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CHAPTER 3.1.12

The Genus *Phenylobacterium*

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The genus *Phenylobacterium* comprises a single species called *P. immobile*, which is remarkable for its extremely limited nutritional spectrum. All strains isolated and described hitherto grow optimally only on artificial compounds like chloridazon, antipyrin, and pyramidon (formulas in Fig. 1). Chloridazon, formerly called pyrazon, is the active ingredient of the herbicide Pyramin®, which is used for the control of broadleaf weeds in sugar beet and beet root cultures. The fact that the breakdown of this herbicide is a microbial process was demonstrated by studies with soil samples, including heat-sterilized soil (Drescher and Otto, 1969; Frank and Switzer, 1969). Engvild and Jensen (1969) described the isolation of bacteria capable of growth on chloridazon as sole source of carbon and energy. At the same time and independently, Fröhner et al. (1970) isolated chloridazon-degrading bacteria that proved to be similar to the organisms of Engvild and Jensen. Meanwhile, more than 20 different strains have been isolated that can grow on the herbicide chloridazon and also on the structurally related analgesics antipyrin and pyramidon. All of these xenobiotic-degrading bacteria show a high degree of similarity with respect to different properties, and they were grouped together in one single species, named *Phenylobacterium immobile* (Lingens et al., 1985).

P. immobile is not closely related to any other Gram-negative bacterium, as demonstrated by 16S rRNA investigations (Ludwig et al., 1984). It was found to be a member of subgroup alpha-2 of the alpha subclass of the proteobacteria, standing phylogenetically isolated in this group (proteobacteria were formerly named "purple bacteria and their nonphotosynthetic relatives"). Lipopolysaccharide analysis (Weisshaar and Lingens, 1983), serological studies (Dorfer et al., 1985), investigations on the polyamine pattern (Busse and Auling, 1988), and ubiquinone analysis (R. M. Kroppenstedt, J. Eberspächer, and F. Lingens, unpublished observations) have confirmed the results on the phylogenetic position of *P. immobile*.

Habitat

P. immobile seems to be a typical inhabitant of the upper aerobic part of the soil. Different strains have been isolated from soil samples originating from various locations all over the world. Attempts to demonstrate the breakdown of chloridazon in soil or in mud samples under anaerobic conditions failed. Although in one case, a slow degradation of chloridazon in river water was observed, efforts to isolate chloridazon-degrading bacteria from this specific water sample were without success. However, we cannot rule out the possibility that phenylobacteria occur also in aquatic habitats.

Isolation and Cultivation

The technique for the enrichment of *P. immobile* is based on selective pressure exerted on a microbial soil population. This technique leads to the isolation of bacteria that are able to utilize synthetic molecules not normally encountered in nature as sole carbon source. As a synthetic substrate for selective enrichment, chloridazon, antipyrin, or pyramidon can be applied.

Isolation Procedure

A convenient method for the isolation of *P. immobile* starts with sampling about 300 g of soil or compost. Air-dried soil or soil with very low humus content was found to be less satisfactory. The soil sample is mixed with 0.5 g of chloridazon, antipyrin, or pyramidon, and the preparation is incubated at 30°C or at room temperature in a flower pot and regularly moistened with water. Degradation of the xenobiotic compound is followed by thinlayer chromatography. From the excess water that drains from the flower pot, about 0.05 ml is applied to a thin layer plate coated with silica gel containing a fluorescent indicator with maximum sensitivity under UV radiation of 254 nm. Decomposition is complete when the spot of the xenobiotic compound is no longer detectable under UV light and a new spot corresponding to the dephenylated heterocyclic moiety of the xenobiotic appears, usually after one to several weeks, depending on the soil. Then a 5-g sample of the active soil is placed into an Erlenmeyer flask containing 50 ml of mineral salts medium (for composition, see recipe below) supplemented with the xenobiotic as carbon source at a concentration of 0.4%. This culture is

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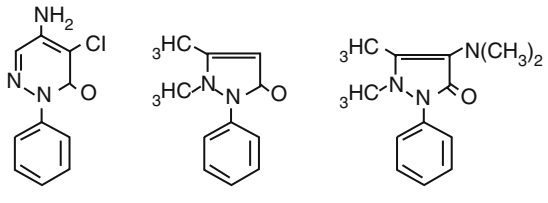

chloridazon antipyrin pyramidon

Fig. 1. The herbicide chloridazon and the structurally related analgesics antipyrin and pyramidon can be used for the enrichment of *P. immobile*. These xenobiotics are also the best growth substrates for the organism.

