casalot L, Sayadi S, Labat M: Isolation and characterization of a novel *Bacillus* sp., strain YAS1, capable of transforming tyrosol under hypersaline conditions. FEMS Microbiol Lett 2005;252: 79–84.

Abdelkafi S, Sayadi S, Ben Ali Gam Z, Casalot L, Labat M: Bioconversion of ferulic acid to vanillic acid by *Halomonas elongata* isolated from table-olive fermentation. FEMS Microbiol Lett 2006;262:115–120.

Abed RMM, Al-Thukair A, de Beer D: Bacterial diversity of a cyanobacterial mat degrading petroleum compounds at elevated salinities and temperatures. FEMS Microbiol Lett 2006;57:290–301.

Alva V, Peyton BM: Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: influence of pH and salinity. Environ Sci Technol 2003;37:4397–4402.

Asad S, Amoozegar MA, Pourbabaee AA, Sarbolouki MN, Dastgheib SM: Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresour Technol 2007;98:2082–2088.

Atlas RM, Bartha R: Degradation and mineralization of petroleum in seawater: limitation by nitrogen and phosphorous. Biotechnol Bioeng 1972;14:309–317.

Azachi M, Henis Y, Shapira R, Oren A: The role of the outer membrane in formaldehyde tolerance in *Escherichia coli* VU3695 and *Halomonas* sp. MAC. Microbiology 1996; 142:1249–1254.

Bertrand JC, Almallah M, Acquaviva M, Mille G: Biodegradation of hydrocarbons by an extremely halophilic archaebacterium. Lett Appl Microbiol 1990;11:260–263.

Brito EM, Guyoneaud R, Goni-Urriza M, Ranchou-Peyruse A, Verbaere A, Crapez MA, Wasserman JC, Duran R: Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. Res Microbiol 2006;157:752–762.

Bromley-Challenor KCA, Caggiano N, Knapps JS: Bacterial growth on N,N-dimethylformamide: implications for the treatment of industrial wastewaters. J Ind Microbiol Biotechnol 2000;25:8–16.

Cohen Y: Bioremediation of oil by marine microbial mats. Int Microbiol 2002;5:189–193.

Colwell RR: Ecological aspects of microbial degradation of petroleum in the marine environment. CRC Crit Rev Microbiol 1977;5:423–445.

Cuadros-Orellana S, Pohlschröder M, Durrant LR: Isolation and characterization of halophilic archaea able to grow in aromatic compounds. Int Biodeter Biodegradation 2006; 57:151–154.

DasSarma S, Arora P: Halophiles; in Encyclopedia of Life Sciences. London, Nature Publishing Group, 2002, vol 8, pp 458–466.

DeFrank JJ, Beaudry WT, Cheng TC, Harvey SP, Stroup AN, Szafraniec LL: Screening of halophilic bacteria and *Alteromonas* species for organophosphorus hydrolyzing enzyme activity. Chem Biol Interact 1993;87:141–148.

DeFrank JJ, Cheng TC: Purification and properties of an organophosphorus acid anhydrase from a halophilic bacterial isolate J Bacteriol 1991;173:1938–1943.

Del Moral A, Prado B, Quesada E, García T, Ferrer R, Ramos-Cormenzana A: Numerical taxonomy of moderately halophilic Gram-negative rods from an inland saltern. J Gen Microbiol 1988;134:733–741.

Deppe U, Richnow HH, Michaelis W, Antranikian G: Degradation of crude oil by an arctic microbial consortium. Extremophiles 2005; 9:461–470.

Díaz MP, Boyd KG, Grigson SJW, Burgess JG: Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. Biotechnol Bioeng 2002;79:145–153.

Díaz MP, Grigson SJW, Peppiatt CJ, Burgess JG: Isolation and characterization of novel hydrocarbon-degrading euryhaline consortia from crude oil and mangrove sediments. Mar Biotechnol 2000;2:522–532.

Eaton RW, Chapman PJ: Bacterial metabolism of naphthalene: construction and use of recombinant bacteria to study ring cleavage of 1,2-dihydroxynaphthalene and subsequent reactions. J Bacteriol 1992;174:7542–7554.

Emerson D, Breznak JA: The response of microbial populations from oil-brine contaminated soil to gradient of NaCl and sodium *p*-toluate in a diffusion gradient chamber. FEMS Microbiol Ecol 1997;23:285–300.

