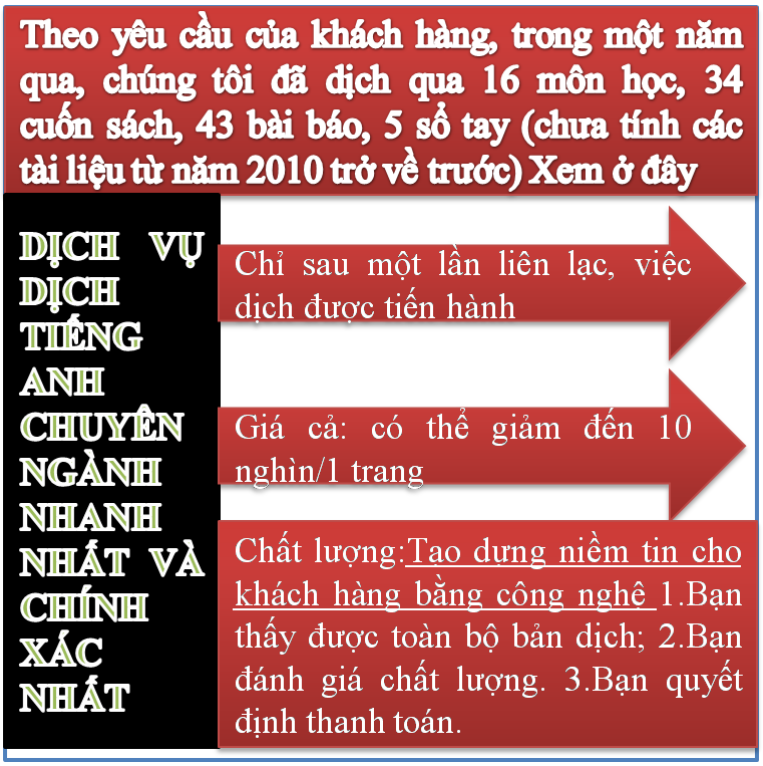

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INTRODUCTION Pseudomonas stutzeri was first described by Burri and Stutzer in 1895 (55). van Niel and Allen, in 1952 (371), precisely defined its phenotypic features and discussed its definitive designation as Pseudomonas stutzeri by Lehmann and Neumann (196). In spite of marked differences from the type strain of the genus, the sequence similarities of the rRNAs, demonstrated initially by DNA-rRNA hybridization, show the legitimacy of the inclusion of P. stutzeri in the genus Pseudomonas. Strains of the species have been identified among denitrifiers found in natural materials. Their inclusion in the phenotypic studies carried out by Stanier et al. in 1966 (340) demonstrated that, in addition to their typical colonies, the strains are nutritionally versatile, using some carbon compounds seldom utilized by other pseudomonads (e.g., starch, maltose, and ethylene glycol). Variations in DNA sequences, as shown by the results of DNA-DNA hybridization experiments, were demonstrated in the early studies of Palleroni et al., in 1970 (251). Work performed in recent years has clearly established firm bases for	GIỚI THIỆU Burri và Stutzer là những nhà khoa học đầu tiên đã mô tả Pseudomonas stutzeri vào năm 1895 (55). Sau đó, vào năm 1952 (371), van Niel và Allen đã xác định chính xác các đặc điểm kiểu hình và Lehmann và Neumann (196) đã ấn định nó là Pseudomonas stutzeri. Mặc dù chúng có sự khác biệt đáng kể với chủng điển hình của chi, sự tương đồng về trình tự rRNA, ban đầu được minh chứng qua sự lai hóa DNA-rRNA đã phần nào khẳng định sự đúng đắn trong việc xếp P. stutzeri vào chi Pseudomonas. Các chủng của loài đã được xác định là những vi khuẩn khử nitơ trong các vật liệu tự nhiên. Các nghiên cứu phát sinh loài của Stanier và các cộng sự vào năm 1966 (340) đã chứng tỏ rằng , cùng với các cụm khuẩn điển hình của chúng, các chủng có sự đa dạng trong hoạt động tổng hợp dinh dưỡng, sử dụng các hợp chất carbon hiếm khi được dùng bởi các pseudomonad khác (ví dụ, tinh bột, maltose, và ethylene glycol). Qua các kết quả thực nghiệm lai hóa DNA-DNA, vào năm1970 (251), Palleroni và các cộng sự đã chứng minh sự thay đổi trình tự DNA trong các nghiên cứu của mình. Các công trình được thực hiện trong những năm gần đây đã tạo một cơ sở vững chắc cho việc xếp các chủng này vào một số biến thể di	

grouping the strains into a number of genomic variants (geno- movars) that are phylogenetically closely related. Some strains have received particular attention because of specific meta-bolic properties (such as denitrification, degradation of aromatic compounds, and nitrogen fixation). Furthermore, some strains have been shown to be naturally transformable and have been studied extensively for their capacities for transformation. P. stutzeri is distributed widely in the environment, occupying diverse ecological niches, and has also been isolated as an opportunistic pathogen from humans. Based on results obtained in recent years, the biology of this species is discussed. DEFINITION OF THE SPECIES AND DIFFERENTIATION FROM OTHER PSEUDOMONAS SPECIES Definition Pseudomonas stutzeri is a member of the genus Pseudomonas sensu stricto. It is in group I of Palleroni's DNA-rRNA homology group within the phylum Proteobacteria (252, 253). P. stutzeri is now recognized as belonging to the class Gamma-	truyền (các Geno-movar) có mối liên hệ chặt chẽ với nhau về mặt phát sinh loài. Một số chủng đã nhận được sự chú ý đặc biệt do có các hoạt động trao đổi chất đặc biệt (chẳng hạn như khử nitơ, phân hủy các hợp chất thơm, và cố định nitơ). Hơn nữa, người ta đã chứng minh rằng một số chất có thể biến đổi tự nhiên và đã nghiên cứu khả năng chuyển đổi của chúng. P. stutzeri phân tán rộng rãi trong môi trường, cư trú trong nhiều loại ổ sinh thái, và đã được phân lập dưới dạng một tác nhân gây bệnh cơ hội ở người. Dựa trên những kết quả thu được trong những năm gần đây, chúng ta sẽ thảo luận về đặc tính sinh học của những loài này. [redacted]	

proteobacteria. Phylogenetic studies of P. stutzeri strains' 16S rRNA sequences and other phylogenetic markers demonstrate that they belong to the same branch, together with related species within the genus, such as P. mendocina, P. alcaligenes, P. pseudoalcaligenes, and P. balearica.

Typically, cells are rod shaped, 1 to 3 ^m in length and 0.5 ^m in width, and have a single polar flagellum.

Under certain conditions, one or two lateral flagella with a short wavelength may be produced. Phenotypic traits of the genus include a negative Gram stain, positive catalase and oxidase tests, and a strictly respiratory metabolism. In addition, P. stutzeri strains are defined as deni- trifiers. They can grow on starch and maltose and have a negative reaction in arginine dihydrolase and glycogen hydrolysis tests.

The G+C content of their genomic DNA lies between 60 and 66 mol%. DNA-DNA hybridizations enable at least

17 genomic groups, called genomovars, to be distinguished. Members of the same genomovar have more than 70% similarity in DNA-DNA hybridizations. Members of different genomovars usually have similarity values below 50%.

Differentiation from Other Species

No fluorescent pigments are produced, which differentiates P. stutzeri from other members of the fluorescent group of Pseudomonas spp. Before the use of genomic approaches to identifying bacteria became widespread, P. stutzeri strains were misidentified with other species. This was due to the intrinsic limitations of exclusively phenotypic identification procedures within the former genus Pseudomonas. P. stutzeri was most commonly confused with other Pseudomonas species (P. men- docina, P.pseudoalcaligenes, P.putida); with species actually in other genera (such as Delftia acidovorans and Ralstonia pick- ettii); or even with the flavobacteria, Alcaligenes or Achro- mobacter. Mandel proposed the species "Pseudomonas sta- nieri" for P.

stutzeri strains with a low G+C content, around 62% (212); however, G+C content alone is a weak parameter for species differentiation. In some collections, P. stutzeri cultures were labeled P. saccharophila. The strain OX1 (ATCC BAA-172) was classified phenotypically as a P. stutzeri strain (13). It has been intensively studied due to its significant phenotypic characteristics. However, when strain OX1 was characterized taxonomically in detail, it turned out to be a member of the P. corrugata phylogenetic branch (73). Pseudomonas sp. strain OX1 may be confused phenotypically with P. stutzeri because P. stutzeri is phenotypically diverse. However, OX1 is genomically distinct. The species most closely related to P. stutzeri is P. balearica (formerly genomovar 6 of the species). It shares many basic phenotypic traits with P. stutzeri strains and belongs to the same 16S rRNA phylogenetic branch. However, it can be differentiated chemotaxonomically from P. stutzeri by its ability to grow above 42°C and by a few other biochemical tests (23).	[redacted]	

P. chloritidismutans is a member of genomovar 3. However, it has been proposed as the type strain of a new species (404) and is discussed below (see "Physiological properties"). There is always a danger of drawing taxonomic conclusions from the properties of metabolic systems that are involved in the metabolism of unusual substrates or molecules. The phylogenies of genes of the rrn operon, considered individually or with other housekeeping genes, demonstrate that all P. stutzeri strains are monophyletic. Such phylogenetic studies are currently another good tool for discriminating P. stutzeri from the rest of the bacterial species. P. xanthomarina has recently been described as a new species (289) with only one representative strain. It is located in the same 16S rRNA phylogenetic branch as P. stutzeri and P. balearica, with sequence similarities above 98%. It can be differentiated phenotypically from both	[redacted]	

species.

DISCOVERY AND NOMENCLATURAL PROBLEMS

In 1952, C. B. van Niel and M. B. Allen stated in their note on the history of P. stutzeri: "During the two decades following the discovery of the denitrification process several notable papers were published on the isolation and characterization of denitrifying bacteria. A study on this literature reveals that Burri and Stutzer (1895) were the first to describe such organisms in sufficient detail to render them recognizable. This applies particularly to their Bacillus denitrificans II, an organism of wide distribution and outstanding characteristics, which has been isolated from straw, manure, soil, canal water, etc., and which students of the denitrification process have considered as a very common and easily identifiable denitrifier" (371).

The different names that this denitrifier has gained since its discovery are well documented in van Niel and Allen's 1952 publication (371). They include Bacterium stutzeri (196), Bacillus nitrogenes (229),

Bacillus stutzeri (68), Achromobacter sewerinii (28), Pseudomonas stutzeri (322), and Achromobacter stutzeri (27). The species "Pseudomonas stanieri" was proposed in 1966 by Mandel for those strains with a G+ C content of around 62% (212). However, no clear differences in phenotype can be found between P. stutzeri and "Pseudomonas stanieri." It is not to be confused with Marinomonas stanieri, formerly considered a Pseudomonas species. The type strain is Lautrop strain AB 201 (equivalent to Stanier 221, ATCC 17588, CCUG11256, DSM 5190, ICMP 12591, LMG11199, NCIB 11358, and WCPPB 1973). In addition, a reference strain has been proposed for each genomovar (Table 1). Some relevant strains that were previously assigned to other species are Pseudomonas perfectomarina strain ZoBell (19), Alcaligenes faecalis A15 (380), and Flavobacterium lute-scens strain ATCC 27951 (24). Many, but not all, strains have been deposited in publicly recognized culture collections, are available for scientific research, and should be used as	[redacted]	

reference strains.

OCCURRENCE AND ISOLATION PROCEDURES

Detection of P. stutzeri basically relies on two methods: (i) enrichment and isolation of pure cultures and (ii) direct analysis without the need for culturing. Both methods are essential to autoecological studies and to understanding the role of the species in the environment.

An elective culture method for the specific enrichment of denitrifiers and the isolation of P. stutzeri was developed by Iterson in 1902 (described in 1952 by van Niel and Allen [371]). A mineral medium with 2% nitrate under anaerobic conditions and tartrate (or malate, succinate, malonate, citrate, ethanol, or acetate) leads to a predominant population of P. stutzeri, even when some isolates are not able to grow on tartrate in pure culture. Tartrate may be converted anaerobically to an

assimilable substrate by other bacteria in the sample.

A selection of cells producing colonies with the unusual morphology of P. stutzeri permits an efficient isolation procedure from environmental samples. Incubation temperatures of 37°C or above allow a more selective enrichment, which can be combined with denitrifying conditions.

DNA methods based on 16S rRNA sequences have been also designed to detect P. stutzeri in DNA extracted directly from environmental samples. Bennasar et al., in 1998, developed PCR primers that were specific to all known genomovars of P. stutzeri at that time (24). This served as a confirmation test, as did amplicon cleavage using the restriction enzyme HindIII or a specific DNA probe targeted at the amplified product (24).

Amann et al. considered the difficulty of obtaining a DNA probe to cover all of the P. stutzeri strains (5). However, they designed a DNA probe for specific 23S rRNA sequences.

This is useful in fluorescence in situ hybridization techniques to detect and quantify P. stutzeri in environmental samples. Nevertheless, not all strains can be detected, due to the high genetic diversity of the species, including the rrn operon. Besides the rrn genes, other genes are now used for functional analysis of ecosystems. These genes also detect P. stutzeri. They include nirS or nosZ for detecting denitrification (46) and nifH for analyzing diazotrophic bacteria in the rhizo- sphere (93). The usefulness of a conserved nosZ probe for screening the distribution of denitrifying bacteria with similar N2o reductases in the environment has been described elsewhere (65, 386). In 2001, Griintzig et al. developed a very sensitive method based on real-time PCR analysis of DNA isolated from soil and sediment samples (132). However, not all DNAs of the species' strains could be amplified. Specific primers for PCR and an internal probe of the	[redacted]	

denitrification gene nirS enabled less than 100 cells per g of sample to be quantified. In their analysis of P. stutzeri populations in marine waters, Ward and Cockcroft used monoclonal antibodies raised against outer membrane proteins of the strain ZoBell (388). ZoBell originally named this strain "Pseudomonas perfectomarina." Sikorski et al. were able to isolate members of P. stutzeri from aquatic habitats and terrestrial ecosystems in a two-step procedure. Firstly, the occurrence of P. stutzeri cells was assessed by a previously designed, slightly modified PCR procedure (24, 325). Secondly, the positive samples were screened TABLE 1. P. stutzeri strains cited in the text, with relevant characteristics, origins, and references for P. stutzeri by means of plating on an artificial seawater medium with ethylene glycol, starch, or maltose as the carbon source under aerobic conditions (325). The	[redacted]	

characteristic colony morphology of P. stutzeri led to a highly efficient isolation procedure: one P. stutzeri colony was detected among 9,100 colonies of other bacteria.

However, many strains of P. stutzeri that have been studied in detail were isolated by their metabolic peculiarities. They were not specifically isolated for denitrification ability or because P. stutzeri was the target of the study.

PHENOTYPIC PROPERTIES

Apart from the 1952 study by van Niel and Allen, the only papers containing detailed descriptions of P. stutzeri's phenotypic properties are those by Stanier et al. in 1966 and Ros- sello-Mora et al. in 1994 (295, 340, 371).

Strains of P. stutzeri, like most recognized Pseudomonas spp., can grow in minimal, chemically defined media, with ammonium ions or nitrate and a single organic molecule as the sole carbon and energy source. No additional growth factors are required. Some P. stutzeri strains can grow

diazotrophically. This characteristic seems to be rare among the genus Pseudo-monas. None of the strains tolerate acidic conditions: they do not grow at pH 4.5. P. stutzeri has a respiratory metabolism, and oxygen is the terminal electron acceptor. However, all strains can use nitrate as an alternative electron acceptor and can carry out oxygen-repressible denitrification. Denitrification may be delayed or may appear only after serial transfers in nitrate media under semiaerobic conditions (73, 340). Oxidative degradation of aromatic compounds involves the participation of mono- and dioxygenases. Typically, catechol or pro- tocatechuate is the central intermediate in this reaction. Each is cleaved through an ortho pathway when no accessory genes are involved in the degradation. Amylolytic activity is one of the phenotypic characteristics of the species.	[redacted]	

The enzymology of the exo-amylase—which is responsible for the formation of maltotetraose as an end product—has been examined at the molecular level. This enzyme has also been cloned (231). Ob- rador and Aguilar demonstrated that polyethylene glycol was degraded to yield ethylene glycol, a substrate typically used by P. stutzeri strains (241).