incubated on a rotary shaker at 30°C, and degradation is monitored by thin layer chromatography. When the decomposition of the xenobiotic is complete, 1 ml of the culture fluid is transferred into a new Erlenmeyer flask. After 5 to 10 transfers, a sample of the liquid culture is streaked onto agar plates containing the same medium. Single colonies, which normally appear after 1 to 3 weeks, are picked and again streaked onto agar. After 5 to 10 transfers, pure cultures can usually be obtained. In several cases, the isolation of a pure culture was more difficult than usual because *P. immobile* was closely associated with other nondegrading bacteria. In one case the other organism was identified as *Pseudomonas cepacia*.

Medium for *Phenylobacterium immobile*

Fröhner et al. (1970) found, for the first isolates of *P. immobile*, that vitamin B_{12} was an essential growth factor. This vitamin is thus routinely added to the mineral salts medium at 30 µg/liter. When grown on chloridazon or antipyrin *P. immobile* acidifies the culture fluid. Therefore a medium with reasonable buffer capacity was developed that due to the organism's osmotic sensitivity had to be kept at a low total salt concentration.

Medium for the Culture of *P. immobile*

The mineral salts medium has the following composition per 1 liter of deionized water:

$Na_2HPO_4 \cdot 12H_2O$	0.7 g
KH_2PO_4	0.3 g
$(NH_4)_2HPO_4$	0.7 g
$(NH_4)H_2PO_4$	0.3 g
$(NH_4)_2SO_4$	0.1 g
Trace element solution (see below)	1 ml
Vitamin B_{12} solution, 0.03 mg/ml	1 ml
$MgSO_4 \cdot 7H_2O$	0.25 g
$CaCl_2 \cdot 6H_2O$	0.05 g

To avoid precipitates the magnesium and calcium salts are each dissolved separately.

Trace element solution per 1 liter of deionized water:

$MnSO_4 \cdot 4H_2O$	400 mg
$ZnSO_4 \cdot 7H_2O$	400 mg
$FeCl_3 \cdot 6H_2O$	200 mg
$CuSO_4 \cdot 5H_2O$	40 mg
H_3BO_3	500 mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	200 mg
KI	100 mg
Biotin	100 mg

As carbon source, chloridazon, antipyrin, or pyramidon is added at a concentration of 0.4 to 1 g per liter. The pH of the medium is 7.0.

Cultivation

Optimum growth and maximum cell yield are achieved when *P. immobile* is grown at 30°C in mineral salts medium with chloridazon or antipyrin at 0.4 to 1 g per liter. Under these conditions, a doubling time of 7 to 8 hours is observed, and, depending on the strain, a yield of about 0.4 to 1.0 g bacteria (wet weight) per liter of culture fluid is obtained.

Complex media used for routine cultivation in bacteriology with 10 to 20 g peptone per liter, or the same amount of yeast extract plus meat extract, do not support the growth of *P. immobile*. These bacteria were found to be osmotically sensitive, as demonstrated by NaCl addition to the chloridazon-mineral salts medium. At a NaCl concentration of 5 to 7 g per liter, considerable growth inhibition was observed with total inhibition at 10 g per liter. *P. immobile*, however, does grow on complex media with 0.5 to 2 g per liter peptone plus yeast extract, but growth is considerably slower than on antipyrin or chloridazon.

The strictly aerobic bacteria are cultivated on a rotary shaker, and in large-scale fermentations they are well aerated with 50 liter per min of air in a 100-liter-fermentor. For large-scale fermentation conducted with the type strain *P. immobile* strain E, a scale-up ratio of 1:10, starting with a 1-liter culture inoculated from an agar plate, was found to yield good results. In this case, a fermentation time of 24 h (from inoculation of the 100-liter fermentor to the late log phase) and a cell yield of 1 g per liter were obtained.