Emerson D, Chauchan S, Oriel P, Breznak JA: *Haloferax* sp. D1227, a halophilic archaeon capable of growth on aromatic compounds. Arch Microbiol 1994;161:445–452.

Fairley DJ, Boyd DR, Sharma ND, Allen CC, Morgan P, Larkin MJ: Aerobic metabolism of 4-hydroxybenzoic acid in *Archaea* via an unusual pathway involving an intramolecular migration (NIH shift). Appl Environ Microbiol 2002;68:6246–6255.

Fairley DJ, Wang G, Rensing C, Pepper IL, Larkin MJ: Expression of gentisate 1,2-dioxygenase (*gdoA*) genes involved in aromatic degradation in two haloarchaeal genera. Appl Microbiol Biotechnol 2006;73:691–695.

Field JA, Stams AJ, Kato M, Schraa G: Enhanced biodegradation of aromatic pollutants in co-cultures of anaerobic and aerobic bacterial consortia. Antonie Van Leeuwenhoek 1995; 67:47–77.

Fu W, Oriel P: Gentisate 1,2-dioxygenase from *Haloferax* sp. D1227. Extremophiles 1998;2: 439–436.

Fu W, Oriel P: Degradation of 3-phenylpropionic acid by *Haloferax* sp. D1227. Extremophiles 1999;3:45–53.

Fulthorpe RR, McGowan C, Maltseva OV, Holben WE, Tiedje JM: 2,4-Dichlorophenoxyacetic acid-degrading bacteria contain mosaics of catabolic genes. Appl Environ Microbiol 1995;61:3274–3281.

Fuse H: Oxidation of organic compounds by bacteria. Patent JP10128385. 1998.

García MT, Gallego V, Ventosa A, Mellado E: *Thalassobacillus devorans* gen. nov., sp. nov., a moderately halophilic, phenol-degrading, Gram-positive bacterium. Int J Sys Evol Microbiol 2005b;55:1789–1795.

Garcia MT, Mellado E, Ostos JC, Ventosa A: *Halomonas organivorans* sp. nov., a moderate halophile able to degrade aromatic compounds. Int J Syst Evol Microbiol 2004;54: 1723–1728.

García MT, Ventosa A, Mellado E: Catabolic versatility of aromatic compound-degrading halophilic bacteria. FEMS Microbiol Ecol 2005a;54:97–109.

García MT, Ventosa A, Ruiz-Berraquero F, Kocur M: Taxonomic study and amended description of *Vibrio costicola*. Int J Syst Bacteriol 1987;37:352–360.

Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, Bertrand JC: *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. Int J Syst Bacteriol 1992;42:568–576.

Gorshkova NM, Ivanova EP, Sergeev AF, Zhukova NV, Alexeeva Y, Wright JP, Nicolau DV, Mikhailov VV, Christen R: *Marinobacter excellens* sp. nov., isolated from sediments of the Sea of Japan. Int J Syst Evol Microbiol 2003;53:2073–2078.

Grant WD, Gemmel RT, McGenity TJ: Halophiles; in Horikoshi K, Grant W (eds): Extremophiles: Microbial Life in Extreme Environments. New York, Wiley-Liss, 1998, pp 93–132.

Green DH, Bowman JP, Smith EA, Gutierrez T, Bolch CJ: *Marinobacter algicola* sp. nov., isolated from laboratory cultures of paralytic shellfish toxin-producing dinoflagellates. Int J Syst Evol Microbiol 2006;56:523–527.

Grötzschel S, Köster J, Abed RMM, Beer D: Degradation of petroleum model compounds immobilized on clay by a hypersaline microbial mat. Biodegradation 2002;13:273–283.

Gu J, Cai H, Yu SL, Qu R, Yin B, Guo YF, Zhao JY, Wu XL: *Marinobacter gudaonensis* sp. nov., isolated from an oil-polluted saline soil in a Chinese oilfield. Int J Syst Evol Microbiol 2007;57:250–254.

Guo J, Zhou J, Wang D, Tian C, Wang P, Uddin MS: A novel moderately halophilic bacterium for decolorizing azo dye under high salt condition. Biodegradation 2008;19:15–19.

Harayama S, Kasai Y, Hara A: Microbial communities in oil-contaminated seawater. Curr Opin Biotechnol 2004;15:205–214.