The arginine deiminase system ("dihydrolase") catalyzes the conversion of arginine to citrulline and of citrulline to ornithine. It has been used by taxonomists to differentiate species.

All P. stutzeri strains give a negative test result for this reaction. They also fail to use glycogen and do not liquefy gelatin.

Colony Structures/Types

Colonies can be distinguished by their unusual shape and consistency (Fig. 1). Freshly isolated colonies are adherent, have a characteristic wrinkled appearance, and are reddish brown, not yellow, in color. They are typically hard, dry,

and tenaciously coherent.

It is easy to remove a colony in its entirety from a solid surface. Colonies generally resemble craters with elevated ridges that often branch and merge, and they have been described as tenacious, with a coral structure. There may be more mucoid protuberances at the periphery than in other areas. The frequent occurrence of irregular polygon-like structures or concentric zones has also been noted (371). The

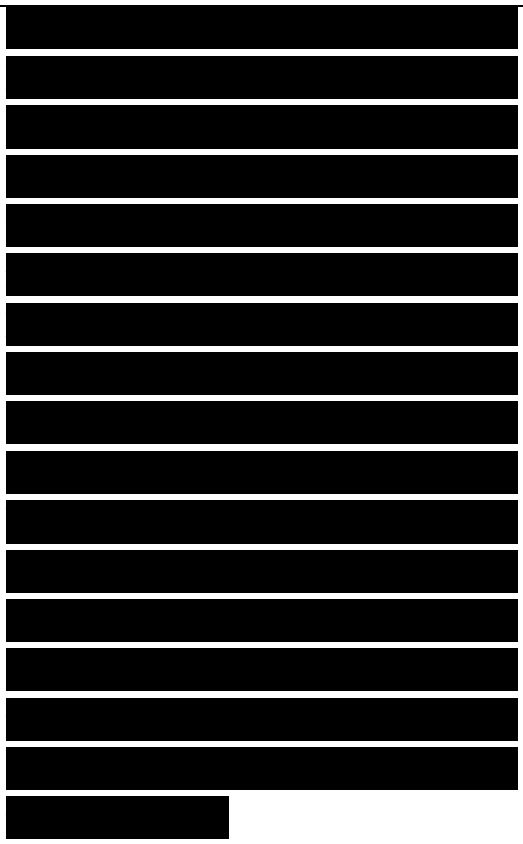


FIG. 1. Colonial morphology. Several typical colonial morphologies of P. stutzeri strains. (The image in panel A was taken from reference 371.)

shapes of colonies are neither uniform nor necessarily constant: they change appearance with time. After repeated transfers in laboratory media, colonies may become smooth, butyra- ceous, and pale in color. This has been described as colonial dissociation. Strain CMT.9.A hydrolyzes agar. This is a rare property and is mainly

restricted to marine bacteria. However, the attack may be limited to what is known as "pitting" of the agar (3). Sorokin et al. give a very detailed description of the colonial morphology, differentiating between R-type and S- type colonies (337). The R-type colonies are stable, but the S type produces both colony types under appropriate conditions. Smooth colonies grown on plates at 30°C and stored at 4°C for 24 h often develop a characteristic wrinkled appearance (A. Cladera, personal communication). P. stutzeri is grouped with the nonpigmented species of the genus, even though many strains' colonies become dark brown. This is due to the high concentration of cytochrome c in the cells. No diffusible pigments are produced on agar plates. Morphological Characterization (Cells, Reserve Materials, Flagella, and Pili) and Chemotaxis Cells are typically motile and predominantly monotrichous. In some strains, lateral flagella	[redacted]	

with a short wavelength are also produced. This particularly occurs in young cultures on complex solid media. These lateral flagella could easily be shed during manipulations incidental to flagellar staining (251). It has been suggested that lateral flagella might be involved in the population's swarming or twitching motility on solid surfaces (319). However, type IV pili may also be responsible for this movement. Statistically, the highest number of flagellated cells is reached at the beginning of the exponential growth phase (192). Seventy percent of cells were flagellated in strain AN11: 38% had only one flagellum, and 31% had one or more additional flagella inserted laterally (80).

Caution should be exercised when only phenotypic traits are used for classification. This can clearly be seen in the case of strain ZoBell. This strain (ATCC 14405) was isolated as a marine bacterium and described by ZoBell and Upham as "Pseudomonas perfectomarinus" in 1944 (412). Subsequently, this organism became the only member of the species P. per-

fectomarina. Its lack of flagella was emphasized by its assignation to a new species, although the authors who first described this strain stated that it was motile (19, 412). After three passages, enrichment for flagellated bacteria on semisolid tryp- tone agar enabled a population in which over 80% of cells were flagellated to develop. This revertant strain is motile by means of a single polar flagellum (294). In a recently published chapter on chemotaxis in Pseudomonas, Parales et al. stated, "All Pseudomonas species are motile by one or more polar flagella and are highly chemotactic" (258). P. stutzeri is no exception. Chemotaxis machinery has not been studied in detail for any Pseudomonas species. Moreover, the ranges of attractants or repellents and environmental conditions to which Pseudomonas spp. respond remain largely unexplored. They seem to be attracted to virtually all of the organic compounds they can use as growth substrates.	[redacted]	

However, they are also attracted to other compounds that they are unable to metabolize. ortega-Calvo et al. studied the chemo- tactic response of several pseudomonads to polycyclic aromatic hydrocarbon-degrading bacteria (243). Strain 9A of P. stutzeri was included in the study. This strain degrades naphthalene, phenanthrene, and anthracene. It was concluded that chemo-taxis was positive to naphthalene and to the root exudates of several plants. Chemotaxis may enhance the biodegradation of pollutants in the rhizosphere, at least in laboratory-scale mi-crocosms. Strain KC mineralizes carbon tetrachloride, and motility-enhanced bioremediation in aquifer sediments has been demonstrated (401, 402). Pseudomonas species have a range of different adhesins that function during initial attachment to a substratum. This leads to biofilm formation. Both flagella and pili seem to be important in the colonization of biotic and abiotic surfaces, particularly in the initial formation of microcolonies. P. aeruginosa's initial biofilm development	[redacted]	

appears to be conditionally dependent on type IV pili. P. stutzeri possesses both flagella and pili but has not been described as a member of consortia that form natural biofilms. Type IV pili confer twitching motility to P. stutzeri strains (a bacterial movement based on pilus extension/ retraction). This is probably at least partly responsible for many colonies' diffuse borders (J. Sikorski, personal communication). These colonies also correspond to strains that have natural transformation ability. Chemical Characterization and Chemotaxonomy DNA base composition. The G+C content of DNA is a useful characteristic in taxonomy for delineating species. It has been proposed that if two strains differ by more than 5% in G+C content, then they should not be allocated to the same species (297). The limit for genus differentiation may be 10%. G+ C content in P. stutzeri strains has been determined by the thermal denaturation temperature of the DNA and by enzymatically hydrolyzing the	[redacted]	

DNA and subsequently analyzing it by high-performance liquid chromatography. Reported values vary widely: 60.7 to 66.3 mol% (251) and 60.9 to 65 mol% (291). However, variations are within the accepted limits for members of the same species. The distribution of values was initially considered to be bimodal. This led to the suggestion that P. stutzeri might be split into two species (212). Nevertheless, the inclusion of novel strains resulted in a Gaussian distribution. Protein patterns. Whole-cell protein patterns obtained by denaturing polyacrylamide gel electrophoresis (PAGE) are highly characteristic at the strain level. They have been used for typing and classification purposes (265). P. stutzeri strains have been found to be particularly heterogeneous (271, 295). Computer-assisted analysis of the protein bands creates a dendrogram that is in good agreement with the genomovar subdivision of the species (366). This result is not surprising, as whole-cell protein patterns reflect the protein-encoding genes in the whole genome and the geno-	[redacted]	

movars were defined by the similarity values of total DNA-DNA hybridizations.

LPS and immunological characteristics.

Lipopolysaccharide (LPS) is the main antigenic molecule on the cell surface. This is considered to be the heat-stable o-antigen of the genus. The specificity of antibodies is related to the composition of the poly-saccharide chains projecting outside the cells. Representative P. stutzeri strains of the seven known genomovars on which experi-ments were done showed marked serological diversity. This par-allels the LPS O side-chain heterogeneity between strains. In the study by Rossello et al., antigenic relatedness was observed only between closely related strains of the same genomovar (292).

Outer membrane proteins analyzed by sodium dodecyl sul- fate-PAGE gave very similar results for all strains tested, regardless of genomovar ascription. Likewise, similar results were attained for immunoblotting using polyclonal antisera against six representative strains' whole

cells. However, a similar procedure, based on Western blotting and immunological fingerprinting of whole-cell proteins using the polyclonal antibody Ab160, raised against Pseudomonas fluorescens MT5— called Westprinting (360)—produced a typical protein profile for each strain. Computer-assisted comparisons revealed a distribution in groups that agreed with the strains' genomovar distribution at different similarity levels (25). Fatty acid composition. Fatty acid composition is a very good taxonomic marker for distinguishing the genus from other genera formerly included in Pseudomonas (e.g., Burk-holderia). These chemotaxonomic characteristics are very useful for identification purposes. Studies of the fatty acid composition of Pseudomonas species (158, 246, 341, 367) revealed that the straight-chain saturated fatty acid C16:0 and the straight-chain unsaturated fatty acids C16:1 and C18:1 were the most abundant. These	[redacted]	

account for 82.3% of total fatty acids in P. stutzeri. Minor quantities of the hydroxylated fatty acids 3-OH 10:0 and 3-OH 12:0 were also detected (295). There were no significant differences between genomovars in the other fatty acids. Members of genomovar 6 had a higher content of cis-9,10-methylenehexadecanoate (17:0) and cis-9,10-methyleneoctadecanoate (19:0). This chemotaxonomic partic-ularity, together with other characteristics, helped to distinguish genomovar 6 as a new species, Pseudomonas balearica (23). Fatty acid composition must be determined under strictly controlled growth conditions, as it is highly dependent on growth substrates. Mrozik et al. describe the changes in fatty acid composition in strains of P. putida and P. stutzeri during naphthalene degradation (232, 233). The reaction of both strains to the addition of naphthalene was an increase in the saturated/unsaturated ratio and alterations in the percentage of hydroxy, cyclopropane, and branched fatty acids. New fatty acids were detected when the strains were exposed to naphthalene.	[redacted]	

Quinone and polyamine composition. The determination of polyamine and quinone composition is a rapid chemotaxo- nomic identification tool. Putrescine is the main component of all members of the genus Pseudomonas (57). Two major polyamines were detected in P. stutzeri: putrescine (35.0 to 92.7 ^mol/g [dry weight]) and spermidine (8.9 to 29.2 ^mol/g [dry weight]). Other polyamines were detected in very small amounts only (1,3-diaminopropane, cadaverine, and spermine) (293). Ubiquinone Q-9 is the only quinone present in all of the P. stutzeri strains studied. PHA. P. stutzeri cells do not accumulate polybetahydroxybu- tyrate. However, the production of novel polyhydroxyalkano- ates (PHA) by one strain of the species (strain 1317) has been demonstrated (141). This strain was isolated from oil-contaminated soil in an oil field in northern China. Another P. stutzeri strain, YM1006, has been isolated from seawater as	[redacted]	

a poly(3- hydroxybutyrate)-degrading bacterium, although it does not seem to be able to accumulate this reserve material. The extracellular polybetahydroxybutyrate depolymerase gene (phaZPst) has been well characterized (242). Some combinations of unusual phenotypic properties can be very helpful in the preliminary assignment of newly isolated strains to certain species. Alternatively, the absence of one or more of the set's properties suggests that the strain should be excluded from the taxon. For example, in addition to the basic characteristics of a Pseudomonas species, the following characteristics strongly suggest that a culture is a strain of Pseudo- monas stutzeri: denitrification with copious gas emission; the formation of dark, folded, coherent colonies; and the capacity to grow at the expense of starch, maltose, or ethylene glycol. However, in our laboratories we have found that enrichment conditions frequently yield cultures lacking one or more of the key characteristics mentioned above. Such enrichment	[redacted]	

conditions included the use of aromatic compounds and some of their halogenated derivatives as the sole carbon and energy sources. Although the general phenotypic properties of these cultures could be used a priori as an argument for excluding them from the species, it was surprising to find that some of them were phylogenetically very similar to P. stutzeri. This is probably true in the case of a strain ascribed to Pseudomonas putida in a patent for the mineralization of halogenated aromatic compounds (U.S. patent no. 4,803,166, 7 February 1989). Its DNA sequences most probably indicate its affiliation to P. stutzeri. Detailed analysis of atypical phenotypes (such as the absence of either motility or denitrification) demonstrated in some cases that the characteristic was cryptic and could be expressed when the cells were adapted. An interesting example of variation to be taken into consideration may be the lack of folded colonies, which, in principle, is taken as an important primary criterion for	[redacted]	

the isolation. In fact, the discovery of P. mendocina at the University of Cuyo, Mendoza, Argentina, was linked to isolations of smooth colonies of Pseudomonas which at first were taken to be biovars of P. stutzeri.

GENOMIC CHARACTERIZATION AND PHYLOGENY DNA-DNA Hybridizations

The genomovar concept was originally defined for P. stutzeri as a provisional taxonomic status for genotypically similar strains within a bacterial species. Two strains classified phenotypically as members of the Pseudomonas stutzeri species were included in the same genomovar when their DNA-DNA similarity values were those generally accepted for members of the same species (more than 70% similarity or less than 5°C difference in thermal denaturation temperature [ATm] values). Members of two different P. stutzeri genomovars have 15 to 50% DNA-DNA similarity values or ATm value differences greater than 5°C. Subsequently, this concept has been used taxonomically to group genotypically similar strains in

other species, such as Burkholderia cepacia and species in the genera Xanthobacter, Azoarcus, and Shewanella, etc. It provides a useful provisional level of classification. The methods used to calculate DNA-DNA similarity values have differed from one laboratory to another. Palleroni used 125I labeling and/or membrane filters (251). Rossello et al. used the ATm method, as described previously (291). Sikorski et al. used the method described by Ziemke et al. (411), with digoxi- genin and biotin labeling and quantification of the binding ratio in microtiter plates (327). Vermeiren et al. used DNA- DNA thermal reassociation, measured photometrically (380). The results were consistent with the genomovar subdivision of the species, regardless of the method used to estimate the similarity value. To date, nine different genomovars have been well documented. Eight new genomovars in the species P. stutzeri were put forward recently (327). one reference	[redacted]	

strain has been proposed for each genomovar and deposited in culture collections. Most strains studied so far are included in genomovar 1 (along with the species' type strain). The genomovars 8 (strain JM300), 9 (strain KC), 10 (strain CLN100), and 18 (strain MT-1) each have only one representative strain.