Growth on Agar Plates

Mineral salts medium with either 0.2% chloridazon or 0.1% antipyrin as carbon source and supplemented with 15 g agar per liter allows good growth of *P. immobile*. However, it takes at least 4 to 7 days until the first tiny colonies are visible on the agar even when the plate is inoculated with a large number of bacteria. After 2 to 3 weeks, the colonies reach a size of 1 to 2 mm in diameter. A concentration of 2 g chloridazon per liter leads to the precipitation of fine chloridazon

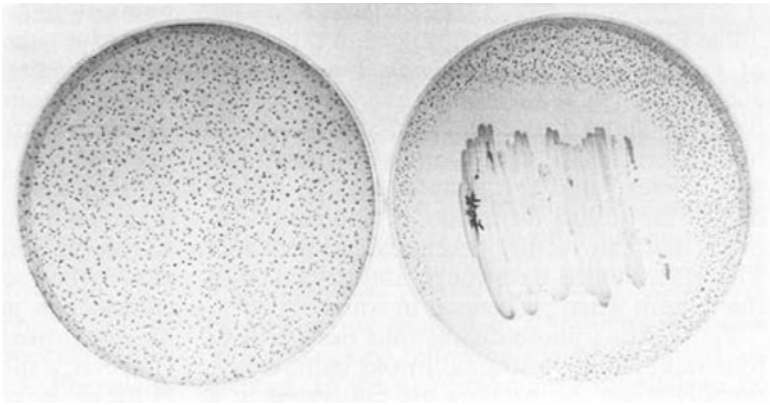

Fig. 2. Agar plates containing mineral salts medium with 2 g chloridazon per liter. On the plate at the right chloridazon crystals have been metabolized by *P. immobile*. The large crystal at the left margin of the bacterial smear is the dephenylated heterocyclic moiety of chloridazon, the main metabolite of chloridazon degradation.

crystals in the agar (Fig. 2). During growth on this agar, *P. immobile* removes the crystals by degradation, and as a result of bacterial growth, a clearance zone around the smear develops. In agar cultures of 4 weeks and older, new, and in most cases, relatively large crystals form within the bacterial smear. These crystals were identified as the dephenylated heterocyclic moiety of chloridazon, which is a dead-end metabolite of chloridazon degradation.

Purity of Cultures

Since none of the *P. immobile* strains grows on an ordinary complex medium, an inoculation of an agar plate containing the following medium is routinely used for testing purity:

Testing Medium

The complex medium contains per liter deionized water:

Nutrient broth (dehydrated)	10 g
Yeast extract	5 g
NaCl	5 g

Growth on this medium indicates contamination of the culture.

Preservation of Cultures

For short-term preservation, bacteria are regularly transferred on agar at intervals of 2 to 3 weeks. For long-term preservation, the bacteria suspended in skimmed milk are dropped onto silica gel grains and stored at 4°C. Good results were obtained with this method, when transfer is repeated every 2 to 3 years. However, even after a period of 10 years some of the strains were viable. Storage at –80°C of a concentrated bacterial suspension in fresh chloridazon-mineral salts medium supplemented with 15% glycerol also resulted in good viability after 2 years.

The following strains have been deposited in the Deutsche Sammlung für Mikroorganismen (DSM) and at the American Type Culture Collection (ATCC): strain E, type strain (DSM 1986, ATCC 35973); strain A_{12} (DSM 2115, ATCC 35972); strain J_1 (DSM 2116, ATCC 35974); strain K_2 (DSM 2117, ATCC 35975); strain N (DSM 2113, ATCC 35976); and strain Z_6 (DSM 2114, ATCC 35977). Two further strains have been deposited at the Czechoslovak Collection of Microorganisms (CCM): strain C_2 (CCM 3864) and strain R (CCM 3865).

Identification

The morphology and physiological properties of *P. immobile* are not especially remarkable (see Table 1). Nearly all biochemical tests are negative, and, with regard to this fact, *P. immobile* shows similarities with *Acinetobacter*. In fact, *Phenylobacterium immobile* was originally identified as an *Acinetobacter* species (Fröhner et al., 1970) but determination of the GC content clearly ruled out this identification.

The most distinguishing feature of *Phenylobacterium immobile* is its high nutritional specialization. This property is shared, although in a less pronounced way, by certain members of the pseudomonads, together with some morphological, physiological, and biochemical characteristics. The general definition of *Pseudomonas*, however, excludes nonmotile organisms, and all strains of *Phenylobacterium immobile* are nonmotile.