Harayama S, Kishira H, Kasai Y, Shutsubo K: Petroleum biodegradation in marine environments. J Mol Microbiol Biotechnol 1999;1:63–70.

Hayes VE, Ternan NG, McMullan G: Organophosphonate metabolism by a moderately halophilic bacterial isolate. FEMS Microbiol Lett 2000;186:171–175.

Head IM, Jones DM, Roling WF: Marine microorganisms make a meal of oil. Nat Rev Microbiol 2006;4:173–182.

Head IM, Swannell RP: Bioremediation of petroleum hydrocarbon contaminants in marine habitats. Curr Opin Biotechnol 1999;10:234–239.

Hebert AM, Vreeland RH: Phenotypic comparison of halotolerant bacteria: *Halomonas halodurans* sp. nov., nom. rev., comb. nov. Int J Syst Bacteriol 1987;37:347–350.

Hedlund BP, Geiselbrecht AD, Staley JT: *Marinobacter* strain NCE312 has a *Pseudomonas*-like naphthalene dioxygenase. FEMS Microbiol Lett 2001;201:47–51.

Hinteregger C, Streischsberg F: *Halomonas* sp., a moderately halophilic strain, for biotreatment of saline phenolic waste-water. Biotechnol Lett 1997;19:1099–1102.

Huu NB, Denner EB, Ha DT, Wanner G, Stan-Lotter H: *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. Int J Syst Bacteriol 1999;49:367–375.

Ivanova EP, Kurilenko VV, Kurilenko AV, Gorshkova NM, Shubin FN, Nicolau DV, Chelomin VP: Tolerance to cadmium of free-living and associated with marine animals and eelgrass marine gamma-proteobacteria. Curr Microbiol 2002;44:357–362.

Jiang HL, Tay ST, Maszenan AM, Tay JH: Physiological traits of bacterial strains isolated from phenol-degrading aerobic granules. FEMS Microbiol Ecol 2006;57:182–191.

Jones BE, Grant WD, Duckworth AW, Owenson GG: Microbial diversity of soda lakes. Extremophiles 1998;2:191–200.

Kamekura M: Diversity of extremely halophilic bacteria. Extremophiles 1998;2:289–295.

Kargi F, Dincer AR: Biological treatment of saline wastewater by fed-batch operation. J Chem Tech Biotechnol 1997;69:167–172.

Keenan SL, Chapman PJ: Carboxy-migration facilitated by bacterial hydroxylation of 4-hydroxybenzoate. J Chem Soc Chem Commun 1978;17:731–732.

Kerr RP, Capone, DG: The effect of salinity on the microbial mineralization of two polycyclic aromatic hydrocarbons in estuarine sediments. Marine Environ Res 1988;26:181–198.

Khmelina VN, Kalyuzhnaya MG, Starostina NG, Suzina NE, Trotsenko YA: Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes. Curr Microbiol 1997;35:257–261.

Kim SG, Bae HS, Oh HM, Lee ST: Isolation and characterization of novel halotolerant and/or halophilic denitrifying bacteria with versatile metabolic pathways for the degradation of trimethylamine. FEMS Microbiol Lett 2003;225:263–269.

Kim BY, Weon HY, Yoo SH, Kim JS, Kwon SW, Stackebrandt E, Go SJ: *Marinobacter koreensis* sp. nov., isolated from sea sand in Korea. Int J Syst Evol Microbiol 2006;56:2653–2656.

Kleinsteuber S, Muller RH, Babel W: Expression of the 2,4-D degradative pathway of pJP4 in an alkaliphilic, moderately halophilic soda lake isolate, *Halomonas* sp. EF43. Extremophiles 2001;5:375–384.

Kleinsteuber S, Riis V, Fetzer I, Harms H, Müller S: Population dynamics within a microbial consortium during growth on diesel fuel in saline environments. Appl Environ Microbiol 2006;72:3531–3542.

Kulichevskaya IS, Milekhina EI, Borzenkov IA, Zvyagintseva IS, Belyaev SS: Oxidation of petroleum hydrocarbons by extremely halophilic archaebacteria. Microbiology 1992;60:596–601.

Kuznetsov VD, Zaitseva TA, Vakulenko LV, Filippova SN: *Streptomyces albiaxialis sp.* Nov. A new petroleum hydrocarbon degrading species of thermo and halotolerant *Streptomyces*. Microbiology 1992;61:62–67.