These might be considered genomospecies, sensu Brenner et al. (50). As an example, we can consider strain CLN100, of genomovar 10. It is a representative of a new species from a genomic perspective, sharing many substantial phenotypic and phylogenetic characteristics with members of the P. stutzeri phylogenetic branch. Some phenotypic traits can be used to discriminate CLN100 from the P. stutzeri and P. balearica strains described to date (simultaneous degradation of chloro- and methyl-de- rivatives of naphthalene and absence of ortho cleavage of cat-echol, etc.). These characteristics could be the basis for describing CLN100 as the type strain of a new species. However, some of these phenotypic traits could be

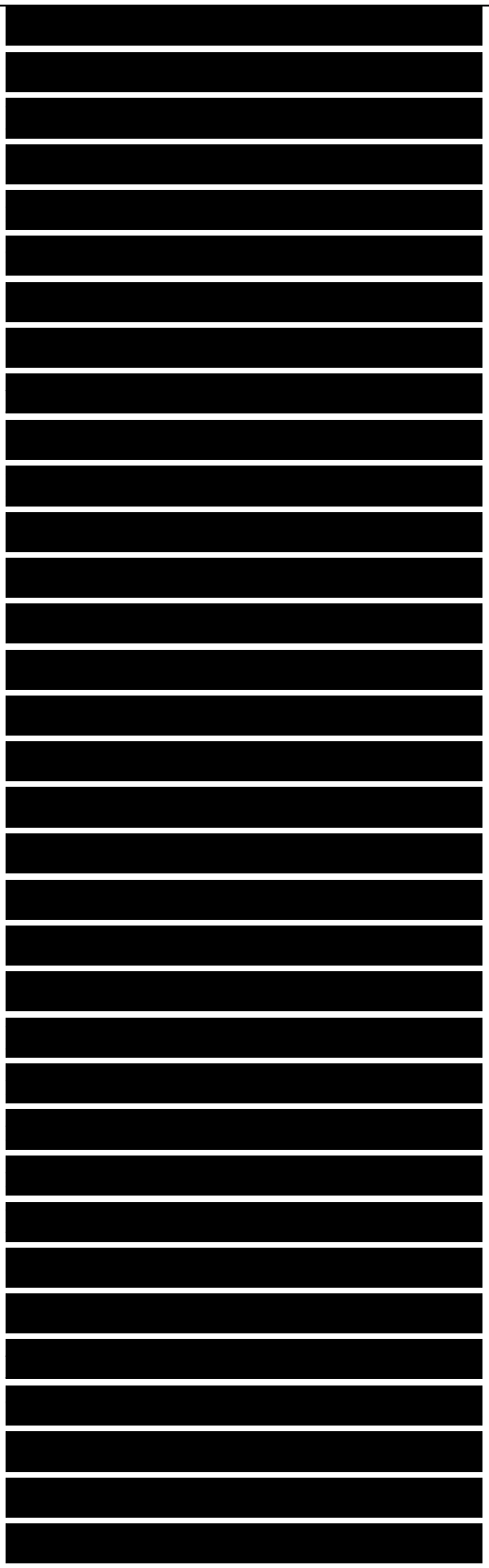
strain specific; therefore, it was preferred not to define a new species until more strains that are genomically and phenotypically similar to strain CLN100 have been described (114).

Genome Size and Organization

Information on genome structure is a very important component of any comprehensive bacterial description. The comparative analysis of bacterial chromosomes on intra- and interspecies levels can provide information about genomic diversity, phylogenetic relationships, and chromosome dynamics. In the genus Pseudomonas, genome structure has been studied only for P. aeruginosa, P. fluorescens, P. putida, and P. stutzeri. Ginard et al. studied 20 strains of P. stutzeri in 1997, representing the seven genomovars known at that time (121). They also studied P. stutzeri's closest relative, P. balearica. The genome of P. stutzeri strains is made up of one circular chromosome. It ranges from 3.75 to 4.64 Mb in size (20% difference in size). In comparison, P. aeruginosa genome sizes, calculated by macrorestriction analysis,

range from 6.345 to 6.606 kb, a fluctuation of only about 4%.

However, a more recent report on P. aeruginosa genome sizes indicates a 20% fluctuation (from 5.2 to 7.1 Mb) (310). The I-CeuI, PacI, and Swal low- resolution map of P. stutzeri's type strain enabled 12 genes— including four rrn operons—and the origin of replication to be located (121). The 20 strains' enzyme digests were used to compare rrn backbone organization within the genomovars. The four rrn operons seemed to be at similar locations with respect to the origin of replication, as did the rest of the six genes analyzed. In most genomovar reference strains, rrn oper- ons are not arranged around the origin of replication but are equally distributed along the chromosome. Large chromosomal rearrangements and differences in genome size seem to be responsible for the differences in genome structure. This suggests that they must have played an important role in P. stutzeri diversification and niche colonization. Strains belonging to the same genomovar have similar genome architectures that are well correlated with



phylogenetic data (121). From one to four plasmids were detected in 10 of the 20 strains analyzed in this study (121). The Eckhardt method, using both conventional and pulsed-field gel electrophoresis, turned out to be the most reliable and useful technique for plasmid detection. Seventy-two percent of the plasmids observed were smaller than 50 kb, one plasmid was between 50 and 95 kb, and four plasmids were larger than 95 kb. No two strains shared the same plasmid profile, and no relation was found between genomovars and the distribution of plasmids among the strains. Seven of the 10 plasmid-containing strains were isolated from polluted environments. This is not uncommon in plasmid analyses. A correlation between the degree of contamination and the incidence of plasmid occurrence was found in an environmental study by Baya et al. (20). Naphthalene degradation plasmids are common in Pseudomonas spp. However, in eight of the nine naphthalene-degrading strains of P. stutzeri studied, the catabolic genes were inserted	[redacted]	

into an I-CeuI chromosomal fragment, as demonstrated by Southern blot hybridizations with nahA and nahH probes. The naphthalene genes seem to be plasmid encoded only in strain 19SMN4 (120, 296).

Genotyping

Genotypic intraspecies relationships in P. stutzeri strains have been determined by various genotyping methods. These are based on restriction fragment length polymorphism (RFLP) analysis of total DNA, PCR amplification of selected genes, or PCR amplification and restriction analysis. These analytical methods differ in discrimination level between strains. They have been applied simultaneously to all P. stutzeri genomovars' reference strains; to P. balearica, the strains most closely related to P. stutzeri; and to related type strains of the genus Pseudomonas. In all methods, computer-assisted analysis generates dendrograms that confirm the consistency of strain clustering with the genomovar subdivisions of the species.

Additional typing by multilocus enzyme electrophoresis (MLEE) and multilocus sequence typing (MLST) is discussed below.	[redacted]	
Methods based on the electrophoretic patterns of macrorestriction fragments (low-frequency restriction fragment analysis) have been used by two independent groups to examine representative strains (121, 271). The restriction enzymes XbaI and SpeI cut the P. stutzeri genome of the strains studied into 20 to 48 fragments. These fragments were resolved by pulsed- field gel electrophoresis. They are useful for generating fingerprints, which can be used to explore genome structures and to determine the degree of relatedness of strains. No correlation was found between the similarity of macrorestriction patterns and the subdivision of the species into genomovars. This was due to the high discriminatory power of the two enzymes and the heterogeneity of the restriction patterns. However, some patterns allowed clonal	[redacted]	

variants between strains to be distinguished. In these cases the related strains belonged to the same genomovar. The marked heterogeneity was attributed, at least in part, to large chromosomal rearrangements (121).

In the ribotyping procedure, total DNA is purified and then cleaved by restriction endonucleases. Brosch et al. (51) used the enzymes SmaI and HincII in their study of Pseudomonas strains. Restriction fragments were separated by electrophoresis, transferred to a nylon membrane, and hybridized with a 16S-23S rRNA probe. Nine strains of P. stutzeri clustered together in the dendrogram, which also showed 217 other strains from different Pseudomonas species. Two identical bands were detected by HincII in P. stutzeri. SmaI profiles were more discriminative, distinguishing from four to eight bands. Members of a single genomovar were grouped in the same branch.

Bennasar et al. (25) revealed genetic diversity and the relationships among P. stutzeri strains by rapid molecular typing methods. Repetitive

extragenic palindromic PCR and enterobacterial repetitive intergenic consensus PCR analyses, based on DNA consensus sequences, generated fingerprints that were then computer analyzed. Groupings were consistent with the genomic groups that had previously been established by DNA-DNA hybridizations or 16S rRNA sequencing. Members of other Pseudomonas species were clearly different. Sikorski et al. (325) carried out random amplified polymorphic DNA (RAPD) PCR analysis in their study of P. stutzeri isolates from marine sediments and soils in geographically restricted areas (local populations). The results demonstrated the complex composition and high strain diversity of the local populations studied. Similar genomic relationships have been revealed by PCR amplification of several genes (16S rRNA, internal transcribed spacer region 1 [ITS1], ITS2, and rpoB) and by analyzing the RFLPs generated by several restriction enzymes (25,133, 325). These methods have confirmed the high	[redacted]	

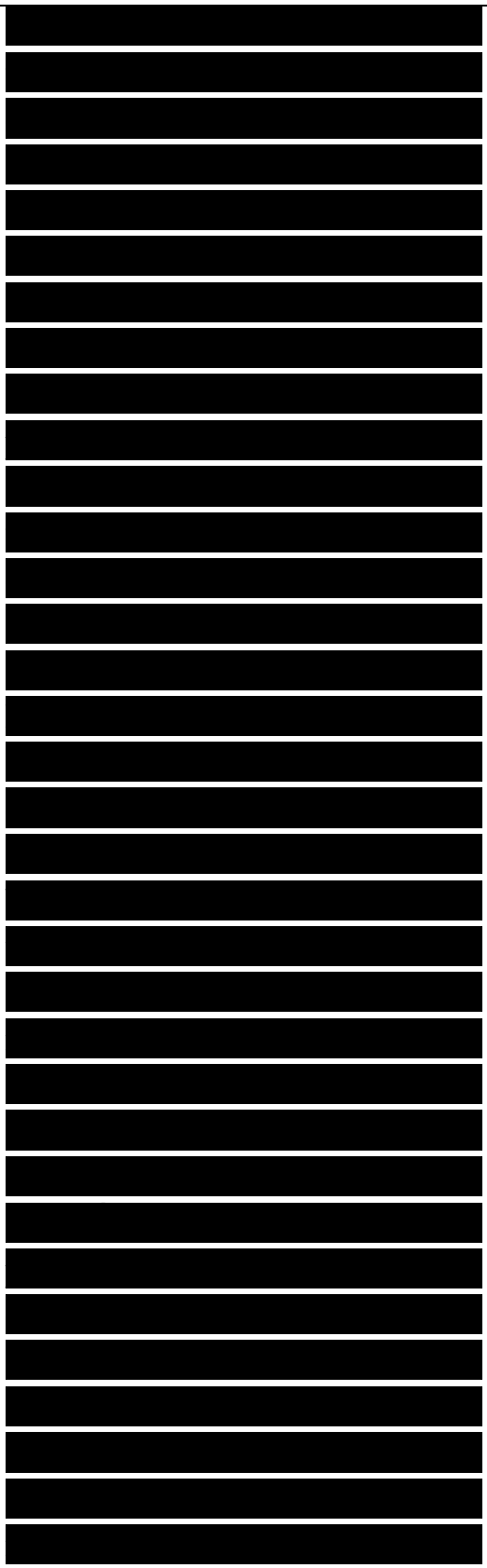
genetic diversity of the species, the consistency of genomic groups (genomovars), and the usefulness of the patterns generated for strain identification. Genetic Diversity: MLEE Knowledge of the genomic structure of a population is essential to thoroughly understanding a species' characteristics. Such knowledge is particularly important in studies of population dynamics or habitat colonization, as it is used to elucidate genetic exchange in natural populations. The MLEE technique involves determining allozyme variation in a variety of housekeeping enzymes. Codon changes within enzyme genes, leading to amino acid substitutions, are detected electro- phoretically by this technique (314). Thus, the variation in chromosomal genes is recorded, and the degree of gene transfer within a species is estimated. This enables relationships between bacterial isolates to be determined and a phylogenetic framework to be constructed. Two independent research groups have used the MLEE approach in studies of P.	[redacted]	

stutzeri (284, 324). In Sikorski's study, 16 P. stutzeri strains belonging to eight different genomovars were analyzed for the allelic profiles of 21 enzymes. A distinctive multilocus genotype was detected in all strains, and up to 11 alleles were detected per locus. In Rius's analysis, 42 P. stutzeri strains from nine genomovars (including 9 strains previously studied by Sikorski et al.) and 20 enzymes were studied.	[redacted]	
The highest number of different alleles found per locus was 32, and all multilocus genotypes were represented by a single strain. Forty-two electrophoretic types were detected. In both analyses, P. stutzeri was shown to have a highly polymorphic structure. If both groups' results are combined, 49 different P. stutzeri strains have been studied with MLEE. A total of 33 different enzymes were analyzed from these strains. An analysis of this set of 49 strains again demonstrates that all of the multilocus genotypes were represented by a single strain. MLEE studies reveal that P. stutzeri is highly polymorphic.	[redacted]	

The highest genetic diversity described for a species is revealed (284) by an analysis of the members of genomovar 1 only. An analysis of source and place of isolation showed no clear association in clusters. When two subgroups of P. stutzeri populations (clinical and environmental isolates) were compared, the mean levels of genetic diversity were not significantly different. This indicates that clinical strains come from the same populations as environmental isolates. This may have important epidemiological implications for the microbiology of P. stutzeri infections. However, when two strains were grouped at moderate genetic distances (below 0.55), each pair of strains belonged to the same genomovar.

Genetic Diversity: MLST

MLST has been proposed as a good method for population genetic analysis and for distinguishing clones within a species (98). This method employs the same principles as MLEE, as it detects neutral genetic variation from multiple

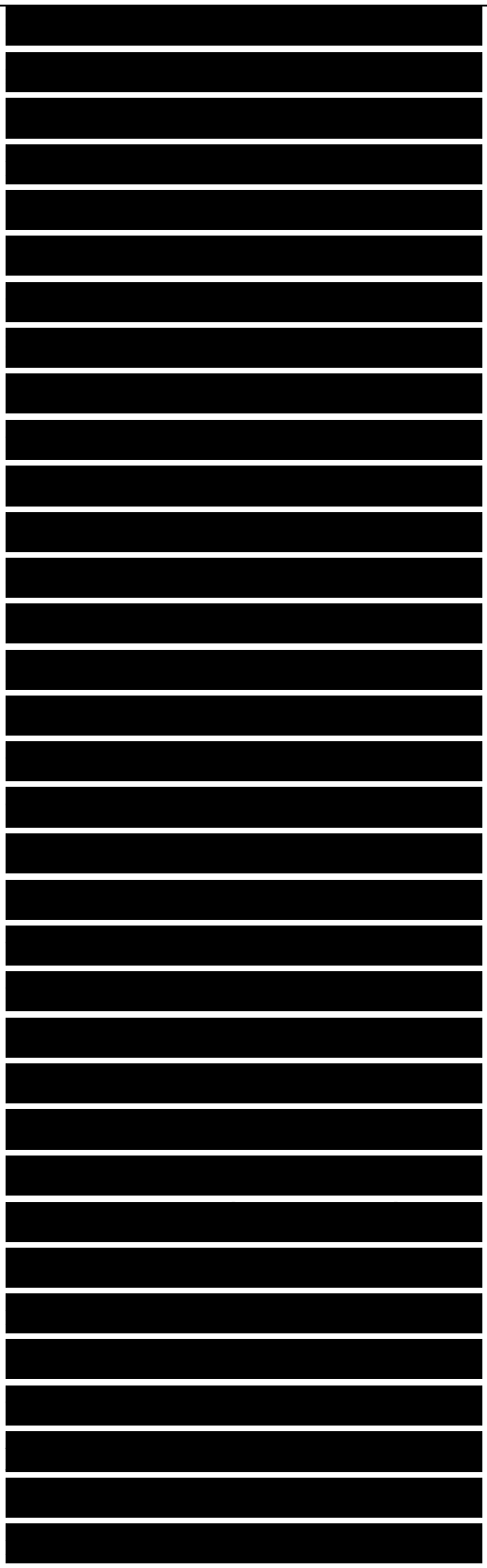
chromosomal locations. This variation is identified by nucleotide sequence determination of selected loci. Cladera et al. (72) attempted to differentiate P. stutzeri populations and to establish the genetic diversity and population structure of the species clearly. They carried out a comparative analysis of gene fragments, using the principles of multilocus sequence analysis. The genes were selected from 26 strains belonging to nine genomovars of the species and from P. balearica strains, the species most closely related to P. stutzeri. Seven representative chromosomal loci were selected, corresponding to three kinds of genes: (i) housekeeping genes that are universally present in bacteria (16S rRNA and ITS1 region, representing the rrn operon, and the gyrB and rpoD genes, which interact with nucleic acid metabolism, coding for gyrase B and DNA-directed RNA polymerase, respectively) and which have been included in previous Pseudomonas taxonomic studies (408); (ii) genes that are characteristic of the species (catA, coding for catechol 1,2-dioxyge-nase, an enzyme responsible for the ortho cleavage of catechol in		

species of RNA group I of Pseudomonas, and nosZ, nitrous oxide reductase, a metabolically characteristic gene defining this denitrifying species); and (iii) nahH, coding for catechol 2,3-dioxygenase, responsible for the meta cleavage of catechol, a gene that is considered to be plasmid encoded in the genus Pseudomonas but chromosomally encoded in most naphthalene-degrading P. stutzeri strains studied to date (296). All loci were highly polymorphic in the 26 strains studied. The number of nucleotide substitutions per nucleotide site varied from 44.2% for catA to 21.8% for nahH. The number of alleles varied in the different loci: 4 in nahH (16 strains), 18 in catA (24 strains), 20 in gyrB (26 strains), 17 in rpoD (26 strains), 18 in nosZ (26 strains), 15 in 16S rRNA (26 strains), and 20 in ITS1 (26 strains). Apart from nahH (a gene that is probably acquired through lateral transfer), the mean number of alleles per locus in the 26 strains was 18.7, an extremely high value. The average number of alleles per locus and strain was 0.72.	[redacted]	

