The genus *Sphingomonas*: physiology and ecology

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Exploitation of the metabolic capabilities of the genus *Sphingomonas* could provide important commercial benefits to biotechnology. Recent advances have demonstrated that these organisms have unique abilities to degrade refractory contaminants, to serve as bacterial antagonists to phytopathogenic fungi, and to secrete the highly useful gellan exopolysaccharides. Unfortunately, *Sphingomonas* are also animal pathogens and can readily degrade the copper pipes in drinking water distribution systems. The closely related *Zymomonas* could be important for commercial ethanol production. These Gram-negative aerobic bacteria are characterized by an outer membrane that contains glycosphingolipids, but lacks lipopolysaccharide. Their distribution in environmental samples has not been systematically examined as yet.

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Abbreviation
2,4-D 2,4-dichlorophenoxyacetic acid

Introduction

On the basis 16S rRNA sequence homology, the genus *Sphingomonas* forms a phylogenetically tight group in the α-4 subclass of the Proteobacteria [1••]. The type species, *Sphingomonas paucimobilis*, is a Gram-negative, aerobic, single polar flagellated, yellow-pigmented bacterium that masqueraded for years as *Pseudomonas* [2]. Some *Sphingomonas* are non-motile and non-fermentative, but all contain a suite of unusual 'signature' components: 18–21 carbon straight chain saturated dihydrosphingosines, monounsaturated dihydrosphingosines, and cyclopropane-containing dihydrosphingosines in a ceramide glycolipid containing uronic acid and amide-linked 2-hydroxy straight-chain saturated fatty acids. In addition, they contain a long-chain respiratory benzoquinone with a side chain of 10 isoprenoid units (ubiquinone Q-10) [2]. The guanine plus cytosine content of genomic DNA ranges from 61% to 67% [2] and most species contain a nostoxanthin carotenoid pigment [3]. *Sphingomonas* do not contain detectable ester or amide-linked 3-OH fatty acids and lack the lipopolysaccharide components or structures that are characteristic to Gram-negative bacteria. Despite the fact that *Sphingomonas* have membranes with an unusual structure, they are widely distributed in nature and have important properties that can be exploited for use in biotechnology.

In this review, we focus on the biotechnological potential of *Sphingomonas* to cause microbiologically induced corrosion, to cause disease in plants, to produce valuable exopolysaccharide polymers and to biodegrade refractory organic compounds. The phylogenetic relationships, unusual lipid composition and ecology of the genus are also discussed.

Phylogenetic relationships

As stated above, the genus *Sphingomonas* is a homogeneous group of organisms in the α-4 subclass of the Proteobacteria [1••]. A group of organisms that are closely related phylogenetically and have the same profile of unusual lipid components has been isolated from the plant rhizosphere and classified in a separate genus, *Rhizomonas*. These organisms are identical in most phenotypic properties, except that they are plant pathogens [1••,4]. A second group with close phylogenetic relationships and similar lipid structures are the commercially important ethanol-producing *Zymomonas* [5•].

Historically, the formation of 3-ketolactose from lactose has been a useful feature in the identification of biovar 1 in the genus *Agrobacterium*, which is in the α-2 subclass of the Proteobacteria, but recent studies have indicated that *Sphingomonas* species have this trait as well. Taxonomic studies performed on a selection of 3-ketolactose positive strains — including *Agrobacterium rhizogenes*, *Chromobacterium lividum*, and strains recovered from *Prunus persica* and the rhizosphere of apple trees — indicate that they are more closely related to *Sphingomonas* species. These organisms have lipid patterns and 16S rRNA sequences characteristic of the genus, but differ enough in other physiological characteristics to justify the proposal of four new species [6••]. The excretion of 3-ketolactose, as detected by high-pressure liquid chromatography, has been identified in 17 of 21 strains from eight species tested [7••]. The aerobic photosynthetic bacterium *Erythrobacter longus*, which contains bacteriochlorophyll *a*, also contains the unusual lipid constellation and secretes 3-ketolactose [7••].