P. immobile is not closely related to any other Gramnegative eubacterium, as shown by partial sequence analysis of 16S rRNA from strain E (Ludwig et al., 1984). Phylogenetically, it was found to belong to subgroup alpha-2 of the proteobacteria (see "Phylogenetic Position" in this Chapter), with an isolated position in this group.

Table 1. Important characters for the identification of *Phenylobacterium immobile*.

Cells: Rods, coccal rods, or cocci; 0.7 to 1.0 by 1.0 to 2.0 μm; singly, in pairs or short chains; nonmotile; nonsporeforming; nonpigmented; no sheaths; no prosthecae.

Colonies: Develop slowly on chloridazon-mineral salts agar, small, colorless, circular, entire edges, convex; smooth or rough colonies possible.

Staining: Gram negative, not acid-fast, no capsule.

Physiology: Strictly aerobic, catalase positive, weakly oxidase positive; no growth at 4°C and 37°C, optimum growth at 28–30°C; no growth at pH 4 and 9, growth between pH 6.5 and 8, optimum pH 6.8–7.0; osmotically sensitive; vitamin B_{12} is a growth factor; NH_4^+ and NO_3^- used as N sources, no denitrification, no N_2 fixation.

Biochemical tests: Negative results in the indole reaction, methyl red, Voges-Proskauer, litmus milk, and urease test; no hydrolysis of gelatin, starch, agar, or esculin; weakly positive for H_2S from thiosulfate or cysteine; no acid or gas from 34 different sugars.

Carbon sources: Optimum growth on chloridazon, antipyrin, pyramidon, and L-phenylalanine (most strains after long lag phase only); no growth on simple carbon sources like sugars, alcohols, carboxylic acids (31 compounds tested), and amino acids (except phenylalanine and glutamate); poor growth on glutamate, pyruvate, fumarate, succinate, and malate; no growth on routine complex media unless medium is diluted (0.5 to 2 g peptone per liter).

GC content: 65–68.5 mol%.

Morphology

Table 1 summarizes some important properties of *Phenylobacterium immobile*. On chloridazon-mineral salts agar, after 2 to 3 weeks colonies are about 1 to 2 mm in diameter and do not adhere to the agar (see also "Growth on Agar Plates," and this chapter Fig. 2). Nearly half of the strains form smooth and shiny colonies that can readily be emulsified in water. The other strains have rough and dry colonies that clump when suspended in water.

Fig. 3 shows photomicrographs from cells of four representative strains. In old cultures, especially when the bacteria are cultivated in a medium that allows only poor growth, such as a dilute complex medium, pleomorphic forms, such as long rods, long chains of cells connected by small filaments, or club-shaped and elliptical forms, sometimes occur.

Gram staining, Ziehl-Neelsen staining, and capsule staining are negative, and electron microscopy of thin sections also reveals the typical Gram-negative cell wall pattern (Lingens et al., 1985). Strain K_2, which forms smooth colonies on agar, was treated with ruthenium red and electron microscopy of ultrathin sections revealed the presence of a microcapsule—or according to the definition of Costerton et al. (1981), a "flexible" capsule—surrounded by a slime layer of acid polysaccharides (Lingens et al., 1985). No microcapsule was detected in ruthenium-red-stained cells of strain E, which forms rough colonies on agar.

Physiological and Biochemical Characteristics

The osmotic sensitivity and nutritional specialization of *Phenylobacterium immobile* do not allow the use of routine media for biochemical characterization. Therefore, tests were performed in modified media (Lingens et al., 1985). The characteristics determined for all strains are shown in Table 1.

Utilization of Carbon Sources

All the different strains of *Phenylobacterium immobile* do not utilize all three xenobiotic substrates. Whereas chloridazon and antipyrin are well utilized by most strains, pyramidon is a growth substrate for only 7 of the 22 isolates. When pyramidon is added to media containing chloridazon or antipyrin, growth inhibition is observed among all of the isolates.

The pathway for the degradation of the three xenobiotics was elucidated by metabolic and enzymatic studies (Blecher et al., 1981; Eberspächer and Lingens, 1978; Miller et al., 1977; Sauber et al., 1977a; Sauber et al., 1977b). The pathway follows the well-known route for the oxidative dissimilation of aromatic compounds, and in the case of these xenobiotics only the aromatic nucleus is used as a carbon source, the heterocyclic moiety remaining unchanged.