In this MLST study (72), the dN/dS ratio—the ratio of non-synonymous substitutions per nonsynonymous site which resulted in an amino acid replacement (dN) to synonymous substitutions per synonymous site that did not change the amino acid (dS)—was calculated for the genes encoding proteins as a measure of the degree (amount and type) of selection in P. stutzeri populations. Changes are selectively neutral when they are independent of the overlying phenotype and the selection pressure dictated by the phenotype's function. The ratio was less than 0.1 in three genes (gyrB, rpoD, and nosZ). The highest dN/dS ratio corresponded to catA (0.18). All ratios were much less than 1, indicating that these gene fragments are not under selection. In other words, most of the sequence variability identified is selectively neutral. Synonymous substitutions were at least 5.5 times (1/0.18) more frequent than amino acid changes at any locus. The number of nucleotide substitutions per nucleotide site was higher than in Campylobacter jejuni,	[redacted]	

Neisseria meningitidis, Streptococcus pneumoniae, Enterobacterfaecium, and species of the Bacillus cereus complex. To our knowledge, the number of nucleotide substitutions described for P. stutzeri is the highest recorded to date (145). The average numbers of alleles per locus and strain analyzed in the protein-coding genes were 0.72 for P. stutzeri (an average of 18.7 alleles per locus in only 26 strains), 0.18 for C. jejuni, and 0.43 for the B. cereus complex. These values are in good agreement with previous observations made in MLEE studies of most of the strains analyzed by the MLST technique. In such MLEE studies the genetic diversity was the highest described for a species (284). Therefore, the extremely high genetic diversity of the species manifested by MLEE was corroborated by the MLST study.

Figure 2 shows an analysis of the sequence types (STs) identified among 26 independent strains of P. stutzeri. This analysis led to the assumption that one different ST per strain can be detected. This is the highest possible number of STs. Remarkably,

when two strains had an allele in common they belonged to the same genomovar. There was only one exception: strain JM300 (genomovar 8) has an rpoD allele that is identical to strain JD4, one of the two members of genomovar 5. This can be explained by genomovars 5 and 8 having a common ancestor or by a possible lateral gene transfer to JM300, a strain intensively studied due to its natural transformation (206). Another strain, AN10 of genomovar 3, presents a possible recombination event with members of the same genomovar. Strains 19SMN4 and ST27MN3, of genomovar 4, were very closely related in the multilocus sequence analysis. They had identical 16S rRNA, rpoD, and gyrB genes. Both strains were isolated as naphthalene degraders from samples taken in a wastewater treatment lagoon. However, they were from different habitats (water column and sediment). Molecular typing methods (25, 121, 133) and MLEE (284) had previously demonstrated that both strains were genetically related but different.		

Again, the enormous genetic diversity of the species was demonstrated in this study. Inclusion of nahH in the analysis modifies the topography of the graph, indicating more possible events of lateral gene transfer (Fig. 2).

Phylogeny

Several genes have been used as phylogenetic markers in P. stutzeri studies. The most extensively used are the rRNAs, 16S rRNA in particular. However, other genes with different degrees of sequence variation have been studied, because they provide useful information for analyzing different phylogenetic levels. Internal transcribed spacer regions ITS1 and ITS2, between the 16S and 23S rRNAs and between the 23S and 5S rRNAs, respectively, in the rrn operon present more-variable positions and are most useful in determining close relationships. Recently, Yamamoto et al. (408) studied the sequences of other housekeeping genes (gyrB and rpoD). These genes are assumed to be less constant than the 16S rRNA molecule

among species of the genus Pseudomonas. In most cases, the study confirmed the phylogenetic branches that were previously defined by the 16S rRNA sequences in the genus.

Phylogenetic tree reconstructions of the same genes used in the MLST method (16S rRNA, ITS1, gyrB, rpoD, nosZ, and catA) were undertaken by Cladera et al. (72). Stability analysis using bootstrap resampling showed that the trees were stable and well defined. Most strains of P. stutzeri clustered in the same phylogenetic branch in the gene trees analyzed. They were usually separated from the other closely related species considered, P. balearica and P. mendocina. Strains belonging to the same genomovar were usually located in the same branch. There were only a few exceptions, which varied depending on the gene analyzed. A consensus phylogenetic tree was constructed for the six genes to deduce a composite molecular phylogeny for P. stutzeri. All P. stutzeri strains are located in the same phylogenetic branch, and members of each genomovar are clustered together, maintaining the

genomovar subdivision of the species. This tree is based on a sequence of no less than 4,551 nucleotides, representing at least 9,546 nucleotides from the respective genomes, as there are four copies of the rrn operon in P. stutzeri. Therefore, between 0.2 and 0.25% of the chromosome (depending on the strain's genome size) has been compared pairwise in 24 independent isolates.

Clonality

There is enormous genetic diversity in P. stutzeri. Despite this, the topologies of the trees and the values of the housekeeping genes' association indices, calculated from MLEE and MLST analyses, indicate that horizontal gene transfer and recombination processes are not enough to disrupt allele associations. This is because there is still a strong linkage disequilibrium among the P. stutzeri isolates. These results suggest that the population structure of P. stutzeri is strongly clonal, indicating that there is no significant level of recombination through independent assortment that might destroy linkage disequilibrium. Some authors

have suggested that recombination events explain some of the diversity found in P. stutzeri (324).

However, results of studies by Rius et al. (284) and Cladera et al. (72) are clear on this point. They use evidence from linkage disequilibrium analysis to argue strongly against the presence of detectable recombination. In a study on the potential for intraspecific horizontal gene exchange by natural genetic transformation, Lorenz and Sikorski (207) concluded that, with regard to transformation, there is sexual isolation from other Pseudomonas species and other genomovars. Gene transfer between genomovars by transformation is limited by sequence divergence at least; heterogamic transformation was reduced in competent cells. The potential to receive genes can also vary greatly among strains. It appears that some strains have a greater potential than others for gene acquisition. It seems that genomovars are free to diverge in neutral sequence characters as a result of sexual isolation mechanisms. These mechanisms prevent randomization of alleles. Nevertheless, the authors consider this border to not be

absolute, and foreign sequences may be acquired and fixed.

A careful analysis of some genes, based on incongruences in the phylogenetic trees and/or what is known as relative codon usage, the codon bias index, or the G+C content of the genes, can help to define some metabolic pathways as genes acquired through horizontal transfer. The following examples are considered below: the aromatic degradative pathway, the nitroge- nase system, the ability to use chlorate as a terminal electron acceptor, and the energy-yielding reactions in the oxidation of thiosulfate.

TAXONOMIC RANKS: GENOMOVARS

Strains ascribed to the species P. stutzeri share some phenotypic traits that distinguish them from other species. In this respect, P. stutzeri is a well-defined species that is relatively easy to recognize. However, several intraspecific groups can be delineated genomically and phylogenetically, even when they are monophyletic. In previous polyphasic taxonomic approaches, groups that are

phenotypically similar but genotypically different have been referred to as "genospecies," "genomospecies," or "genomic species." A genospecies has been defined in bacteriology as a species that can be discerned only by comparison of nucleic acids. If a specific genospecies cannot be differentiated from another genospecies on the basis of any known phenotypic trait, it should not be named until such a differentiating trait is found (392). Brenner et al. (50) proposed that the term "genospecies" be replaced by "genomospecies." This would avoid confusion with the earlier definition of genospecies, which was a group of strains able to exchange genetic materials. The term "genomic species" is also in use: it is a group of strains with high DNA-DNA hybridization values (76, 297). Subspecies designations can be used for organisms that are genetically close but phenotypically divergent. In this way, the infraspecific level seems to be phylogenetically valid. It can be distinguished from the infrasubspecific concept of variety. This concept is based solely on selected "utility" attributes that	[redacted]	

cannot be demonstrated by DNA reassociation (392). Ranks below subspecies are often used to indicate groups of strains that can be distinguished by some special characteristic. Such ranks have no official standing in nomenclature but often have great practical usefulness. An infrasubspecific taxon is one strain or a set of strains that have the same or similar properties and are treated as a taxonomic group. The "genomovar" concept was coined (291, 363) to clarify the taxonomic status of P. stutzeri genomic subgroups. Therefore, the concept was first applied to P. stutzeri. It is a useful pragmatic approach to classifying individual strains when they are genomically different from phenotypically closely related strains. It is also of use when phenotypic intragroup variability cannot be clearly established. This occurs when only a small set of strains (or just one) has been isolated. There is no clear phenotypic or biochemical relationship, or a common geographical origin or source of isolation, between members of the same genomovar in P. stutzeri.		

The suffix "-var" refers to a taxonomic rank below the species level. Nine genomovars (114) have been intensively studied within the species. Members of two different genomovars are genomically distant enough to be considered different genomic species. However, due to the lack of discriminative phenotypic traits, the strains are included in the same nomenspecies. Recent studies undertaken by Sikorski et al. (327) and Romanenko et al. (289) have described some additional P. stutzeri isolates that belong to previously described genomovars and others that represent at least eight new genomovars. These results were obtained by 16S rRNA phylogenetic analysis, RAPDs, and DNA-DNA hy-bridizations (327).	[redacted]	
Since its definition, the genomovar concept has been applied to other genomic groups in different bacterial species, such Burkholderia cepacia (368) and Azoarcus spp. (336). It could be applied to other well-defined genomic	[redacted]	

groups in species such as Shewanella putrefaciens and Bacillus cereus, etc. Other authors (e.g., J. P. Euzeby [http://www.bacterio.cict.fr/]) consider “genomovar” to be an unfortunate term, as it assumes that genomic differentiation should be the basis for differentiating bacterial species.

Due to the high genomic diversity of P. stutzeri strains, other authors prefer to use supraspecific terms to refer to all of them. Examples are the P. stutzeri “group” (337), the P. stutzeri “su-perspecies” (337), and the P. stutzeri “complex” (408).

Cộng tác viên đang dịch:

IDENTIFICATION

Phenotypic Identification

Phenotypic identification based on the characteristics given in the species definition and following dichotomous keys is usually satisfactory (26,115). At present, studies of nutritional properties are frequently carried out with commercial kits designed to reduce the labor involved in traditional methods. Commercial procedures, such as the API 20NE, Microbact NE, and Biolog GN tests, usually identify P. stutzeri strains correctly. The

đặc điểm trong định nghĩa loài

identification manuals consider important distinguishing characteristics, such as denitrification or maltose utilization, to not be universal (denitrification is 94% positive, maltose is 69% positive, arginine dihydrolase is 2% positive, and gelatin liquefaction is 1% positive in the API strips). It is assumed that some tests may not be in accordance with the species' typical features. The strain sometimes has to be "adapted" to the test, by growing it under similar, but not strictly selective, conditions prior to the test. Denitrification is a good example of this and is considered below. In a study of the presence and identification of P. stutzeri in clinical samples, Holmes (154) stated that routine clinical laboratories have difficulty identifying this species.	phải Trong xét nghiệm y tế truyền thống	
A microbial cell expresses some 200 different proteins that can be separated by PAGE. This yields complex banding patterns, which are considered to be highly specific fingerprints (265). Strains with at least 70% DNA similarity tend to have similarities in protein electrophoretograms. Therefore, PAGE is thought to be a sensitive technique for	có những nét đặc trưng rất riêng	

gaining information on the similarities between strains within the same species or subspecies. Individual strains can often be recognized by protein pattern. Under standard growth and PAGE conditions, the patterns are reproducible. Computer-assisted analysis enables the information to be normalized and stored. This method has been used to identify P. stutzeri strains when a wide database is available (366). The Sherlock microbial identification system is based on analyzing total fatty acid profiles. It gives satisfactory results within the genus Pseudomonas, including P. stutzeri, if the cells are cultured under strictly controlled conditions. Molecular DNA-Based Identification A PCR and an oligonucleotide probe method have been developed specifically for detecting and identifying P. stutzeri. The amplification primers and the probe were designed from the analysis of available 16S rRNA sequences. Positions that were specific for P. stutzeri and differed from the rest of Pseudomonas species were selected from variable regions in the Pseudomonas 16S rRNA.	[redacted]	

Positions 743 (G) and 746 (A) fulfilled both criteria, and a 21-nucleotide primer was designed (rps743). A second oligonucleotide, fps158 (17-mer), at positions 142 to 158, was selected as a second specific primer. It produced a 625-bp amplicon in PCR. The specificity of the amplicon was further identified with a DNA probe (17-mer) that included 12 bases of the 5' end of primer rps743 (25). A second set of primers, fps158 and rps1271, was developed by Bennasar et al. (24). These primers produced a 1,159-bp amplicon containing a BamHI restriction site. The specificity of the amplicon for P. stutzeri was then corroborated by restriction, giving two fragments, of 695 and 465 bp, respectively. A slightly modified set of primers in the same region was used successfully by Sikorski et al. (325). The three methods permit good molecular differentiation of P. stutzeri from other species. They have been used to identify P. stutzeri and to detect it in environmental samples, as indicated below (see "Occurrence and Isolation Procedures"). Polyphasic Identification	[redacted] [redacted] [redacted] [redacted] nhiều giai đoạn	

The species is well-defined phenotypically and chemo-taxonomically. However, some of its distinguishing traits are lacking in well-documented strains (starch hydrolysis, arginine dihydrolase activity, and motility, etc.). In addition, many biochemical properties are extremely variable within the species and are not correlated with the genomovar groupings. DNA-DNA similarity values of more than 70% (or less than 5°C difference in thermal denaturation temperatures) are required to definitively assign a strain to a given species. In P. stutzeri, a polyphasic taxonomic approach is needed for assigning a new strain to the species: the strain has to agree with the basic phenotypic traits of the species, has to be placed in the same branch as P. stutzeri reference strains in the phylogenetic trees of one or more housekeeping genes, and has to show DNA-DNA similarity values of more than 70% with a reference strain of a recognized genomovar. If the last condition is not fulfilled, the strain can be proposed as a new genomovar within the species. If it can be phenotypically distinguished from P. stutzeri strains, it can be proposed as a new species.	[redacted]	

PHYSIOLOGICAL PROPERTIES Temperature, Pressure, pH, and O2 Relationships

The species has a wide range of growth temperatures. Temperatures from 4°C (strain NF13 grew at 4, 22, and 35 but not 55°C [297]) to 45°C (CMT.9.A grows at 45°C) have been cited for individual strains. However, growth at these extreme temperatures seems to be limited to selected strains. Strains that grow at low temperatures are mainly those isolated from cold habitats. Most strains grow at 40°C and 41°C, some at 43°C. The optimum temperature for growth is approximately 35°C. Palleroni et al. (251) subdivided P. stutzeri into two biotypes: one clustered around 62% G+C that does not tolerate a temperature of 43°C, and a second of around 65 to 66% G+C that grows at 43°C or higher.