Microbially influenced corrosion

Localized corrosion of copper cold-water pipes results in surface erosions, covered tubercles, and through-wall pinhole pits on the pipe inner surface. One recent study has investigated the causes of this corrosion in the plumbing

system of a large building [8•]. The localization of the pits appeared to be related to stagnation.

In a test system, heating the water to 64°C resulted in a sharp decrease in copper liberated, oxygen utilization, and bacterial colony forming units in the water system. These findings were not detected in the unheated control. When the temperature was lowered, the bacteria, the copper ions, and the oxygen depletion resumed. Dosing the water system with cefoxitin antibiotic at 30 ng ml^{-1} resulted in a decrease in oxygen utilization, in copper ion concentration, and in microbial population growth, as measured by colony counts on selective agar in the water. Two organisms were consistently isolated from the water samples, *Pseudomonas fluorescens* and a *Sphingomonas* species. Both organisms are able to accumulate copper in their cell wall, and the binding of copper locally facilitates the anodic reactions in microbially influenced corrosion of copper. These organisms can be a significant problem in drinking water distribution systems with copper pipes if the regrowth biofilms contain the copper-binding *Sphingomonas* species.

Interactions with plants

Sphingomonas species are often found in association with plants. Many strains have been isolated from the rhizosphere and, as described above, share the secretion of 3-ketolactose [6••,7••]. The closely related *Rhizomonas* species [1••,4] (e.g. *Rhizomonas suberifaciens)* are significant pathogens of lettuce, causing corky root disease [9]. Amongst other organisms, *S. paucimobilis* has been shown to exhibit antagonism against the phytopathogenic fungus *Verticillium dahliae* [10•]. This is of particular significance as verticillium wilt is widely distributed and affects a number of commercially important plant species.

Exopolymer production

Gellan-related exopolysaccharides, which are produced by certain *Sphingomonas*, consist of a repeating unit of (β1→3) glucose–(β1→4) glucuronic acid–(β1→4) glucose–(β1→4) i-rhamnose/(β1→4) i-mannose, with side groups of i-glycerate, *O*-acetyl groups, monosaccharides or disaccharides, all of which readily form gels after deacylation [11]. These polysaccharides have important food and industrial applications. The exopolysaccharide of *S. paucimobilis* GS1 is 5.5-fold as viscous as xanthan gum and is stable over a pH range of 2–10 (as opposed to a pH range of 4–8 for xanthan gum). It maintains its strength at 90°C, whereas xanthan gum has only 26% of its original viscosity at this temperature [12••]. The exopolysaccharide is stable in solutions of 50 g l^{-1} NaCl and is unaffected by other salts, such as $CaCl_2$, $CoCl_2$, KCl, $MgCl_2$, $ZnCl_2$, and NH_4Cl or NH_4SO_4. The viscosity of this exopolysaccharide is also superior to starch, alginate, carboxymethyl cellulose, and gum arabic. When the polymer is deacylated, it forms a firm gel. It is clear, stiff, and thermoreversible in the presence of the cations Ca^{2+}, Na^+, K^+ and Mg^{2+}, with a gel strength fourfold that of agar. The gellan-like deacylated polymer withstands two cycles of autoclaving. It melts at 90°C and sets at 50°C at a concentration of 10 g l^{-1} in 5 g l^{-1} NaCl [12••].

Recent studies examining the biosynthesis of gellan produced by *Sphingomonas elodea* have provided interesting insights into the glucose and central carbon metabolism of this organism. Vartak *et al.* [13••] have created mutants deficient in the enzyme 6-phosphogluconate dehydrogenase in an attempt to increase the gellan yield by decreasing the proportion of glucose transformation to CO_2. They found that this genetic alteration had no effect on either CO_2 production or gellan yield and, in conjunction with other enzymatic analyses, they showed that *S. elodea* utilizes the Entner–Doudoroff and pentose-phosphate pathways for glucose catabolism [13••].