More than 20 different heterocyclic or aromatic compounds that are structurally related to chloridazon or antipyrin were tested as possible carbon sources (Lingens et al., 1985). Most chloridazon analogs with altered heterocyclic moiety proved to be good growth substrates. However, a substitution at the aromatic nucleus, as for *o*-, *m*- or *p*-methylchloridazon, made the compound nondegradable by the organism. Of the aniline derivatives tested, N-methylacetanilide and N-methylformanilide were found to be poor growth substrates. Of 18 aromatic compounds that usually are utilized by various bacterial species, none

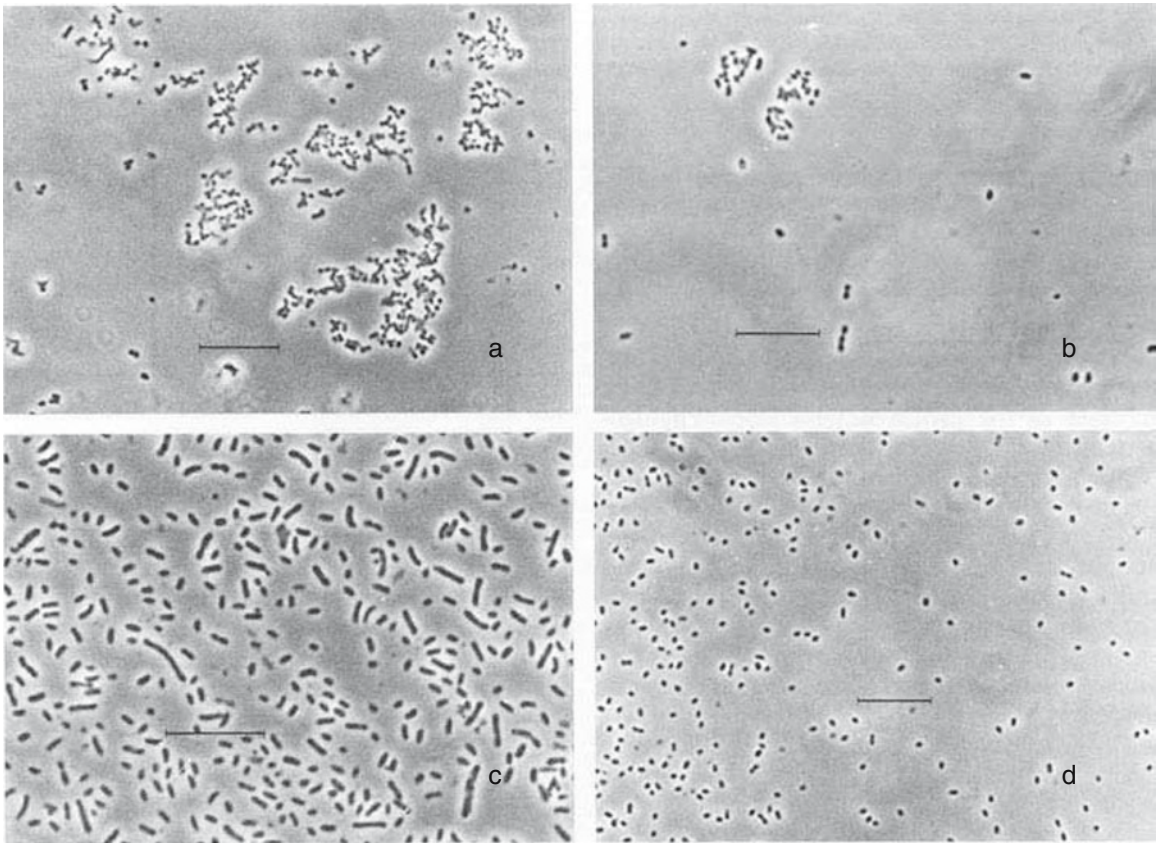

Fig. 3. Phase contrast photomicrographs of cells of *Phenylobacterium immobile*. (a) Strain A_{13}; (b) strain E, the type strain; (c) strain K_2; and (d) strain N. Bars = 10 µm.

supported growth of *Phenylobacterium immobile*. Among these compounds were benzene, toluene, phenol, catechol, benzaldehyde, benzoate, and a number of mono- and dihydroxylated benzoates.