Some strains (NF13, MT-1) have been isolated from the deep-sea bottom. organisms adapted to the deep-sea environment have to grow under conditions of 2°C and 100-MPa pressure. On 28 February 1996, a sediment sample was obtained from the Mariana Trench by the

unmanned submersible Kaiko. It seems likely that this was the first time sediment samples were collected from the world's deepest point without any microbiological contamination from other depths (351). The analysis of amplified 16S rRNA sequences from DNA directly extracted from these sediment samples demonstrated the presence of bacteria belonging to the P. aeruginosa branch (Mariana bacteria no. 2 [D87347] and no. 11 [D87346]). Pressure-regulated gene clusters were also amplified. Therefore, in addition to being barotolerant, the bacteria from the Mariana sediment may be barophilic microorganisms. Barophilic microorganisms were isolated by maintaining the conditions of 100 MPa and 4°C. Twenty-eight strains were selected. Strain MT-1, isolate HTA208, was grown on marine agar at 28°C and pH 7.6. Its 16S RNA sequence affiliates the strain with the P. stutzeri phylogenetic branch. It was able to grow at a hydrostatic pressure of 30 to 60 MPa, and slight growth occurred at 100 MPa. The growth rate of the P. stutzeri type strain was strongly affected by hydrostatic pressure. It must be clarified whether the isolated bacteria	[redacted]	

are active or inactive under high hydrostatic pressure and low temperature or whether their presence is simply a result of settling of flocculated organic matter.

As mentioned above, no strain tolerates acidic conditions: all fail to grow at pH 4.5. This is probably the reason why there is a negative reaction to the oxidation/fermentation test for the use of carbohydrates. Many P. stutzeri strains give a neutral result, as the medium is not buffered and acidification inhibits further growth, even when the strain might be able to use the added sugar.

P. stutzeri strains grow well under atmospheric oxygen. How-ever, microaerophilic conditions have to be established when nitrogen-fixing strains are cultured as diazotrophs. All strains described to date are facultatively anaerobic with nitrate. Some strains are also anaerobic, with chlorate or perchlorate as terminal electron acceptors. Both anaerobic properties are discussed in the following section.

Denitrification

The denitrification process carried out by bacteria makes use of N oxides as terminal electron acceptors for cellular

bioenergetics under anaerobic, microaerophilic, and occasionally even aerobic conditions (for reviews, see references 45,77,184, 263, and 420). During the denitrification process, which involves a pathway of four successive steps, several metallo- proteins catalyze the reduction of nitrate to nitrite, nitric oxide (NO), and finally nitrous oxide (N2O) to dinitrogen (N2). The metalloenzymes include nitrate reductase (EC 1.7.99.4), nitrite reductase (EC 1.7.2.1 and EC 1.9.3.2), nitric oxide reductase (EC 1.7.99.7), and nitrous oxide (N2O) reductase (EC 1.7.99.6) (152). In contrast to the assimilatory reduction of nitrate or nitrite to ammonia for biosynthetic purposes, denitrification in bacteria is a dissimilatory transformation, associated with energy conservation (420). In other words, the enzymatic electron transfer is coupled to ATP synthesis via proton translocation and the formation of a membrane potential (347). The bacterial process of denitrification is normally a facultative trait. It provides bacteria with a respiratory pathway for anaerobic life (184, 420). The distribution of denitrification capabilities among the prokaryotes does	[redacted]	

not follow a clear pattern (263). The former genus Pseudomonas is one of the largest taxonomic clusters of known denitrifying bacteria. This fact has largely favored the use of species of the genus Pseudomonas as model organisms for studying the denitrification process. Within the genus Pseudomonas, and probably also within the prokaryotes, much of the relevant work, advances in the biochemical characterization of denitrification, and essential genetics using highly interdisciplinary approaches have been achieved with P. stutzeri. Denitrification is a stable trait for P. stutzeri; it is one of the most active denitrifying, hetero- trophic bacteria, and it has been considered a model system for the denitrification process (420).

Structural gene clusters and the nature of denitrification genes. A total of about 50 genes are needed in a single denitrifying bacterium to encode the denitrification apparatus' core structures (384). The genes contain structural information for the nitrogen oxide reductases and functions for metal processing, cofactor synthesis, electron donation, assembly processes, protein

maturation, and regulation. These denitrification genes have a chromosomal location in P. stutzeri. The complete denitrification process that leads to N2 formation starts with nitrate reduction. Therefore, many bacteria have more than one of the three types of nitrate reductases: soluble assimilatory nitrate reductase and two dissimilatory reductases that are further subdivided into respiratory and periplasmic nitrate reductase (230). In P. stutzeri, the genes coding for the dissimilatory reductases, the nar genes, are not linked to the denitrification genes sensu stricto (which include the nir-nor loci and the nos genes; see below). Instead, they form a separate locus in this bacterium (48, 420). As in other denitrifying bacteria that depend on the cytochrome cd1 nitrite reductase, in P. stutzeri the genes encoding functions for nitrite respiration (nir) and nitric oxide (NO) respiration (nor) seem to be preferentially organized in a mixed cluster made up of both types of genes (8, 48, 88, 169, 170, 384, 414). Effectively, this is a single denitrification supercluster of about 30 kb. It contains 33 genes and was located and	[redacted]	

mapped on a 56-kb BamHI fragment in P. stutzeri (strain ZoBell) (48). In addition to the nir-nor genes, this cluster harbors the nos genes, which are needed for the respiratory reduction of nitrous oxide (N_2O) and are about 14 kb from the nir genes. Figure 3 shows that the gene organization in P. stutzeri subclusters is nos-nir-nor. Twenty-three of the 33 genes recognized in the P. stutzeri denitrification cluster are transcribed in the same direction. Two groups of seven (nirO, nirP, nirQ, nirJEN, and nirY) and three (fnrD and its two adjacent open reading frames [ORFs]) genes are transcribed in the opposite direction. At least 10 transcriptional units have been clearly defined with confidence. However, the definitive number remains open, since not all of the promoters have been mapped (420). Furthermore, polycistronic transcripts for norCB and nirSTB in P. stutzeri have been identified experimentally (418, 420).

(i) nar genes. The organization of the genes coding for respiratory nitrate reduction (nar) in P. stutzeri has been almost completely determined (14, 137). Some of

the nar genes have homologs in the Escherichia coli nitrate respiration apparatus. These include narK (encoding a putative nitrite transporter/ facilitator permease), narG, and narXL. The 5' end of narL overlaps the 3' end of an ORF. Other characteristic E. coli genes that are either missing or unidentified in P. stutzeri are narH, narJ, and narI. An additional gene (orf134) that, together with narK, encodes a hypothetical transporter similar to fungal or plant nitrate transporters (narC) is missing in E. coli. An additional ORF immediately follows the narL gene. A sequence encoding an FNR family transcription factor, DnrE (137), follows directly from this ORF. Both the regulatory and the structural genes have opposite orientations in P. stutzeri (137). Moreover, homologs of the structural genes encoding the subunit NapA (a second dissimilatory nitrate reductase in the form of a periplasmic, dissimilatory-type enzyme found in many denitrifiers, such as Cupriavidus necator H16) have been detected by hybridizing a 109-kb SpeI fragment from P. stutzeri genomic DNA. These loci are probably not linked to the P. stutzeri 30-kb denitrification	[redacted]	

cluster (384). Additionally, a partial napA gene for periplasmic nitrate reductase has been detected by PCR in the P. stutzeri strain ZoBell (239). The amplified fragment (380 nucleotides) has 85% identity on a 223-nucleotide stretch of the Cupriavidus necator H16 megaplasmid, encoding key enzymes for H2-based lithoautotrophy and anaerobiosis. The features in this part of the sequence belong to a large subunit of the periplasmic nitrate reductase. Similarly, when a shorter fragment (92 nucleotides) from the complete genome of Erwinia carotovora subsp. atroseptica SCRI1043 is used, the same fragment has 86% identity with the features that are described for this part of the Erwinia carotovora genome sequence as a periplasmic nitrate reductase. (ii) nir genes. The nitrite reductase gene sequence (cytochrome cd1), nirS, in two strains of P. stutzeri has been determined. The cytochrome cd1 from P. stutzeri ZoBell was isolated by a phage expression library, and JM300 was isolated with protein-derived oligonucleotide probes (170, 332). In addition to the nirS gene, the nir gene subcluster contains nirTBM genes. The	[redacted]	

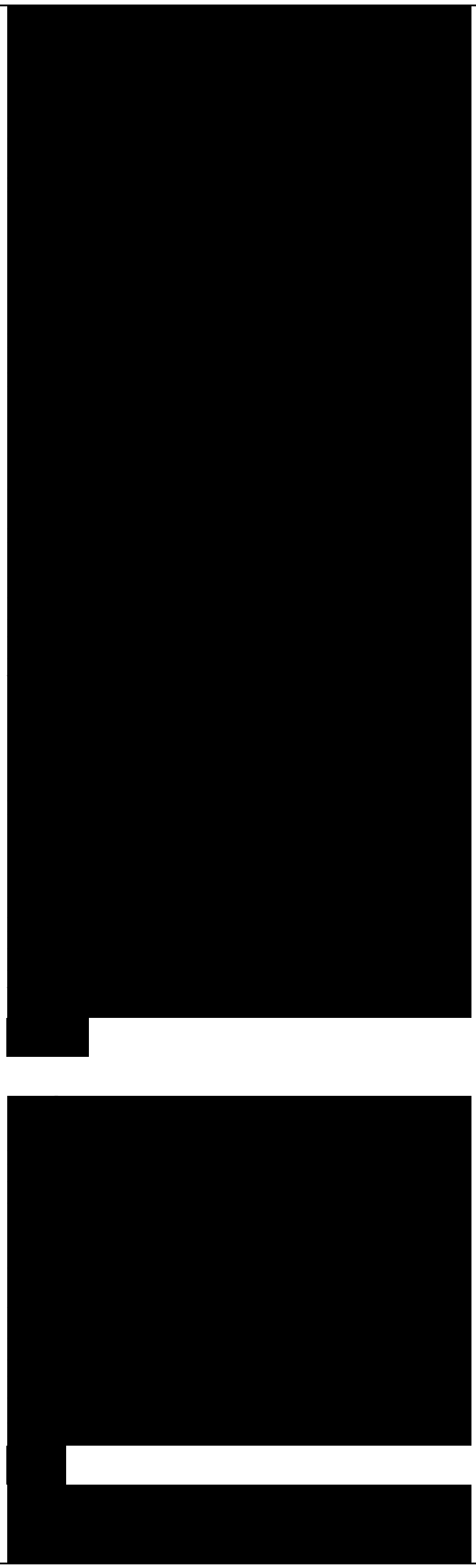
nirT gene encodes tetraheme cytochrome, and nirB encodes diheme cytochrome (cytochrome c552). Tetraheme cytochrome encoded by nirT may have a putative electron donor function (170). However, it seems that in P. stutzeri, nirM encodes the electron donor (cytochrome c551) for NirS. The nirD locus has been established by Tn5 mutagenesis. The function of its gene product(s) may be related to processing of cytochrome cd1 or to heme D1 biosynthesis (169, 413). In addition, the nirS gene is part of clusters that harbor genes for the biosynthesis of heme D1, a cofactor of denitrifi- ers. The cytochrome cd1 nitrite reductase depends on this cofactor. Such genes seem to be distributed over two loci in P. stutzeri: nirJEN and nirCFDLGH (123, 254). As in other Pseudomonas spp., several genes, e.g., nirDLGH, appear to be duplicated in P. stutzeri (254). The gene nirE, found in P. stutzeri upstream of nirS (transcribed in the opposite direction and part of the cluster nirQJENY [384]), encodes a putative methyltransferase for the heme D1 pathway. Two P. stutzeri ORFs, orf393 and orf507, are homologous to the nirJ and	[redacted]	

nirN genes of P. aeruginosa, respectively (123). The gene product of nirN (orf507) seems to affect anaerobic growth and in vivo nitrite reduction (123). The nirF gene is preceded in the nir clusters by nirC (formerly orf5), which probably encodes a putatively periplasmic monoheme cytochrome c. The presumed function of this cytochrome is related to the maturation of NirS (170). Both nirE and the putative nirMCFDLGH operons have recognition motifs for the anaerobic regulator FNR in their promoter regions.

A putative LysR-type regulator has been located in an ORF (nirY) found between the nos and nir operons in P. stutzeri (123). The LysR-type regulator belongs to a family of factors involved in the control of a wide variety of processes, including oxygen stress regulation (309).

Finally, P. stutzeri has a nirR gene encoding a 25.6-kDa protein that affects nitrite reduction, i.e., NirS synthesis (169). This nirR gene is not located in the described denitrification gene cluster and has no significant similarity to known proteins.