Several bacteria, both Gram-positive and Gram-negative, have been isolated that form inducible extracellular eliminase-type endoenzymes which cleave the β-D-glucosyl-(1→4)-β-D-glucuronosyl portion of the repeat unit in gellan-like polysaccharides [14•]. These gellan lyases (also described as sphinganases) are effective in degrading the related exopolysaccharides to varying extents. Such enzymes may be useful in forming lower viscosity derivatives of gellan for use in controlling water loss and air entertainment in cement formulations. Alternative uses include removing Gelrite from plant tissue-culture media or application in structural studies of polysaccharides [15•].

Biodegradative activities

One of the most extraordinary features possessed by many members of *Sphingomonas* is the capacity to degrade refractory pollutants. *Sphingomonas* sp. SS3 isolated from contaminated soil in Germany utilizes diphenyl ether and its 4-fluoro, 4-chloro, and (to a lesser extent) 4-bromo derivatives as a sole source of carbon and energy [16]. Other isomeric monohalogenated derivatives are co-metabolized by SS3. The initial step in diphenyl ether degradation involves a 1,2 dioxygenation and subsequent formation of phenol and catechol as intermediates.

The diaryl ethers dibenzo-*p*-dioxin and dibenzofuran are utilized as sole sources of carbon and energy by *Sphingomonas* sp. RW1, which was isolated from the water of the river Elbe [17]. The initial reaction is an oxygenolytic attack at the carbon adjacent to the ether bridge. Dihydrodiols are transient intermediates and the respective trihydroxy compounds, 2,2′,3-trihydroxyldiphenyl ether or 2,2′,3-trihydroxylbiphenyl, are formed subsequently. The trihydroxy compounds then undergo *meta* cleavage and dibenzofuran metabolites are further degraded by the catechol *meta* cleavage pathway and the gentisate pathway. Catechol produced from the metabolism of dibenzo-*p*-dioxin can be either *ortho* or *meta* cleaved by *Sphingomonas* sp. RW1. 16S rRNA sequence analysis has identified this strain as a new species having

a distinct but close relationship to other *Sphingomonas* sp. [18].

Sphingomonas sp. HH69, isolated from soil, mineralizes 2-acetoxydibenzofuran, 3-acetoxydibenzofuran, and 4-acetoxydibenzofuran, and 2-hydroxydibenzofuran, 3-hydroxydibenzofuran, and 4-hydroxydibenzofuran [19••]. The strain degrades 2-methoxydibenzofuran after adaptation to 5-methoxysalicylic acid. The 3-methoxydibenzofuran and 4-methoxydibenzofuran are co-oxidized. Studies with this organism indicate significant regiospecificity in the dioxygenolytic cleavage of the ether bond.

Sphingomonas macrogoltabidus has been shown to degrade polyethylene glycol (PEG-4000), and a biculture of several types of bacteria and *Sphingomonas terrae* degraded PEG 6000 [20]. The function of the non-*Sphingomonas* component of the biculture was to degrade an inhibitor, glyoxylate.

Several *Sphingomonas* isolates recovered from the Middendorf formation at depths 180–410 m below the surface at a site in the Savannah River (Aiken, South Carolina, in the South-eastern USA coastal plains) were able to degrade toluene, naphthalene, *o*-xylene, *m*-xylene, *p*-xylene, *p*-cresol, salicylate and benzoate [21••]. Most of the strains could produce zones of clearing around colonies grown on agar plates sprayed with fluorene, biphenyl, and dibenzothiophene on agar. Mineralization of ^{14}C-labeled compounds showed that from 6% to 17% of the toluene and from 40% to 69% of the naphthalene was converted to CO_2 within 48 h by five subsurface isolates. Neither authentic *Sphingomonas capsulata*, nor *S. paucimobilis*, nor one subsurface isolate were able to mineralize these compounds. The ability to metabolize both toluene and naphthalene is rare amongst Gram-negative aerobic heterotrophic bacteria and important in bioremediation of subsurface petroleum contamination. The sequence homology for the 16S rRNA of these subsurface sediment isolates showed that they form a distinct cluster most closely related to *S. capsulata*. The lipids of the subsurface isolates contain a smaller proportion of 2-hydroxy (14:0) and *cis* vaccenic acid and a 10-fold higher proportion of 2-hydroxy (15:0). In general, they also have lower amounts of (18:0) dihydrosphingosine and cyclopropane (21:0) sphinganine than *S. capsulata* and *S. paucimobilis*. In addition, they contain a cyclopropane (20:0) sphinganine not found in the two *Sphingomonas* species isolated from the surface [21••]. Many of the subsurface *Sphingomonas* strains also harbor megaplasmids. *Sphingomonas* strain F199 was shown to contain a supercoiled 180 kb plasmid with catechol 2,3-dioxygenase genes linked to two distinct regions of the plasmid [22••]. Therefore, at least some of the aromatic catabolic activity of these subsurface *Sphingomonas* spp. is encoded on plasmids, and this feature deserves further investigation.