Lefebvre O: Application des micro-organismes halophiles au traitement des effluents industriels hypersalins; PhD thesis. Ecole Nationale Supérieure Agronomique de Montpellier, 2004.

Lefebvre O, Moletta R: Treatment of organic pollution in industrial saline wastewater: a literature review. Water Res 2006;40:3671–3682.

Litchfield CD: Survival strategies for microorganisms in hypersaline environments and their relevance to life on early Mars. Meteorit Planet Sci 1998;33:813–819.

Maltseva O, McGowan C, Fulthorpe R, Oriel P: Degradation of 2,4-dichlorophenoxyacetic acid by haloalkaliphilic bacteria. Microbiology 1996;142:1115–1122.

Maltseva O, Oriel P: Monitoring of an alkaline 2,4,6-trichlorophenol-degrading enrichment culture by DNA fingerprinting methods and isolation of the responsible organism, haloalkaliphilic *Nocardioides* sp. strain M6. Appl Environ Microbiol 1997;63:4145–4149.

Margesin R, Schinner F: Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl Microbiol Biotechnol 2001a;56:650–663.

Margesin R, Schinner R: Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles 2001b;5:73–83.

Marhuenda-Egea FC, Piera-Velazquez S, Cadenas C, Cadenas E: Mechanism of adaptation of an atypical alkaline *p*-nitrophenyl phosphatase from the archaeon *Halobacterium salinarum* at low-water environments. Biotechnol Bioeng 2002;78:497–502.

Marquez MC, Ventosa A: *Marinobacter hydrocarbonoclasticus* Gauthier et al. 1992 and *Marinobacter aquaeolei* Nguyen et al. 1999 are heterotypic synonyms. Int J Syst Evol Microbiol 2005;55:1349–1351.

Martinez-Checa F, Toledo FL, Vichez R, Quesada E, Calvo C: Yield production, chemical composition, and functional properties of emulsifie H28 synthesized by *Halomonas eurialina* strain H-28 in media containing various hydrocarbons. 2002;58:358–363.

Maskow T, Kleinsteuber S; Carbon and energy fluxes during haloadaptation of *Halomonas* sp. EF11 growing on phenol. Extremophiles 2004;8:133–141.

Mathrani IM, Boone DR, Mah RA, Fox GE, Lau PP: *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int J Syst Bacteriol 1988;38:139–142.

Nicholson CA, Fathepure BZ: Biodegradation of benzene by halophilic and halotolerant bacteria under aerobic conditions. Appl Environ Microbiol 2004;70:1222–1225.

Nicholson CA, Fathepure BZ: Aerobic biodegradation of benzene and toluene under hypersaline conditions at the Great Salt Plains, Oklahoma. FEMS Microbiol Lett 2005;245:257–262.

Nordlund I, Powlowski J, Shingler V: Complete nucleotide sequence and polypeptide analysis of multicomponent phenol hydroxylase from *Pseudomonas* sp. strain CF600. J Bacteriol 1990;172:6826–6833.

Oesterhelt D, Patzelt H, Kesler B: Decomposition of halogenated hydrocarbons by halophilic bacteria. Patent DE19639894. 1998.

Oren A: Intracellular salt concentrations of the anaerobic halophilic eubacteria *Haloanaerobium praevalens* and *Halobacteroides halobius*. Can J Microbiol. 1986;32:4–9.

Oren A: Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 1999;63:334–348.

Oren A: Halophilic Microorganisms and Their Environments. Amsterdam, Kluwer Academic Publishers, 2002a.

Oren A: Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. J Ind Microbiol Biotechnol 2002b;28:56–63.

Oren A, Gurevich P, Azachi M, Hents Y: Microbial degradation of pollutants at high salt concentrations. Biodegradation 1992;3:387–398.

Oriel P, Chauhan S, Maltseva O, Fu W: Degradation of aromatics and haloaromatics by halophilic bacteria, in Horikoshi K, Fukuda M, Kudo T (eds): Microbial Diversity and Genetics of Biodegradation. Tokyo, Japan Scientific Societies Press, 1997, pp 123–130.

Perkins EJ, Gordon MP, Caceres O, Lurquin PF: Organization and sequence analysis of the 2,4-dichlorophenol hydroxylase and dichlorocatechol oxidative operons of plasmid pJP4. J Bacteriol 1990;172:2351–2359.