(iii) nor genes. Generally, two groups of NO reductases

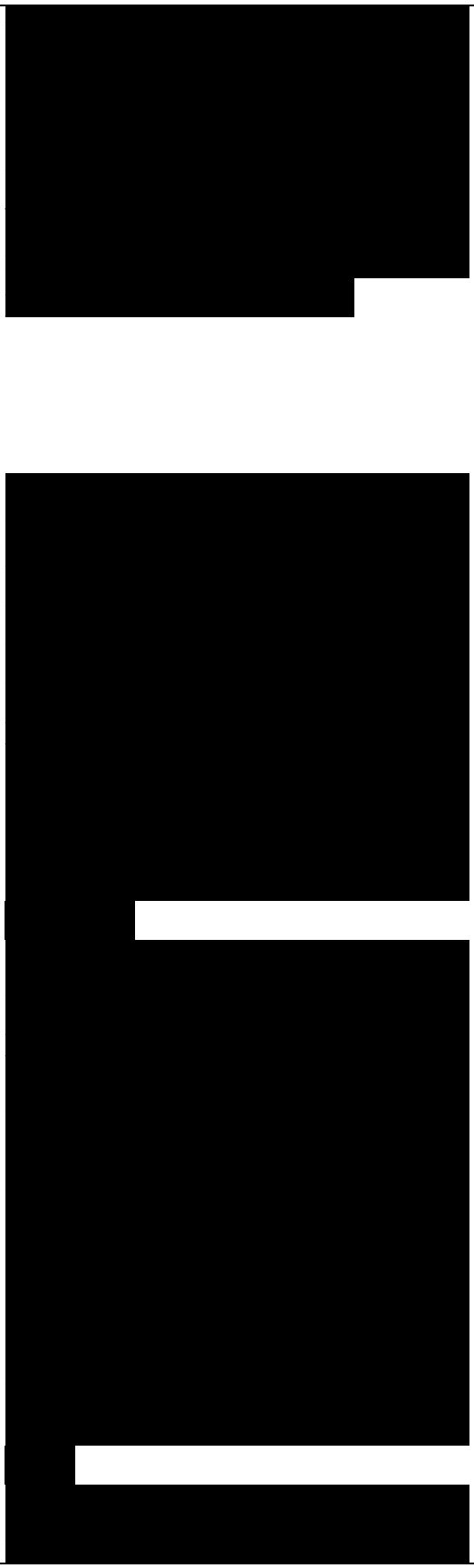


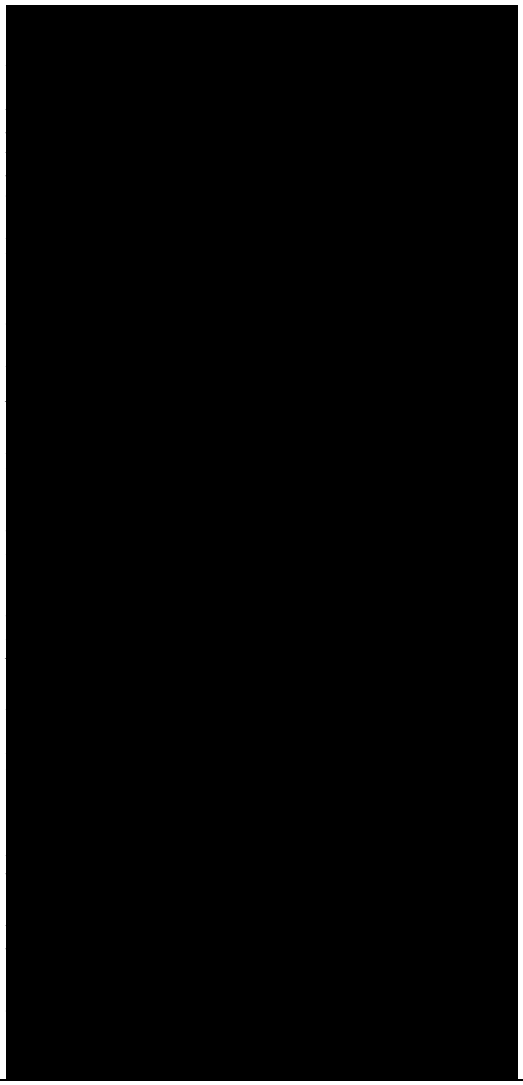
(NORs) can be distinguished from a comparison of primary structures: the so-called short-chain NORs (scNORs) (450 amino acids) and the long-chain NORs (lcNORs) (about 760 amino acids). Furthermore, this division corresponds to electron donor specificities: scNORs are part of a cytochrome c complex, whereas lcNORs derive electrons from quinol. Thus, these two enzyme forms have been named "cNOR" and "qNOR," respectively, in some studies (146). The genes encoding the NO reductase (cytochrome bc-type complex), norCB, were the first scNOR genes to be identified. They were established by reverse genetics on purified NOR from P. stutzeri (47, 48). A cosmid library of P. stutzeri was mapped by using oligonucleotide probes derived from the N terminus of the purified NorC subunit (47).

The nor genes in P. stutzeri are arranged in three transcriptional units that consist of a norCB operon, a nirQOP operon, and a monocistronic norD transcript. The dnrN operon follows norD downstream. It includes (encoded on the cDNA strand) a gene for the nor regulator, DnrD (385). The norC gene

product functions as a cytochrome c subunit of scNORs. Immediately downstream from norC is an ORF (norB) that encodes a strongly hydrophobic protein. In fact, the norB gene encodes the catalytic subunit of the scNOR and oxidizes cytochrome c. Evidence that this ORF represents the structural gene of the cytochrome b subunit came from a deletion-re- placement mutation of this region. This rendered the Nor~ P. stutzeri strain MK321 immunonegative for an antiserum against cytochrome b (47). The norD gene is downstream of norCB. This gene presumably encodes a cytoplasmic protein that affects the expression and function of both NirS and NorCB.

The P. stutzeri accessory genes nirQOP are situated upstream from norCB and are separated by approximately 7 kb of the nir genes, encoding the cytochrome cd1 nitrite reductase and the components for heme D1 biosynthesis. The nirQ gene (norQ, formerly orf8) is positioned immediately upstream of nirS. The nirQ gene has a certain sequence similarity to regulators included in the NtrC family (its product has been predicted to

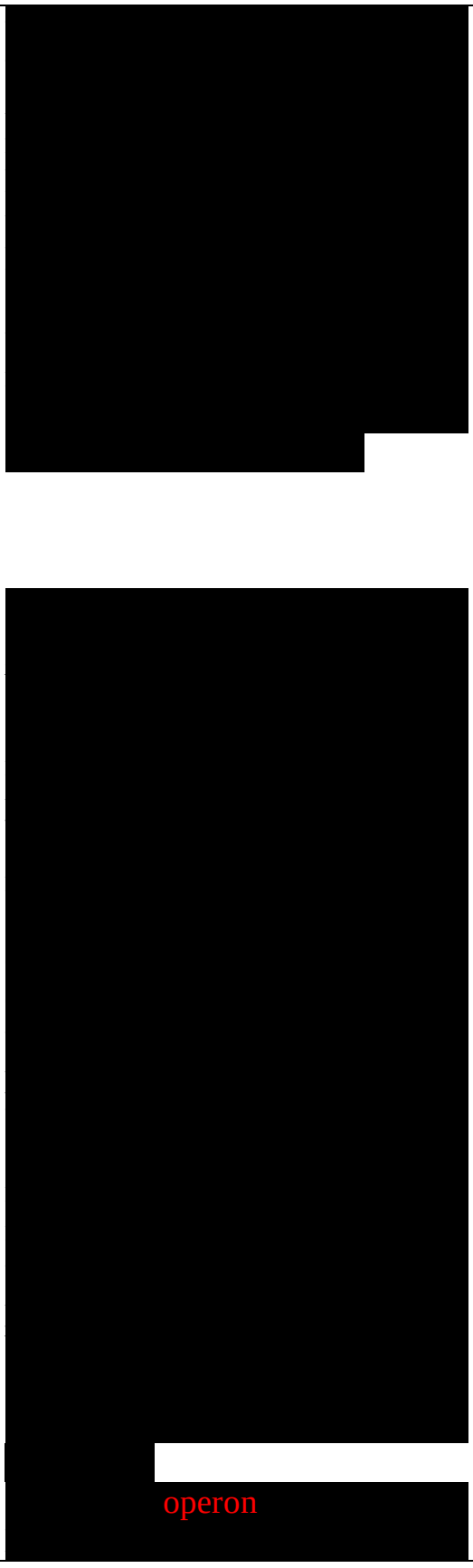
be a potential denitrification regulatory component), and it is transcribed in an orientation opposite that of nirS (48). Both nitrite and No respiratory reduction processes are affected by the mutagenesis of nirQ (it affects the catalytic functions of NirS and NorCB). This shows that there is a dependency between these processes (171). The nirO gene (formerly orf175) encodes a five-span membrane protein that affects the yield and rate of anaerobic growth. It is similar to the cytochrome c oxidase subunit III. Finally, nirP (norF, formerly orf82) encodes a two- or three- span membrane protein and is involved in No and nitrite reduction. (iv) nos genes. The nos gene subcluster is located 9 kb upstream of the nir cluster. P. stutzeri nos genes are needed for the anaerobic respiration of nitrous oxide. This is the final part of the whole denitrification process. The nos-encoding region is approximately 8 kb and contains nosZ (the structural gene for the copper-containing enzyme nitrous oxide reductase), genes for copper chromophore biosynthesis, and a supposed regulatory region (382, 414).		

The nosR gene, located upstream from nosZ, encodes a membrane-bound transacting regulatory component that is necessary for the transcription of nosZ (84-86, 416). The codon usage for NosR shows the characteristics of a typical Pseudomonas gene. There is a high overall G+C content (62.4 mol%) and a preference for G or C at the third codon position (84). Furthermore, inverted repeats at the end of nosR are not prominent (84). The synthesis of the functional multicopper enzyme nitrous oxide reductase (N2OR) needs an assembly apparatus and a maturation process to insert the prosthetic copper (155, 414). To achieve this, P. stutzeri has some genes immediately downstream of the N2OR structural gene. They encode several of the accessory proteins required in the biosynthesis of the catalytically active enzyme. Three maturation genes, nosDFY, encode the corresponding proteins. These products are involved in acquiring or processing copper to form a catalytically active N2O reductase (155, 414). The process includes the formation of a putative ABC transporter complex (consisting of NosD, NosF, and NosY) that extends	 	

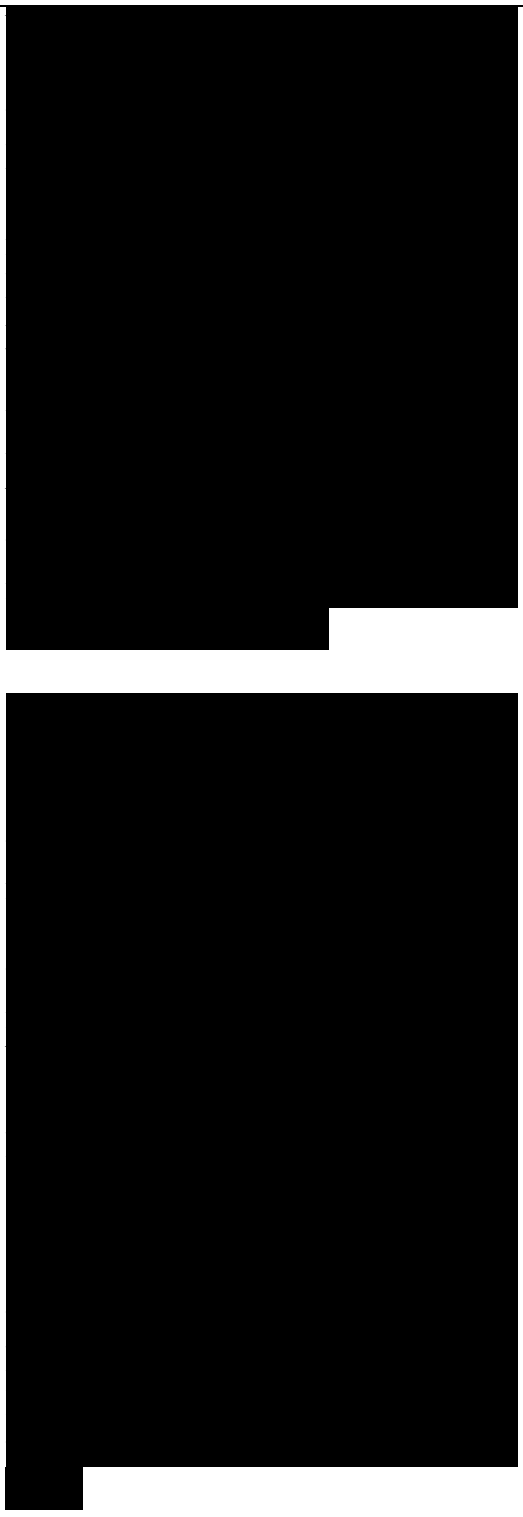
to both sides of the cytoplasmic membrane (155). Sequence similarity leads to the deduction that nosF may encode an ATP/GTPase (155, 414). Moreover, the expressed and purified NosF protein has a structural similarity to the ATPase of maltose or histidine ABC transporters. If any of the nosDYF maturation genes are mutationally inactivated, the CuZ center is missing in the resulting enzyme (155, 274, 420).

In addition, the nos operon encodes a Cu chaperone, NosL. The predicted presequence of the gene product derived from nosL (formerly orf4) is similar to that of lipoproteins (48). Furthermore, like many other bacteria, P. stutzeri has a Tat (twin-arginine) (143, 305) translocation system for exporting proteins (in addition to the Sec system), which seem to be transported in a folded form (30, 285, 405). Downstream of nosL , the nos region contains information that encodes a component of the Tat translocon, TatE (155). It is of note that the P. stutzeri tatE locus (formerly identified as orf57) is unlinked to the rest of the tat genes (123, 143).

Finally, the nnrS operon is situated immediately

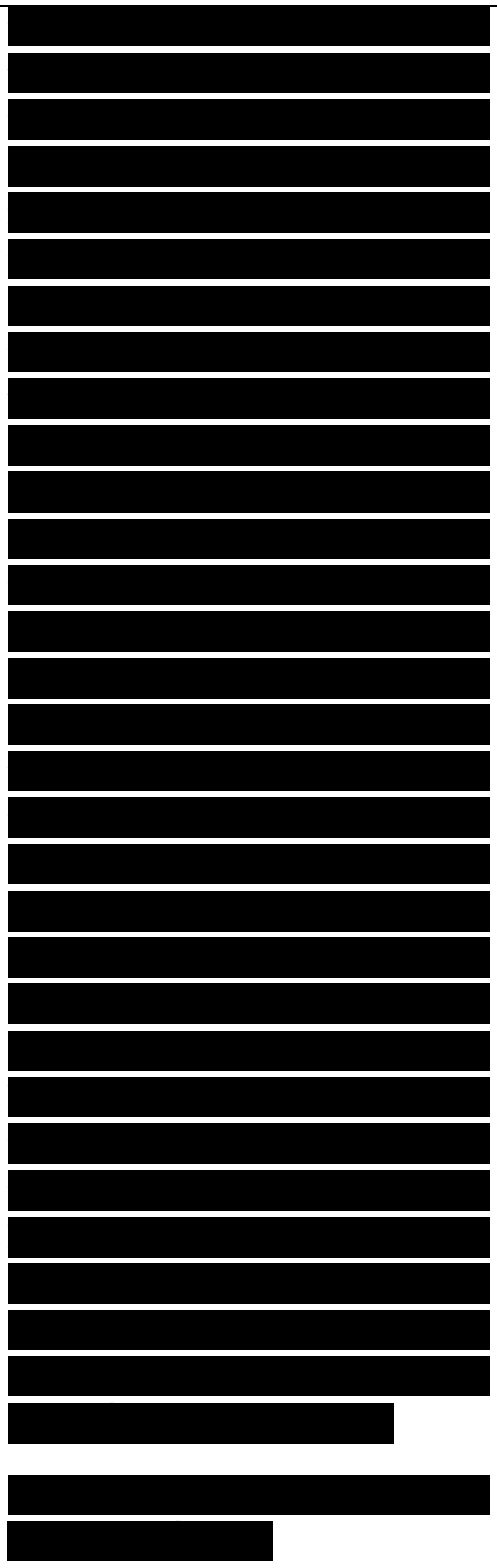


downstream of the nos operon. The nnrS operon includes a gene (orf396) that encodes a putative heme-Cu protein (NnrS) and a member of the short-chain dehydrogenase family (Orf247) (155). Additionally, in this region the orf378 gene codes for a putative membrane-bound protein (Orf378). Metalloenzymes involved in the denitrification process. (i) Nitrate respiration and NaRs. Respiratory nitrate reductases (NaRs) are complexes that have either two or three subunits, depending on the isolation method. Pseudomonas stutzeri strain ZoBell (ATCC 14405) has a membrane-bound nitrate reductase (EC 1.7.99.4) containing three subunits (apy). It can be prepared by detergent extraction (148). The heterotrimeric enzyme has the electronic spectrum and absorbance intensity of a diheme protein. Nitrate reductase contains, per Mr 172,000, about 13 iron-sulfur groups and one atom of molybdenum bound to a pterin cofactor (39). In P. stutzeri dissimi- latory nitrate reductases, the organic moiety of the molybdenum cofactor is a molybdopterin guanine	[redacted] kim loại [redacted]	

dinucleotide (109). The name "bactopterin" was initially proposed for the modified bacterial Mo cofactor (226). The cofactor in the molybdoenzymes is thought to modulate the redox behavior of the metal. It also aids in electron transfer to or from other redox centers without the pterin cofactor and undergoes a redox process itself (149). The pterin unit connects the electron flow between the molybdenum and the iron-sulfur center (148). Furthermore, the cofactor has a structural function: it fixes the molybdenum core in the center of the protein matrix (94,148). In addition, a two-subunit (ap) form of dissimilatory nitrate reductase can be separated from the membrane-residing y subunit by a heat solubilization step. The ap unit alone has the same catalytic center as apy, the a subunit (NarG), which consists of molybdenum and two pterin cofactors. The ligand environment of molybdenum in the active center seems to be unaltered by heat treatment of ap and apy preparations, as the electron paramagnetic resonance spectrum properties for the catalytically active molybdenum center signal, Mo(V), are almost identical in		