Ecological localization

Sphingomonas have been isolated from hospital water supplies, respirators, stocked distilled water, blood, wounds, hospital dialysis equipment, patients with meningitis, septicemia, bacteremia, peritonitis and wound infections, soil, river water, deep subsurface sediments, corroding copper pipes, drinking water, and the rhizosphere and surfaces of plants [2,6••,8•,12••,17,21••,23•]. Members of the inter-related genus *Rhizomonas* occur as pathogens of plants [1••,4,9].

Membrane structure

The chemical structure of novel glycosphingolipids of *S. paucimobilis* has been elucidated. Kawahara *et al.* [24] have characterized the tetrasaccharide α-D-mannose-*p*-(1→2)-α-D-galactose-*p*-(1→6)-α-D-glucosamine-*p*-(1→4)-α-D-glucuronic acid-1-α-(18:0)/(18:1) ω5 dihydrosphingosine with an amide-linked 2-hydroxy (14:0) at the 2 position of the long-chain base. The cell envelope consists of a cell membrane containing proteins, respiratory quinones and phospholipids and an outer membrane containing the glycosphingolipids with the carbohydrate portions directed outwards [25••]. The glycosphingolipid occupies a position (and presumably provides many of the functions) analagous to the lipopolysaccharide found in most Gram-negative bacteria. Earlier studies showed that the predominant phospholipids in these bacteria are cardiolipin, phosphatidylethanolamine, and phoshphatidyl glycerol [2].

Conclusions

We have, as yet, only a distorted picture of the microbial ecology of the genus *Sphingomonas* as most of the known species were isolated and later found to have an unusual and characteristic lipid constellation. *Sphingomonas* species have a widespread distribution in water, in soil, and in association with plants. They have also been identified as agents of infectious disease. Of the 244 culturable Gram-negative aerobes in the Department of Energy Subsurface Science Culture Collection isolated from subsurface tunnel walls at Yucca Mountain (Nevada, USA) whose fatty acid profiles have been examined, 11% have lipid compositions suggestive of *Sphingomonas*. In recent studies investigating the diversity of 2,4-dichlorophenoxyacetic acid-degrading bacteria isolated from control and treated soils, 18 of 47 strains were identified as *S. paucimobilis* by fatty acid analysis and shown to be closely related by PCR amplification of extragenic palindromic sequences. These *S. paucimobilis* isolates constitute a new class of 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacteria as they do not hybridize with the commonly utilized *tfd* functional gene probe [26•,27•,28••]. *Sphingomonas* clearly represent a prominent population in the environment because each strain was isolated from different field samples (usually those with high 2,4-D loadings) at different sampling times. Ex-

periments using separate soil microcosms experiments have shown that 2,4-D-treated soils from the Michigan site described above were enriched with a degrading population of *S. paucimobilis*. These populations may not have been detected had the investigators screened using only the *tfd* functional gene probe. A third set of experiments using four of the 2,4-D degraders showed *S. paucimobilis* 1443 ranked second in relative fitness when organisms were inoculated into non-native 2,4-D amended soil. Studies of axenic cultures of *S. paucimobilis* showed a low relative fitness coefficient when compared with other strains tested.