One strain (strain N) was found to grow well on L-phenylalanine with a normal lag phase of 1 day. All other strains had lag phases of 2 to 3 weeks, but then they also grew well on phenylalanine. The long lag phases were only observed when the strains were transferred from chloridazon or antipyrin to phenylalanine for the first time, since after additional transfers the bacteria grew immediately. In liquid cultures during growth on mineral salts medium with phenylalanine, especially at higher concentrations (3 to 5 g per liter), a yellowish-green fluorescent pigment is produced. On chloridazon or antipyrin mineral salts media, a greenish-yellow nonfluorescent pigment is formed. Phenylalanine-induced cells also grow well on phenylpropionate, phenylpyruvate, and phenyllactate.

Nucleic Acid Data

GC content of the DNA of *P. immobile* was found to be between 65 and 66.5 mol%, although for one strain (C_2) a somewhat higher value of 68.5 mol% was determined. DNA hybridization tests revealed 100% homology of DNA preparations of four different strains with DNA of strain R. No homology was found using DNA from *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, or calf thymus.

Depending on the strain, between one and six different plasmids were found, which varied in size from 8 to 300 megadaltons (Kreis et al., 1981).

From the type strain, 16S rRNA was isolated, and oligonucleotides of hexamer size and larger were sequenced (Ludwig et al., 1984). With this oligonucleotide catalog, similarity coefficients (S_{AB} values) to more than 400 microorganisms were calculated, allowing the phylogenetic allocation of *Phenylobacterium immobile* to be determined (see "Phylogenetic Position" in this Chapter).

Lipopolysaccharide, Peptidoglycan, and Polyamine Pattern

The composition of the carbohydrate moiety and of the lipid A from the lipopolysaccharide of the

type strain has been described by Weisshaar and Lingens (1983). Remarkable is the presence of one mole of 2,3-diamino-2,3-dideoxy-D-glucose as a constituent of the lipid A backbone. This diaminosugar was only found in members of subgroup alpha-2 of the alpha subclass of the proteobacteria (see "Phylogenetic Position" in this Chapter), whereas the lipid A of most Gram-negative bacteria contains glucosamine.

The detection of ester-linked 3-hydroxy-5-*cis*-dodecanoic acid as a major substituent in the lipopolyaccharide is also remarkable (Bellmann and Lingens, 1985a). This unusual fatty acid, not found in nature before, was used to demonstrate the presence of *Phenylobacterium immobile* in soil samples (Bellmann and Lingens, 1985b).

The peptidoglycan composition of the type strain was identical to that of a normal Gram-negative bacterium (Lingens et al., 1985). The type strain was found to contain only sym-homospermidine as a polyamine component (Busse and Auling, 1988).

Toxicity

Bacteria of *P. immobile* are harmless when tested in rats and rabbits (Kaiser et al., 1981). Tests included oral administration, intracutaneous and intraperitoneal injections of the bacteria, and inhalation experiments.

Enzymes

The type strain was found to possess all of the enzymes of the citric acid cycle. Properties of citrate synthase, of rhodanese (Layh et al., 1982), of arogenate (pretyrosine) dehydrogenase (Keller et al., 1982), and of *meta*-cleaving chloridazon-catechol-dioxygenases (Schmitt et al., 1984) were studied in more detail, and the taxonomic significance of these enzymes has been discussed.

Serology

Agglutination and immunofluorescence tests revealed the serological uniformity of the different strains (Layh et al., 1983). Slight differences in immune reactions allowed a classification of the strains into 5 serological subgroups. No relationship was found between *Phenylobacterium immobile* and 40 representative Gram-negative bacteria, including *Acinetobacter calcoaceticus*, *Azospirillum brasiliense*, *Paracoccus denitrificans*, different *Pseudomonas* species, *Rhizobium* species, *Rhodomicrobium vannielii,* and *Rhodopseudomonas capsulata.* A slight but significant immunofluorescence reaction was observed with *Pseudomonas vesicularis*, *Gluconobacter oxydans*, *Aquaspirillum itersonii*, and *Rhodospirillum rubrum* (Dorfer et al., 1985). Crossed immunoelectrophoresis revealed a serological relationship between *Phenylobacterium immobile* and *Pseudomonas diminuta* (J. Dorfer, C. Löffler, J. Eberspächer, and F. Lingens, unpublished observations).