both cases (39, 148). The iron-sulfur complexes in the p subunit (NarH) seem to participate in electron transport from the membrane's quinol pool. The small y subunit (NarI), which spans the membrane, is a cytochrome b protein that contains two b-type heme groups (148). The topology of the y subunit was predicted (by means of an analogy with the NarI subunit from E. coli [89]) to be a transmembrane anchor which holds a two-subunit (ap) form on the cytoplasmic side of the membrane (29). NarI is attributed with quinol oxidation and electron transport to the p subunit. The purified, soluble, ap form of the enzyme from P. stutzeri has been seen to have high specific activity (71 U/mg, when one unit of nitrate reductase activity is defined as the production of 1 ^mol nitrite per min). The enzyme (ap-NaR) has a pH range for optimum activity of 7.5 to 8.0, regardless of the NaCl concentration (1 mM to 1 M). NaR activity is strongly dependent on temperature. The maximum temperature is 76°C. The enzyme is competitively inhibited by azide but not by chlorate (no inhibition was found in concentrations of up	[redacted]	

to 100 mM NaClO3) (148). The ap-NaR form of the enzyme has Km values of 3.2 to 3.8 mM for nitrate (148). However, the Km affinity of the P. stutzeri apy nitrate reductase, determined with exogenous redox mediators, was 0.49 ± 0.07 mM at saturating methyl viologen concentrations (41). These differences could be explained by the fact that electron donors first have to reduce the y subunit in the holoform. In contrast, electron donors have easier access to the iron-sulfur centers in the ap form. As a result, the apy form reaches substrate saturation at a lower nitrate concentration (148). Cộng tác viên dịch tới đây Many denitrifiers have a second dissimilatory nitrate reductase, in the form of a periplasmic dissimilatory-type enzyme (e.g., napA in C. necator H16). There was no initial evidence for such reductases in the denitrifying Pseudomonas species. However, sequence- and hybridization-based analyses have demonstrated their presence in P. stutzeri (see above). In general, these periplasmic dissimilatory-type	[redacted]	

enzymes have a NapA subunit that binds a molybdenum cofactor. They may also have a four-cysteine motif near the N terminus, to attach a 4Fe-4S cluster. In addition, a small-subunit NapB with two potential heme C-binding sites was detected in the sequence. This seems to be needed by these dissimilatory nitrate reductases. Furthermore, a NapC protein belonging to a homologous family of tetraheme c-type cytochromes was first reported as P. stutzeri NirT (170, 420). The putative role of NapC involves electron transfer between a quinol and the periplasmic nitrate reductase. The physiological role of this periplasmic dissimilatory nitrate reductase could be to promote the transition from aerobiosis to anaerobiosis (147, 240). Whereas membrane-bound respiratory nitrate reductase is expressed only under anaerobic growth conditions, periplasmic nitrate reductase is synthesized and active in the presence of oxygen (21, 321). In addition, both enzymes are under nitrate control, exerted via the sensor protein NarX or NarQ.		

(ii) Properties of NarL and NarX proteins. NarL of P. stutzeri is a 218-amino-acid protein, Mr 24,378. It has 51 and 47% positional identity with the E. coli proteins NarL and NarP, respectively (137). NarL regulates the narG operon. It acts at the transcriptional level by activating the narG operon. However, it does not activate the other structural genes of oxidoreductases involved in denitrification (137). Several of its residues (Asp13, Asp14, Asp59, and Lys109) correspond to a set of conserved amino acids found in response regulator proteins. In particular, the aspartic acid residues form an acidic pocket which is part of the phosphoryl acceptor chemistry (185, 236, 259, 343). The P. stutzeri-derived protein NarX consists of 648 amino acids, Mr 71,791. It has 31% positional identity with NarX and NarQ of E. coli. Hydropathy and transmembrane prediction analysis seem to suggest that it has two membrane-spanning regions. A carboxy-terminal	[redacted]	

cytoplasmic domain (C) and an internal periplasmic domain (also known as the P box) have been delimited (137). The C domain has characteristic conserved regions (termed H, N, and D) for the histidine protein kinase family (344) and is therefore a common feature of sensor proteins. It is thought to be important in conferring specificity on sensor response regulator interaction (259). Both the periplasmic P and the cytoplasmic C regions (a stretch of conserved residues intercalated between the aforementioned H and N regions) are conserved in either nitrate- or nitrite- responsive sensory kinases and are specific for NarX-type sensor proteins (137). NarX functions as a sensory component that has an approximately twofold preference for nitrate, i.e., nitrite is about half as active an inducer as nitrate (399). The P region is responsible both for binding nitrate and nitrite and for harboring the essential elements for distinguishing between these ions (137). (iii) Nitrite respiration and NiRs. Nitrite reductases (NiRs) are key periplasmic enzymes in denitrification. They are responsible for catalyzing the first step of a process that leads	[redacted]	

to the formation of a gaseous intermediate, which is no longer available to most living organisms. The nitrite reductase reaction in denitrifying bacteria is usually performed by the activity of two metalloenzymes. These enzymes are different in terms of their structure and prosthetic metal compounds. The metalloenzymes are the cytochrome cd1 (which contains the hemes c and d1 as essential cofactors and is encoded by nirS) (83) and a copper-containing nitrite reductase at the active site (which is encoded by the nirK gene) (420). The two nitrite reductases have never been found within the same cell. Neither of them could be exclusively associated with a particular member of the Proteobacteria (416). The nirS gene in P. stutzeri (186) encodes the 62-kDa subunit of the homodimeric cytochrome cd1 nitrite reductase (EC 1.9.3.2) (393). P. stutzeri mutants that have lost cytochrome cdj can no longer utilize nitrite (413). The enzyme has a quaternary structure a2 and a molecular mass of 119 to 134 kDa. The prosthetic groups are heme C and heme D1. Both of these	[redacted]	

groups are present in each subunit to render cytochrome cd1, a tetraheme protein. Heme D1 is noncovalently bound and is extractable from the enzyme by acidified acetone. Reinsertion of heme D1 leads to restored activity and to spectroscopic properties of the protein (393). Heme D1 of cytochrome cdj consists of an unusual macrocycle with a set of oxo, methyl, and acrylate substituents. This has been proven by chemical synthesis (228, 406). Cytochrome cd1 catalyzes the reduction of nitrite to No through the oxidation of cytochrome c551 (electron donor) (420). Its activity level with nitrite is 4.15 ^mol ■ min-1 ■ mg-1 (420).	[redacted]	
The biogenesis of the periplasmic protein cytochrome cd1 involves translocation of the protein across the membrane. This is accompanied by the simultaneous or separate transport of prosthetic groups into the periplasm, covalent binding of heme C and insertion of the noncovalent heme D1, and finally folding of the protein into its mature	[redacted]	

form. The translocation of cytochrome cd1 to the periplasm proceeds in the absence of heme D1 (123, 413) and, probably as it occurs in other microorganisms, heme C (248, 249). Heme attachment occurs on the periplasmic side of the membrane (420). Heme C biosynthesis in P. stutzeri proceeds via the glutamate (C-5) pathway (214). The central metabolite is glutamyl- tRNAGlu, formed by glutamyl-tRNA synthetase (EC 6.1.1.17) (162). A functional denitrification apparatus is dependent on the expression of genes for heme D1 biosynthesis. It is assumed that NirE catalyzes the methylation of uroporphyrinogen III during heme D1 synthesis, yielding precorrin-2. Dehydrogenation of this intermediate gives sirohydrochlorin. Despite being homologous to CysG, NirE of P. stutzeri (123) needs an N-terminal domain to catalyze dehydrogenation. In addition, Fe chelation in the siroheme pathway is missing. The conversion of precorrin-2 to heme D1 is probably catalyzed by nirD locus products (NirCFDLGH). Indeed, mutations in each particular gene of this locus result either in the absence of heme D1	[redacted]	

from the enzyme or in a nonfunctional cytochrome cd1 (254, 413). NirJ and the orf393 gene product have a certain similarity to the PqqE/PqqIII/Pqq proteins, which have unknown functions in the biosynthetic pathway of pyrroloquinoline quinone; NifB is involved in nitrogenase Mo cofactor biosynthesis. The N-terminal region of these proteins has a conserved cysteine motif: CXXXCXYC. This motif is different in NirJ: CXXXC XXCY. Moreover, the C-terminal domain of NirJ is rich in cysteines. Therefore, a metal-binding site has been postulated. An additional protein, NirN (formerly the orf507 product), has an overall similarity to NirF and NirS, and its heme C-binding domain is comparable to that of NirC (123). Finally, several of the nir gene products involved in biosynthetic or auxiliary functions have potential export signals, which indicates that periplasmic	[redacted]	

compartmentalization takes part in the final maturation steps of NirS. (iv) Nitric oxide respiration and NORs. The respiratory reduction of nitric oxide (No) is part of a biogeochemical process sustained by prokaryotes. No is an essential substrate for nitrate and nitrite denitrifiers that release nitrous oxide or dinitrogen as products. Thus, NO is of bioenergetic importance to denitrifying bacteria as both a respiratory substrate and an electron acceptor in anaerobic environments. The NORs are integral membrane proteins. They are responsible for the reduction of No to N2o and are members of the heme-copper oxidase superfamily. The search for NORs in P. stutzeri led to the identification of the first purified and biochemically characterized enzyme, in the form of a two-subunit cytochrome bc complex (144). The hypothesis that NORs and heme-copper oxidases (369, 418, 420) have a common ancestor was based on the following evolutionary considerations: the reasonable association of oxygen (aerobic respiration) and nitrate respiration (bacterial denitrification) and the	[redacted]	

relationship between nitrous oxide reductases and cytochrome c oxidases (421), proposed on the basis of unexpected structural similarities between the P. stutzeri NOR (NorB sequence) and the cbb3 terminal oxidase. The high sequence similarity of the catalytic subunits of NOR and cbb3 oxidases has enabled an accurate three-dimensional structural model of the transmembrane helix and cofactor arrangement of NorB to be constructed. This was achieved by combining the amino acid sequence of the NorB protein from P. stutzeri with crystallographic data from Paracoccus denitrifi- cans oxidase (161, 173). The proposed three-dimensional model of the membrane topology is characterized by a core catalytic subunit spanning the membrane 12 times (227, 417,418). The NOR catalytic subunit is a binuclear center (high and low spin). The high-spin center contains a heme iron and a second nonheme metal, which is Fe in NOR and Cu in oxidases. The NOR nonheme Fe is referred to as FeB, which is clearly analogous to the oxidase CuB. The presence of this second metal (Cu or Fe) is a prerequisite for the catalytic

activity of both NOR and O2 reductases (122,173). The second metal center is a six-coordinate low-spin heme. It acts as an electron transfer center between the donor and the binuclear center. The catalytic subunit of NOR has six topologically conserved histidine residues, for coordinating the two heme groups and a nonheme Fe (161). The resulting NorB model is a compact hydrophobic molecule that limits the exposure of polar surface areas on either side of the membrane.

The structural organization of the P. stutzeri NOR genes indicates that there is a single 2.2-kb transcript from the norCB operon, rendering a two-subunit core complex (418). In fact, when NOR from P. stutzeri is purified, a two-subunit composition and the results of activity assays showed that a minimal composition of two subunits is sufficient for catalysis (91, 176).

The absorption spectrum shows the characteristic spectral features of both heme c and heme b (421). The isolated NorB subunit, in its reduced form, has absorption maxima at

428, 531, and 560 nm. The isolated, reduced NorC subunit has absorption maxima at 418, 523, and 551.5 nm. NorC is a membrane-bound, monoheme, c-type cytochrome. Its N terminus is oriented toward the cytoplasm, and it has a large heme c- binding domain of 120 amino acids residing in the periplasm. A single sequence motif, CXXCH, for covalent heme c attachment is located in the periplasmic domain. This motif follows the single transmembrane helix for anchoring the protein in the membrane (418, 421). The exposure of the heme c-binding domain toward the periplasm was shown by a topologically sensitive reporter gene fusion into Leu67. This was carried out in the immediate vicinity of the heme attachment site, 61-CIGCH-65, of P. stutzeri NOR (173). The orientation of NorC toward the outside allows this protein to interact with a periplasmic cytochrome or (pseudo)azurin and supply electrons to the membrane-bound NorB. (v) Nitrous oxide respiration and N2ORs. Nitrous oxide reduction is the final step in the denitrification pathway and is catalyzed by the enzyme N2OR. The gene encoding N2OR (nosZ) is largely unique	[redacted]	

to denitrifying bacteria. The diversity of nosZ has been used to detect denitrifier-specific DNA in environmental samples (307, 308).

N2OR from P. stutzeri has probably been more intensively studied than N2ORs from other species (420). It is a periplasmic dimer enzyme that exists in several forms. These forms are distinguished by their redox and spectroscopic properties. Form I can be isolated anaerobically in high-activity "purple" species. What is known as form II ("pink" species) is obtained when N2OR is purified under aerobic conditions. Form II has low activity and a low Cu content, presumably due to oxygen affecting the catalytic center (283, 420). Form I is converted to the blue form III when dithionite is added. Form IV can be prepared from the apoenzyme by incubation with Cu(II). This form is catalytically inactive. Finally, form V of N2OR carries only CuA. It was obtained from the P. stutzeri mutant MK402, which is unable to create the catalytic center (283, 419).

Each subunit of the enzyme, which is encoded by nosZ, contains two binuclear copper

centers. The metal ion content is at least six Cu ions per subunit. The two binuclear centers are termed CuA, the entry site for electrons, and CuZ, the substrate-binding site (4). The model of the CuA center is well described. Its properties are unique among proteins' Cu centers. It is thought to be restricted for cytochrome c oxidase (COX) by a mononuclear Cu(Cys)2(His)2 structure (for a detailed review, see reference 420). In the model, certain conserved histidine residues are involved in the mature protein's copper-binding activity (153, 420). The histidines at positions 583 and 626 coordinate with two cysteines (at positions 618 and 622) and a methionine (at position 629) to bind the copper atoms in P. stutzeri (308). Regarding the CuZ, or catalytic, center, eight conserved histidine residues that are likely to be involved in the coordination of copper in the protein have been observed (416). The issue of whether Cu ligation is carried out by amino acids other than histidine is crucial to explaining the N2OR	[redacted]	

catalytic site (99, 106). It seems that only one region in the amino acid sequence has the spacing and sulfur-containing amino acid side chains needed to coordinate the additional binuclear copper center (308). For this reason, the region between amino acids 132 and 178 has been proposed as the catalytic, or CuZ, site. This site contains four of the eight conserved histidines (308).