Culturable *Sphingomonas* may represent 0.1–20% of the bacteria detected by direct microscopy in subsurface materials. To date, no ecological studies have examined the distribution and predominance of these organisms. The presence of an amide-linked 2-hydroxy fatty acid with no 3-hydroxy fatty acids after acid hydrolysis and subsequent extraction is often the first indication of a novel lipid composition. A much more effective method to screen for *Sphingomonas* sp. in environmental samples would be to detect directly in the lipid extract the sphinganine long-chain bases with an amide-linked 2-hydroxy fatty acid and a carbohydrate side chain.

Sphinganine-containing lipids are novel in bacteria. Besides the *Sphingomonas* group, there is the genus *Sphingobacterium* from the *Cytophaga/Flavobacterium* group that has a much lower guanine plus cytosine content and contains menaquinones [29]. Appropriately, *Rhizomonas* [1••,4] and *Zymomonas* [5•] are in the same subclass (α-4 of the Proteobacteria) as *Sphingomonas* because they have the same novel lipid composition. *Zymomonas* has an important commercial role in ethanol production from low-cost plant-derived substrates [5•]. The photosynthetic organism *Chlorobium limicola* contains a neuraminic acid in its glycosphingolipid [30]. The highly pigmented, bacteriochlorophyll *a* containing genus *Erythrobacter* contains the characteristic 2-hydroxy fatty acids and the Q-10 respiratory quinone of *Sphingomonas* and falls into the α-4 subclass of the Proteobacteria [20,31]. Some of the anaerobic *Bacteroides* now renamed *Prevotella* contain ceramide phospholipids with no glucuronic acids, and their dihydrosphanganines show terminal methyl branching [32].

Members of the genus *Sphingomonas* have an aerobic heterotrophic soil-based life-style somewhat akin to the *Pseudomonas* with which they were confused for much of their history. They have an additional ability to degrade extraordinarily recalcitrant carbon sources with slow growth and to produce gellan and related exopolysaccharides, which gives then an important role in biotechnology. The cardinal feature that defines the genus is the substitution of the uronic acid containing ceramides in the outer membrane for the lipopolysaccharides of classic Gram-negative bacteria. Were they selected to make the uronic acid ceramide lipopolysaccharide because it makes their association with plants more effective? Did they borrow genes from eukaryotes to form this eukaryote-like acidic carbohydrate surface as a part of their outer membrane, rather than as an exopolysaccharide slime like many of the pseudomonads? Does their close phylogenetic association with *Erythrobacter*, which carries out photosynthesis without CO_2 fixation, imply an ancient origin with the first eukaryotes? The structural sequence of the *Sphingomonas* 6-phosphogluconate dehydrogenase suggests an age of at least 1.6 billion years [11]. Further exploration of the physiology and ecology of these bacteria may provide insights into their natural history as well as enabling more effective exploitation of their highly useful metabolic capabilities.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Takeuchi M, Sawada W, Oyaizu H, Yolota A: **Phylogenetic evidence for *Sphingomonas* and *Rhizomonas* as nonphotosynthetic members of the alpha-4 subclass of the *Proteobacteria*.** *Int J Syst Bacteriol* 1994, 44:308–314.
This is an excellent overview of the 16S rRNA phylogenetic sequence relationships of the α-4 subclass of the Proteobacteria. The results indicate that *Rhizomonas* and *Sphingomonas* are interrelated and that the genus and species definitions should be re-examined.

2. Yabuuchi E, Yano I, Oyaizu H, Hashimoto Y, Ezaki T, Yamamoto Y, Ezaki T, Yamamoto H: **Proposals of *Sphingomonas paucimobilis* gen nov. and comb. nov. *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov. and two genospecies of the genus *Sphingomonas*.** *Microbiol Immunol* 1990, 34:99–119.

3. Jenkins DL, Andrews AG, McQuade TJ, Starr MP: **The pigment of *Pseudomonas paucimobilis* is a carotenoid (nostoxanthin), rather than a brominated arylpolyene (xanthmonadin).** *Curr Microbiol* 1979, 3:1–4.