Phylogenetic Position

Partial sequence analysis of 16S rRNA has revealed the isolated phylogenetic position of *Phenylobacterium immobile* (Ludwig et al., 1984). The highest S_{AB} values (0.51) were found with *Pseudomonas diminuta* and *Rhizobium leguminosarum*. Similarity coefficients of this magnitude indicate a rather remote relationship that would not be detectable by DNA-DNA hybridization. A comparison of the 16S rRNA nucleotide catalogs showed that *Phenylobacterium* is a member of subgroup alpha-2 of the alpha subclass of the proteobacteria (in the nomenclature of Stackebrandt et al., 1988). These organisms have also been called the "subgroup alpha-2 of the alpha purple bacteria" (Woese, 1987) or the "subgroup Ib of the purple nonsulfur bacteria and their nonphotosynthetic relatives." Members of this group belong to the "4th rRNA superfamily," (de Vos and de Ley, 1983). S_{AB} values of 0.49 and 0.46 were found with *Rhodopseudomonas viridis*, *R. capsulata*, *R. sphaeroides*, *Rhodomicrobium vannielii*, and *Aquaspirillum itersonii*. For representatives of the other major groups of Gram-negative bacteria, including *Acinetobacter calcoaceticus* and the two phylogenetically defined clusters of the genus *Pseudomonas*, S_{AB} values of 0.23 to 0.32 were obtained, indicating a wide phylogenetic distance.

In accordance with this finding, weak serological reactions of *Phenylobacterium immobile* were found only with members of the alpha subclass of the proteobacteria (Dorfer et al., 1985) and not with any other Gram-negative bacteria.

Furthermore, the demonstration of 2,3-diamino-2,3-dideoxy-D-glucose as a lipid A constituent of *Phenylobacterium immobile* supports its relationship to subgroup alpha-2 of the proteobacteria. This unusual sugar was also detected in *Rhodopseudomonas viridis*, *R. palustris*, *R. sulfoviridis*, *Pseudomonas diminuta*, *P. vesicularis*, and *Nitrobacter winogradskyi*, which are all members of the same phylogenetic group (Weckesser and Mayer, 1987).

Busse and Auling (1988) have shown that polyamines may serve as a useful chemotaxonomic marker within the proteobacteria. They found that the species of the alpha-2 subgroup have sym-homospermidine as the dominant

component of the polyamine pattern. Consistent with its phylogenetic position, *Phenylobacterium immobile* contains sym-homospermidine exclusively.

Ubiquinones are also useful chemotaxonomic markers, and members of the alpha subgroup of the proteobacteria were shown to contain ubiquinone composed of 10 isoprenoid units. Strains E, J_1, and Z_6 of *P. immobile* contain a ubiquinone of the Q-10 type (R. M. Kroppenstedt, J. Eberspäher, and F. Lingens, unpublished observations).

Literature Cited

Bellmann, W., F. Lingens. 1985a. Structural studies on the core oligosaccharide of *Phenylobacterium immobile* strain K_2 lipopolysaccharide. Biol. Chem. Hoppe-Seyler 366:567–575.

Bellmann, W., F. Lingens. 1985b. Nachweis herbizidabbauender Bakterien durch die Erfassung einer spezifischen Fettsäure in Bodenproben. Naturwissenschaften 72:599.

Blecher, H., R. Blecher, W. Wegst, J. Eberspäher, F. Lingens. 1981. Bacterial degradation of aminopyrine. Xenobiotica 11:120–123.

Busse, J., G. Auling. 1988. Polyamine pattern as a chemotaxonomic marker within the Proteobacteria. System. Appl. Microbiol. 11:1–8.

Costerton, J. W., R. T. Irvin, K. J. Cheng. 1981. The bacterial glycocalyx in nature and disease. Ann. Rev. Microbiol. 35:299–324.

De Vos, P., J. de Ley. 1983. Intra- and intergeneric similarities of *Pseudomonas* and *Xanthomonas* ribosomal ribonucleic acid cistrons. Internat. J. System. Bacteriol. 33:487–509.

Dorfer, J., G. Layh, J. Eberspäher, F. Lingens. 1985. Relationships of *Phenylobacterium immobile* and purple nonsulfur bacteria on the basis of surface antigens. FEMS Microbiol. Lett. 28:151–155.