Pernetti M, Di Palma L: Experimental evaluation of inhibition effects of saline wastewater on activated sludge. Environ Technol 2005;26:695–703.

Piedad Diaz M, Grigson SJ, Peppiatt CJ, Burgess JG: Isolation and characterization of novel hydrocarbon-degrading euryhaline consortia from crude oil and mangrove sediments. Mar Biotechnol 2000;2:522–532.

Pieper D, Reineke W: Engineering bacteria for bioremediation. Curr Opin Biotechnol 2000;11:262–270.

Purdy KJ, Cresswell-Maynard TD, Nedwell DB, McGenity TJ, Grant WD, Timmis KN, Embley TM: Isolation of haloarchaea that grow at low salinities. Environ Microbiol 2004;6:591–595.

Razo-Flores E, Donlon B, Lettinga G, Field JA: Biotransformation and biodegradation of N-substituted aromatics in methanogenic granular sludge. FEMS Microbiol Rev 1997;20:525–538.

Riis V, Kleinsteuber S, Babel W: Influence of high salinities on the degradation of diesel fuel by bacteria consortia. Can J Microbiol 2003;49:713–721.

Robertson DE, Lai MC, Gunsalus RP, Roberts MF: Composition, variation, and dynamics of major osmotic solutes in *Methanohalophilus s*train FDF1. Appl Environ Microbiol 1992;58:2438–2443.

Romanenko LA, Schumann P, Rohde M, Zhukova NV, Mikhailov VV, Stackebrandt E: *Marinobacter bryozoorum* sp. nov. and *Marinobacter sediminum* sp. nov., novel bacteria from the marine environment. Int J Syst Evol Microbiol 2005;55:143–148.

Rontani JF, Gilewicz MJ, Michotey VD, Zheng TL, Bonin PC, Bertrand JC: Aerobic and anaerobic metabolism of 6,10,14-trimethylpentadecan-2-one by a denitrifying bacterium isolated from marine sediments. Appl Environ Microbiol 1997;63:636–643.

Rontani JF, Mouzdahir A, Michotey V, Caumette P, Bonin P: Production of a polyunsaturated isoprenoid wax ester during aerobic metabolism of squalene by *Marinobacter squalenivorans* sp. nov. Appl Environ Microbiol 2003;69:4167–4176.

Rosenberg A: *Pseudomonas halodurans* sp. nov., a halotolerant bacterium. Arch Microbiol 1983;136:117–123.

Rothschild LJ, Mancinelli RL: Life in extreme environments. Nature 2001;409:1092–1101.

Sette LD, Simioni KC, Vasconcellos SP, Dussan LJ, Neto EV, Oliveira VM: Analysis of the composition of bacterial communities in oil reservoirs from a southern offshore Brazilian basin. Antonie Van Leeuwenhoek 2007;91:253–266.

Shieh WY, Jean WD, Lin YT, Tseng M: *Marinobacter lutaoensis* sp. nov., a thermotolerant marine bacterium isolated from a coastal hot spring in Lutao, Taiwan. Can J Microbiol 2003;49:244–252.

Shivaji S, Gupta P, Chaturvedi P, Suresh K, Delille D: *Marinobacter maritimus* sp. nov., a psychrotolerant strain isolated from sea water off the subantarctic Kerguelen islands. Int J Syst Evol Microbiol 2005;55:1453–1456.

Singh1 BK, Walker A: Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 2006;30:428–471.

Sørensen SR, Arbeli Z, Aamand J, Ronen Z: Metabolism of diphenylurea by a *Marinobacter* sp. isolated from a contaminated ephemeral stream bed in the Negev Desert. FEMS Microbiol Lett 2002;213:199–204.

Sørensen SR, Bending GD, Jacobsen CS, Walker A, Aamand J: Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields. FEMS Microbiol Ecol 2003;45:1–11.

Sorokin DY, Jones BE, Kuenen JG: An obligate methylotrophic, methane-oxidizing *Methylomicrobium* species from a highly alkaline environment. Extremophiles 2000;4:145–155.

Sorokin DY, Tourova TP, Antipov AN, Muyzer G, Kuenen JG: Anaerobic growth of the haloalkaliphilic denitrifying sulfur-oxidizing bacterium *Thialkalivibrio thiocyanodenitrificans* sp. nov. with thiocyanate. Microbiology 2004;150:2435–2442.