4. Van Bruggen AH, Jochimsen KN, Steinberger EM, Segers P, Gillis M: **Classification of *Rhizomonas suberifaciens*, an unnamed *Rhizomonas* species, and *Sphingomonas* spp. in rRNA superfamily IV.** *Int J Syst Bacteriol* 1993, 43:1–7.

5. • Tahara Y, Kawazu M: **Isolation of glucuronic acid-containing glycosphingolipid from *Zymomonas mobilis*.** *Biosci Biotechnol Biochem* 1994, 58:586–587.
This paper presents the structure of the novel glycosphingolipid isolated from *Zymomonas mobilis*.

6. •• Takeuchi M, Sakane T, Yanagi M, Yamasato K, Hamana K, Yokota A: **Taxanomic study of bacteria isolated from plants: proposal of *Sphingomonas rosa* sp. nov., *Sphingomonas pruni* sp. nov., *Sphingomonas asaccharolytica* sp. nov., and *Sphingomonas mali* sp. nov.** *Int J Syst Bacteriol* 1995, 45:334–341.
This paper proposes the reclassification of two previously existing species (*A. rhizogenes* and *C. lividum*) as *Sphingomonas* sp. on the basis of pheno-

typic and 16S rRNA sequence homologies. After the examination of these and several other strains of 3-ketolactose producing rhizosphere isolates, four new *Sphingomonas* species were proposed.

7. Sakane T, Takeuchi M, De Bruyn A, Kersters K, Yokota A:
•• **Distribution of 3-ketolactose formation among *Sphingomonas* spp. and other members of alpha subclass of *Proteobacteria*.** *Int J Syst Bacteriol* 1995, **45**:342–347.

Several bacterial species within alpha subclass of the Proteobacteria are screened for 3-ketolactose production using high-pressure liquid chromatography. The ketosugar was produced by certain species of *Agrobacterium* (α-2 subclass) and eight different species of *Sphingomonas* (α-4 subclass).

8. Arens P, Tuschewitzki GJ, Wollmann M, Follner H, Jacob H:
• **Indicators for microbiologically induced corrosion of copper pipes in a cold-water plumbing system.** *Zbl Hyg* 1995, **196**:444–454.

Reports a series of chemical and microbiological analyses indicating that the corrosion damage in the cold-water plumbing system of a large building was induced by bacteria. Several organisms that are representative of *Sphingomonas* sp. and *P. fluorescens* are shown to accumulate copper in their cell walls under certain culture conditions.

9. Van Bruggen AHC, Jochimsen K, Brown PR: ***Rhizomonas suberifaciens* gen. nov., the causal agent of corky root of lettuce.** *Int J Syst Bacteriol* 1990, **40**:175–188.

10. Berg G, Ballin G: **Bacterial antagonists to *Verticillium dahliae* Kleb.** *J Phytopathol* 1994, **141**:99–110.
•

This paper reports the detection of phytoprotective features provided by a *Sphingomonas* species and shows the potential of these rhizosphere-active bacteria in biological control.

11. Pollock TJ: **Gellan-related polysaccharides and the genus *Sphingomonas*.** *J Gen Microbiol* 1993, **139**:1939–1945.

12. Ashtaputre A, Shah A: **Studies on a viscous, gel-forming**
•• **exopolysaccharide from *Sphingomonas paucimobilis* GS1.** *Appl Environ Microbiol* 1995, **61**:1159–1162.

The physical properties of a novel polymer produced by *S. paucimobilis* GS1 are described. The anionic heteropolysaccharide has a very high viscosity and is stable over a wide range of temperatures. The deacylated polymer is stable after autoclaving and is superior to agar with respect to gel strength.

13. Vartak NB, Lin CC, Cleary JM, Fagan MJ, Saier MH: **Glucose**
•• **metabolism in *Sphingomonas elodea*: pathway engineering via construction of a glucose-6-phosphate dehydrogenase insertion mutant.** *Microbiology* 1995, 141:2339–2350.

The mechanisms of glucose and central carbon metabolism are studied in *S. eldoea*. Enzymatic assays suggest that the Embden–Meyerhof glycolytic pathway is not utilized; instead, glucose is metabolized through the pentose-phosphate and Entner–Doudoroff pathways. Insertion mutants are constructed in an attempt to better understand gellan production by this organism.