Drescher, N., S. Otto. 1969. Über den Abbau von 1-Phenyl-4-amino-5-chlor-pyridazon-6(Pyrazon) im Boden. Z. Pflanzenkr. Pflanzenschutz 76:27–33.

Eberspäher, J., F. Lingens. 1978. Reinigung and Eigenschaften von zwei Chloridazondihydrodiol-Dehydrogenasen aus Chloridazon-abbauenden Bakterien. Hoppe Seyler's Z. Physiol. Chem. 359:1323–1334.

Engvild, K. C., H. L. Jensen. 1969. Microbiological decomposition of the herbicide Pyrazon. Soil Biol. Biochem. 1:295–300.

Frank, R., C. M. Switzer. 1969. Behaviour of Pyrazon in soil. Weed Sci. 17:323–326.

Fröhner, C., O. Oltmanns, F. Lingens. 1970. Isolierung und Charakterisierung Pyrazon-abbauender Bakterien. Arch. Mikrobiol. 74:82–89.

Kaiser, A., H.-G. Classen, J. Eberspäher, F. Lingens. 1981. Acute toxicity testing of some herbicides-, alcaloids-, and antibiotics-metabolizing soil bacteria in the rat. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B 173:173–179.

Keller, B., E. Keller, F. Lingens. 1982. Arogenate (pretyrosine) as an obligatory intermediate of the biosynthesis of L-tyrosine in chloridazon-degrading bacteria. FEMS Microbiol. Lett. 13:121–123.

Kreis, M., J. Eberspäher, F. Lingens. 1981. Detection and characterization of plasmids in chloridazon and antipyrin degrading bacteria. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C 2:45–60.

Layh, G., R. Böhm, J. Eberspäher, F. Lingens. 1983. Serological studies on chloridazon-degrading bacteria. Syst. Appl. Microbiol. 4:459–469.

Layh, G., J. Eberspäher, F. Lingens. 1982. Rhodanese in chloridazon-degrading bacteria. FEMS Microbiol. Lett. 15:23–26.

Lingens, F., R. Blecher, H. Blecher, F. Blobel, J. Eberspäher, C. Fröhner, H. Grösch, H. Grösch, G. Layh. 1985. *Phenylobacterium immobile* gen. nov., sp. nov., a Gram-negative bacterium that degrades the herbicide chloridazon. Internat. J. System. Bacteriol. 35:26–39.

Ludwig, W., J. Eberspäher, F. Lingens, E. Stackebrandt. 1984. 16S ribosomal RNA studies on the relationship of a chloridazon-degrading Gram-negative eubacterium. Syst. Appl. Microbiol. 5:241–246.

Müller, R., S. Haug, J. Eberspäher, F. Lingens. 1977. Catechol-2,3-Dioxygenase aus Pyrazon-abbauenden Bakterien. Hoppe Seyler's Z. Physiol. Chem. 358:797–805.

Sauber, K., C. Fröhner, G. Rosenberg, J. Eberspäher, F. Lingens. 1977a. Purification and properties of pyrazon dioxygenase from pyrazon-degrading bacteria. Eur. J. Biochem. 74:89–97.

Sauber, K., R. Müller, E. Keller, J. Eberspäher, F. Lingens. 1977b. Abbau von Antipyrin durch Pyrazon-abbauende Bakterien. Z. Naturforsch. 32:557–562.

Schmitt, S., R. Müller, F. Lingens. 1984. Chloridazon-catechol dioxygenases, a distinct group of meta-cleaving enzymes. Hoppe Seyler's Z. Physiol. Chem. 365:143–150.

Stackebrandt, E., R. G. E. Murray, H. G. Trüper. 1988. *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the "purple bacteria and their relatives." Internat. J. System. Bacteriol. 38:321–325.

Weckesser, J., H. Mayer. 1987. Lipopolysaccharide aus phototrophen Bakterien. Forum Mikrobiol. 10:242–248.

Weisshaar, R., F. Lingens. 1983. The lipopolysaccharide of a chloridazon-degrading bacterium. Eur. J. Biochem. 137:155–161.

Woese, C. R.1987. Bacterial evolution. Microbiol. Reviews 51:221–271.