Sorokin DY, Tourova TP, Bezsoudnova EY, Pol A, Muyzer G: Denitrification in a binary culture and thiocyanate metabolism in *Thiohalophilus thiocyanoxidans* gen. nov. sp. nov. – a moderately halophilic chemolithoautotrophic sulfur-oxidizing Gammaproteobacterium from hypersaline lakes. Arch Microbiol 2007b;187:441–450.

Sorokin DY, Tourova TP, Lysenko AM, Kuenen JG: Microbial thiocyanate utilization under highly alkaline conditions. Appl Environ Microbiol 2001;67:528–538.

Sorokin DY, Tourova TP, Lysenko AM, Mityushina LL, Kuenen JG: *Thioalkalivibrio thiocyanoxidans* sp. nov. and *Thioalkalivibrio paradoxus* sp. nov., novel alkaliphilic, obligately autotrophic, sulfur-oxidizing bacteria capable of growth on thiocyanate, from soda lakes. Int J Syst Evol Microbiol 2002;52:657–664.

Sorokin DY, van Pelt S, Tourova TP, Takaichi S, Muyzer G: Acetonitrile degradation under haloalkaline conditions by *Natronocella acetinitrilica* gen. nov., sp. nov. Microbiology 2007a;153:1157–1164.

Speight JG: The Chemistry and Technology of Petroleum, ed 3. New York, Marcel Dekker, 1998, pp 1–202.

Takai K, Moyer CL, Miyazaki M, Nogi Y, Hirayama H, Nealson KH, Horikoshi K *Marinobacter alkaliphilus* sp. nov., a novel alkaliphilic bacterium isolated from subseafloor alkaline serpentine mud from Ocean Drilling Program Site 1200 at South Chamorro Seamount, Mariana Forearc. Extremophiles 2005;9:17–27

van den Bosch PL, van Beusekom OC, Buisman CJ, Janssen AJ: Sulfide oxidation under haloalkaline conditions in a fed-batch bioreactor. Biotechnol Bioeng 2007;97:1053–1063.

Ventosa A, Carmen Marquez M, Garabito MJ, Arahal DR: Moderately halophilic grampositive bacterial diversity in hypersaline environments. Extremophiles 1998a;2:297–304.

Ventosa A, Nieto JJ, Oren A: Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 1998b;62:504–544.

Ventosa A, Quesada E, Rodriguez-Valera F, Ruiz-Berraquero F, Ramos-Cormenzana A: Numerical taxonomy of moderately halophilic gram-negative rods. J Gen Microbiol 1982;128:1959–1968.

Ward DM, and Brock TD: Hydrocarbon biodegradation in hypersaline enviroments. Appl Environ Microbiol 1978;35:353–359.

Woolard CR, Irvine RL: Biological treatment of hypersaline wastewater by a biofilm of halophilic bacteria. Water Environ Res 1994;66:230–235.

Woolard CR, Irvine RL: Treatment of hypersaline wastewater in the sequencing batch reactor. Water Res 1995;29:1159–1168.

Yakimov MM, Denaro R, Genovese M, Cappello S, D'Auria G, Chernikova TN, Timmis KN, Golyshin PN, Giluliano L: Natural microbial diversity in superficial sediments of Milazzo Harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons. Environ Microbiol 2005;7:1426–1441.

Yang L, Lai C, Shieh WK: Biodegradation of dispersed diesel fuel under high salinity conditions. Wat Res 2000;34:3303–3314.

Yoon JH, Shin DY, Kim IG, Kang KH, Park YH: *Marinobacter litoralis* sp. nov., a moderately halophilic bacterium isolated from sea water from the East Sea in Korea. Int J Syst Evol Microbiol 2003;53:563–568.

Yoon JH, Yeo SH, Kim IG, Oh TK: *Marinobacter flavimaris* sp. nov. and *Marinobacter daepoensis* sp. nov., slightly halophilic organisms isolated from sea water of the Yellow Sea in Korea. Int J Syst Evol Microbiol 2004;54:1799–1803.

Zvyagintseva IS, Poglasova MN, Gotoeva MT, Belyaev SS: Effect of the medium salinity on oil degradation by nocardioform bacteria. Microbiology 2001;70:652–656.