14. Kennedy L, Sutherland W: **Gellan lyases – novel polysaccharide**
• **lyases.** *Microbiology* 1994, **140**:3007–3013.

Several gellan-degrading bacteria are isolated from soil. Degradation of the deacylated gellan polymer is highly specific and other bacterial polysaccharides are not affected. In addition, the enzymes have very little degradative activity against the native acylated exopolysaccharide of *S. paucimobilis*.

15. Mikolajczak MJ, Thorne L, Pollock TJ, Armentrout RW:
• **Sphinganase, a new endoglycanase that cleaves specific members of the gellan family of polysaccharides.** *Appl Environ Microbiol* 1994, **60**:402–407.

A Gram-positive spore-forming aerobic bacterium capable of degrading several gellan-like polysaccharides is isolated from soil by standard enrichment techniques. A new endoglycanase is isolated and purified and proves effective at degrading both the native and deacylated forms of gellan, rhamsan, and S-198. Potential commercial uses for the enzyme are discussed.

16. Schmidt S, Wittich RM, Erdmann D, Wilkes H, Francke W, Fortnagel P: **Biodegradation of diphenyl ether and its monohalogenated derivatives by *Sphingomonas* sp. strain SS3.** *Appl Environ Microbiol* 1992, **58**:2744–2750.

17. Wittich RM, Wilkes H, Sinnwell V, Francke W, Fortnagel P: **Metabolism of Dibenzo-*p*-dioxin by *Sphingomonas* sp. strain RW1.** *Appl Environ Microbiol* 1992, **58**:1005–1010.

18. Moore ERB, Wittich RM, Fortnagel P, Timmis KN: **16S ribosomal RNA gene sequence characterization and phylogenetic analysis of a dibenzo-*p*-dioxin-degrading isolate within the new genus *Sphingomonas*.** *Lett Appl Microbiol* 1993, **17**:115–118.

19. Harms H, Wilkes H, Wittich RM, Fortnagel P: **Metabolism**
•• **of hydroxydibenzofurans, methoxydibenzofurans, acetoxydibenzofurans and nitrobenzofurans by *Sphingomonas* sp. strain HH69.** *Appl Environ Microbiol* 1995, **61**:2499–2505.

This is the latest in an elegant series of studies of biaryl ether degradation pathways by *Sphingomonas* spp. The complete or partial degradation of 11 hydroxybenzofurans, methoxybenzofurans, acetoxybenzofurans, and nitrodibenzofurans is demonstrated.

20. Takeuchi M, Kawai F,Shimada Y, Yokota A: **Taxanomic study of polyethylene glycol-utilizing bacteria: amended description of the genus *Sphingomonas* and new descriptions of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov. and *Sphingomonas terrae* sp. nov.** *Syst Appl Microbiol* 1993, **16**:227–238.

21. Fredrickson JK, Balkwill DL, Drake GR, Romine MF,
•• Ringelberg DB, White DC: **Aromatic-degrading *Sphingomonas* isolates from the deep subsurface.** *Appl Environ Microbiol* 1995, **61**:1917–1922.

This study shows the presence of a cluster of *Sphingomonas* sp. isolated from the deep subsurface with distinct 16S rRNA and lipid profiles as well as important biodegradative capabilities.

22. Stillwell LC, Thurston SJ, Schneider RP, Romine MF,
•• Fredrickson JK, Saffer JD: **Physical mapping and characterization of a catabolic plasmid from the deep-subsurface bacterium *Sphingomonas* sp. strain F199.** *J Bacteriol* 1995, **177**:4537–4539.

In this study, an important enzyme for *Sphingomonas* hydrocarbon biodegradation is localized to a megaplasmid. This could indicate a critical role for large plasmids in the physiology of *Sphingomonas* (another distinction from *Pseudomonas*).

23. Segers P, Vancanneyt M, Pot B, Torck U, Hoste B, Dewettinck D,
• Falsen E, Kersters K, De Vos P: **Classification of *Pseudomonas diminunata* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Büsing, Döll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminunata* comb. nov. and *Brevundimonas vesicularis* comb. nov., respectively.** *Int J Syst Bacteriol* 1994, **44**:499–510.

A description of the origins of environmental and clinical *Sphingomonas* species is provided.

24. Kawahara K, Seydel U, Matsuura M, Danbara H, Rietschel E:
Chemical structure of glycosphingolipids isolated from *Sphingomonas paucimobilis*. *FEBS Lett* 1991, **292**:107–110.

25. Kawasaki S, Moriguchi R, Sekiya K, Nakai T, Ono E,
•• Kume K, Kawahara K: **The cell envelope structure of the lipopolysaccharide-lacking Gram-negative bacterium *Sphingomonas paucimobilis*.** *J Bacteriol* 1994, **176**:284–290.

This is an elegant study of the structure and anatomic localization of the novel glycosphingolipids of *S. paucimobilis*. Biochemical and immunoelectron microscopic analyses are used to determine the presence of sphingolipids on the surface of the outer membrane.

26. Ka JO, Holben WE, Tiedje JM: **Use of gene probes to aid**
• **in recovery and identification of functionally dominant 2,4-dichlorophenoxyacetic acid degrading populations in soil.** *Appl Environ Microbiol* 1994, **60**:1116–1120.

See annotation [28••].

27. Ka JO, Holben WE, Tiedje JM: **Analysis of competition in soil**
• **among 2,4-dichlorophenoxyacetic acid-degrading bacteria.** *Appl Environ Microbiol* 1994, **60**:1121–1128.

See annotation [28••].

28. Ka JO, Holbin WE, Tiedje JM: **Genetic and phenotypic diversity**
•• **of 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacteria isolated from 2,4-D treated field soils.** *Appl Environ Microbiol* 1994, **60**:1106–1115.

This paper and the two subsequent papers in the same journal [26•,27•] report an elegant series of experiments showing that the addition of 2,4-D to soils repeatedly induces growth of bacteria of which a large proportion are *S. paucimobilis*. *Sphingomonas* are detected both by fatty acid analysis and PCR amplification of extragenic palindromic sequences. These *Sphingomonas* have low relative fitness compared with other strains when grown axenically, but were ranked second in relative fitness when grown in a soil consortium. Some of the *Sphingomonas*-enriched 2,4-D treated soils in microcosms may not have been detected by the specific functional (enzyme) gene probe, which was developed primarily for *Pseudomonas*-type organisms. This serves to emphasize the utility of phenotypic biomarkers, such as the signature lipids, in assessing the diversity of natural communities. Genes provide comprehensive indications of the metabolic potential of a community if, and only if, the gene sequences of all the important enzymes are known. All the lipids are usually extractable and identifiable chemically, but may be ambiguous for species identification because of overlap amongst species.

29. Takeuchi M, Yokota A: **Proposals of *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov.,**

Sphingobacterium heparinum **comb. nov., *Sphingobacterium thalpophilum* comb. nov., and two genospecies of the genus *Sphingobacterium*, and synonymy of *Flavobacterium yabuchiae* and *Sphingobacterium spiritivorum*.** *J Gen Appl Microbiol* 1992, **38**:465–482.

30. Jensen MT, Knudsen J, Olson JM: **A novel aminoglycosphingolipid found in *Chlorobium limicola* f. *thiosulfatophilum* 6230.** *Arch Microbiol* 1991, **156**:248–254.

31. Yurkov V, Stackebrandt E, Holmes A, Fuerst JA, Hugenholtz P, Golecki J, Gadon N, Gorlenko V, Kompantseva E, Drews G: **Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., and *Erythrobacter litoralis* sp. nov.** *Int J Syst Bacteriol* 1994, **44**:427–434.

32. White DC, Tucker AN, Sweely CC: **Characterization of the iso-branched sphinganines from the ceramide phospholipids of *Bacteroides melaninogenicus*.** *Biochim Biophy Acta* 1969, **187**:527–532.