Chlorate and Perchlorate as Terminal Electron Acceptors

For over 50 years, it has been known that bacteria can reduce chlorate (ClO_3^-) and perchlorate (ClO_4^-) under anaerobic conditions. Many nitrate-reducing bacteria in pure cultures reduce chlorate and perchlorate [which is usually referred to as (per)chlorate] by means of membrane-bound respiratory nitrate reductases and assimilatory nitrate reductases. In all cases, chlorite (ClO_2^-) is produced as a toxic end product. For many years, there was no evidence that these bacteria could grow using this metabolism. It is now known that specialized bacteria that can grow by anaerobic reductive dissimilation of (per)chlorate into innocuous chloride have evolved. For a

recent review, see the work of Coates and Achenbach (75). All known dissimilatory (per)chlorate-reducing bacteria (DCRB) are facultatively anaerobic or microaerophilic bacteria. Some, but not all, are able to respire nitrate. The DCRB are phylogenetically diverse. Some isolates belong to the Gammaproteobacteria. The strains PK, CFPBD, PDA, and PDB have been affiliated with the P. stutzeri phylogenetic branch by 16S rRNA sequence analysis. They also seem to be affiliated with genomovars 3 and 1 or 5 in the phylogenetic trees. Strain AW1 is a member of P. stutzeri genomovar 3. Wolterink et al. have proposed this strain as the type and only member of a new species, P. chloritidismutans (404). Chlorate and perchlorate reduction specifically involves a c-type cytochrome in the transfer of electrons to (per)chlorate. A periplasmic oxygen-sensitive perchlorate reductase has been characterized recently (179). In addition to perchlorate, this reductase also reduces chlorate, nitrate, iodate, and bromate.	[redacted]	

The next biochemical step is the quantitative dismutation of chlorite into chloride and O2 by chlorite dismutase. Chlorite dismutase is a highly specific enzyme that does not act on other analogous anions. Studies with a chlorite dismutase-specific immunoprobe indicated that this enzyme is present on the outer membrane of all DPRB and that it is highly conserved among these organisms, regardless of their phylogenetic affiliation. Expression of the chlorite dismutase gene (cld) is constitutive in strains PDA and PK. Genomic organization has been studied in strain PK: a gene encoding a c-type cytochrome lies between cld and a transposase gene. The trans- posase gene is followed by the chlorate reductase operon, with the gene order clrABDC (a subunit, p subunit, chaperone protein, and y subunit). A recent comparison of a phylogenetic tree based on 16S rRNA with a tree developed from the cld gene sequences of 11 diverse DPRB demonstrated significant discrepancies. The results of this comparison supported evolution through horizontal gene transfer (22). As mentioned above, strain AW1 is a member of genomovar 3.

The definition of this strain as a new species (P. chloritidismutans) was based only on its ability to use chlorate, rather than nitrate, as the terminal electron acceptor. However, careful adaptation to nitrate use enabled a denitrifying variant to be isolated. The only difference between this new species and P. stutzeri is its ability to dechlorinate (74). This characteristic is most probably acquired through horizontal gene transfer. This case demonstrates the danger of drawing taxonomic conclusions from homologies that are found in metabolic systems involved in the use of unusual substrates. Organic Compounds Used as the Sole Carbon and Energy Source Extensive nutritional studies of carbon substrates (more than 150) used by P. stutzeri strains have been carried out by Stanier et al (340). Conventional methodologies and commercial kits were later used by other investigators (131,295). These showed that	[redacted]	

intraspecies heterogeneity was high but P. stutzeri strains clustered separately from other phenons in the P. aeruginosa group, as pointed out by Palleroni and Doudoroff in their review on the genus Pseudomonas (253a). In an exhaustive phenotypic study, Rossello-Mora et al. (295) analyzed a total of 327 biochemical characteristics in 48 strains. The expanded physiological analysis did not improve the situation that was previously observed in the 102-charac- teristic study (291). This high degree of intraspecies physiological heterogeneity had already been observed by Palleroni and coworkers and by others (113, 251, 340, 371) and in other later numerical studies (115, 291). Numerical analysis of the data did not lead to genomovar-specific clusters but reflected considerable heterogeneity within genomovars. Characterization of individual genomic groups on the basis of biochemical tests was not possible.	[redacted]	

All strains tested were positive for the following activities with the indicated substrates: growth on gluconate, D-glucose, D-maltose, starch, glycerol, acetate, butyrate, isobutyrate, isovalerate, propionate, fumarate, glutarate, glycolate, glyoxylate, DL-3-hydroxybutyrate, itaconate, DL-lactate, DL-malate, malonate, oxaloacetate, 2-oxoglutarate, pyruvate, succinate, D-alanine, D-asparagine, L-glutamate, L-glutamine, L-isoleucine, and L-proline and hydrolysis of L-alanine-para-nitroanilide. One hundred seven characteristics were variable in the 48 strains tested. The 48 phenotypic tests enabling the best discrimination between genomic groups of Pseudomonas stutzeri were selected. They are listed in the original publication by Ros-sello-Mora and coworkers (295). The mineralization, or cometabolism, of xenobiotics or an-thropogenic substrates by specialized strains merits special at-tention. It is described in "Biodegradation and useful properties for biotechnological applications," below.	[redacted]	

Inorganic Energy Sources (Thiosulfate) The phylogenetic diversity of sulfur- and thiosulfate-oxidizing bacteria has been of interest to several authors. The evolution of such bacteria can be clarified by molecular investigation of the genes encoding these pathways' enzymes, together with an analysis of the 16S rRNA phylogenies of bacteria that have these properties. Strain NF13 was isolated by Ruby and coworkers from the Galapagos rift hydrothermal vents (190, 300). It was assumed that H2S could be the predominant energy source for chemosynthesis in this habitat. Enrichment cultures under selective conditions for sulfur-oxidizing bacteria yielded several physiological groups of strains. One group, represented by strain NF13, was able to grow heterotrophically in media containing peptone or yeast extract either with or without thiosulfate. Acid was produced when thiosulfate was present. This group was unable to utilize CO2 as the sole source of carbon. Of all the sulfur-oxidizing bacteria isolated, only strain NF13 was	[redacted]	

found to fix nitrogen. Strain NF13 was studied phylogenetically by Lane and coworkers using the 16S rRNA sequence (190). It was classified as an unnamed "thio-bacillus" within the Gammaproteobacteria. Recent analysis (A. Cladera, personal communication) clearly placed isolate NF13 in the P. stutzeri phylogenetic branch. The phenotypic properties analyzed by Ruby and coworkers (300) were in accordance with ascription to the species: gram-negative, denitrifier, motile, nonfermentative.

More recently, Sorokin and coworkers analyzed the anaerobic oxidation of thiosulfate to tetrathionate by obligately het- erotrophic bacteria (337). Strains were isolated from seawater and freshwater and from a sulfide-oxidizing bioreactor. All isolates were obligately heterotrophic. In phenotypic and genomic identification analyses, they were classified as members of P. stutzeri (seven strains) and P. balearica (one strain).

Of the seven P. stutzeri isolates, four were ascribed by DNA- DNA hybridizations to genomovars 3, 4, and 5. The remaining isolates seemed to cluster in another genomic group. Strain ATCC 27951, of "Flavobacterium lutescens," was included in this study (it had previously been reclassified as P. stutzeri by Bennasar et al. [25]). All seven strains oxidized thiosulfate to tetrathionate using nitrite, nitrate, or N2O as an electron acceptor. Thiosulfate oxidation under anaerobic conditions was much slower than in the presence of oxygen. In addition, it was controlled by the availability of an organic electron donor. The oxidation of thiosulfate to tetrathionate (yielding one electron) instead of sulfate (yielding eight electrons) does not generate enough energy to support autotrophic growth (a high-energy- requiring process). It is therefore not surprising that tetrathi- onate-forming isolates are obligate heterotrophs (337). They grow under these circumstances as chemolithoheterotrophs, as the provision of thiosulfate increased their growth yield in acetate-limited continuous	[redacted]	

cultures.

Sijderius, in 1946, studied some P. stutzeri strains with this ability in his dissertation, “Heterotrophe Bacterien, die Thio- sulfaat oxydereeren” (322). Whether this chemolithotrophy is restricted to specialized strains or is a general property of the species has not yet been studied. All strains studied were isolated from sulfur-rich environments, with the exception of strain ATCC 27951, which was isolated (surprisingly) from a yogurt in Algeria.

Production of Siderophores

Pyoverdines are important pigments from a taxonomic and physiological perspective, as they function as efficient siderophores. Their production is enhanced under conditions of iron starvation. Although they are not pigmented, some strains of P. stutzeri synthesize siderophores. P. stutzeri ATCC 17588 produces desferriferrioxamines E and D2 (224, 225). A different strain

(RC7) produces a catechol-like siderophore (63). No siderophores have been detected in P. stutzeri YPL-1 (202). Siderotyping is also a powerful technique for discriminating species within the genus Pseudomonas (225). It is interesting to note that the internal heterogeneity of the nonfluorescent species P. stutzeri is also reflected in its siderophore production capacity. The best studied siderophore is produced by strain KC, the reference strain and only member of genomovar 9 (316). It is highly likely that this is a secondary siderophore in this strain (200). P. stutzeri KC can degrade carbon tetrachloride (CT) (trade names, Freon 10 and Halon 104) to carbon dioxide, chloride ions, and other nonvolatile compounds, such as formate. Chloroform is not formed in this process. CT is used in the manufacture of fluorocarbon propellants, as a cleaner and degreasing solvent, and as a fumigant. The EPA estimates that 10% of U.S. groundwater may be contaminated with CT. CT is a toxic, carcinogenic, and ozone-depleting xenobiotic compound detected in groundwater. For CT transformation to occur, P.	[redacted]	

stutzeri KC must be grown in an anaerobic, slightly alkaline medium at around pH 8. The high pH lowers iron solubility, limiting the iron concentration of the growth medium. The medium must also contain nitrate, an electron donor (such as acetate), and trace levels of copper. Strain KC secretes the molecule PDTC (pyridine-2,6-bisthiocarboxylate), which has a siderophore function. PDTC has been implicated in the uptake of other transition metals in addition to iron (200). It is believed to be an iron chelator that is fortuitously involved in CT transformation. Because of its ability to effectively scavenge iron, strain KC has a competitive advantage over bacteria that lack this ability. The iron chelator alone may transform CT. This is indicated by the fact that the supernatant alone (containing the chelator) from washed cells transforms CT. In its active form and in the presence of copper, PDTC transforms CT to carbon dioxide and other nonvolatile prod-ucts, including formate and chloride ions.	[redacted]	

A pdt locus corresponding to the PDTC biosynthetic pathway in the strain KC genome has been mapped and cloned as a 25,746-bp insert in the pT31 cosmid (199, 200, 315). The pdt operon was sequenced, and a low-density PDTC microarray consisting of 17 PCR-amplified ORFs was printed on chemically modified glass substrates. This was the first published attempt to apply microarray technology to the parallel detection of multiple target genes in P. stutzeri for environmental monitoring (235).

Nitrogen Fixation

After years of controversy, it now seems that several strains that are unambiguously classified as true Pseudomonas species can be added to the list of nitrogen fixers, on the basis of physiological properties, nitrogenase assays, phylogenetic studies, and the detection of nifH by hybridization or PCR amplification and sequencing (66). All well-classified nitrogen-fixing Pseudomonas strains described in the literature are members of P. stutzeri (92). Former

Pseudomonas strains from other species able to fix N2 were transferred to other genera in the a- and fi-Proteobacteria (“Pseudomonas paucimobilis,” “P. diazotrophicus,” and “P. saccharophila,” etc.). P. stutzeri’s simultaneous capacity for nitrogen fixation and denitrification may be of relevance to overall nitrogen cycling in several ecosystems. P. azotofigens has only one strain (6H33bT) and has been described recently (140) as a novel nitrogen-fixing species isolated from a compost pile. \ Pseudomonas stutzeri A15 is a member of genomovar 1. It is a particularly predominant diazotrophic strain. It was isolated from the rice paddy rhizosphere and is widely used as a rice inoculant in China (136, 270, 380, 409). Previously identified as an Alcaligenes faecalis strain, it has been widely studied physiologically, biochemically, and genomically. Strain A15 is able to colonize and infect rice roots and to grow endophytically (92). It may provide rice plants with fixed nitrogen and hence promote	[redacted]	

plant growth. Another strain, P. stutzeri CMT.9.A, was isolated from the roots of a Sorghum nutans cultivar in Germany (189). It has not been assigned to any known genomovar. P. stutzeri JM300, the only member of genomovar 8, is a denitrifying soil isolate (18, 60). Strain ZP6b, classified as P. stutzeri "var. mendocina," was isolated from the rhizosphere of capers (Capparis spinosa) in Spain (6). Functional nitrogenase activity was tested by the acetylene reduction assay and by the incorporation of 15N. Rates of acetylene reduction in the ni-trogenase assay are in the range of those reported for other strains that are presently considered to be Pseudomonas species (66). Nitrogen fixation occurs at low oxygen tension under microaerobic conditions, as has been observed in many aerobic diazotrophs. The optimal pO2 for nitrogen fixation by strain CMT.9.A is 0.01 atm. In addition to nitrogen-fixing ability, some P. stutzeri strains, such as JM300 and CMT.9.A, show hydrogenase activity (18), supplying additional energy for me-tabolism.	[redacted]	

The nifH gene, encoding the nitrogenase protein, is considered to be the diagnostic gene for nitrogen fixation. It has been detected in all diazotrophic P. stutzeri strains studied to date. A nifHDK probe from Klebsiella pneumoniae gave a positive signal against the EcoRI-restricted total DNA of strain ZP6b (6). Both nifH and nifDK probes from Azospirillum brasilense also gave a positive signal against strain A1501 (a derivative of strain A15). Partial nifH sequences from strains A15 and CMT.9.A are identical. The nifH phylogeny in the domain Bacteria is largely congruent with phylogenetic trees based on 16S rRNA. They are within the cluster of other strains of the Gammaproteobacteria (380). Some strains have been analyzed for the presence of alternative nitrogenase systems. Genomic DNA from strains CMT.9.A and JM300 did not hybridize with a nifDK probe encoding the large subunit of Mo-nitrogenase in Azotobacter chroococcum (105). In addition, no signal was detected with the genes of the alternative nitrogenases	[redacted]	

anfDGK and vnfDGK. The other known pseudomonad that fixes nitrogen, P. azotofigens 6H33bT, has nifH and nifD genes that are closely related to the corresponding genes of strain A15. Deduced amino acid sequences for the P. stutzeri A15 nifHDK operon had the highest identity (87 to 91%) with the respective A. vinelandii homologs (92). Moreover, the gene organization in the nifH region of P. stutzeri A15 was identical to the gene organiza-tion of A. vinelandii. The close relationship between the species has been revealed by a detailed phylogenetic analysis of A. vine- landii. This led to the suggestion that Azotobacter could be considered a Pseudomonas in disguise (277). The regulation of nif genes has been studied only for strain A1501. This strain is similar to A15 (if not identical). Strain A1501 was reisolated from rice roots inoculated with strain A15. Results suggest that P. stutzeri has a large number of NtrC family response regulators, as does P. aeruginosa. Differences were	[redacted]	

detected in a P. stutzeri NtrB mutant that had impaired nitrogen fixation. No such differences were found in the case of Azotobacter spp. Moreover, NifA controls NtrBC expression. This may reflect the fact that the regulatory circuit is different from other organisms. The on/off switch of nitrogenase activity and the role of the nif genes also reflect interesting features of this particular strain. A study to identify which genes are switched on during rice colonization and switched off during free-living growth on a synthetic medium has been conducted by Rediers et al. (276). Some of the corresponding genes are involved in stress response, chemotaxis metabolism, and global regulation. Others encode putative proteins that have either unknown functions or no significant homology to known proteins. A 50-kb plasmid that may carry some genetic information for nitrogen fixation in strain CMT.9.A has been identified. When this plasmid is cured, there is an associated loss of N2 fixation capability (189). No plasmid was detected in strain A1501. This is consistent with the assumption that nif	[redacted]	

genes have a chromosomal localization in strain A1501. The origin of the nitrogen-fixing genes in some strains of P. stutzeri can be explained by a plausible lateral gene transfer acquisition. This hypothesis seems to be supported by nifH phylogenies (380). Phosphite and Hypophosphite Oxidation A number of bacteria have been shown to be capable of oxidizing reduced phosphorous compounds when these are provided as the sole source of phosphorous. Inorganic phosphite and hypophosphite can be used as such a source. They are oxidized to phosphate by several species, including members of the genus Pseudomonas. In a screening of bacteria that oxidize reduced phosphorous compounds to phosphate, Metcalf and Wolfe, in 1998, were able to isolate 10 bacterial strains after enrichment under selective conditions (223). Strain WM88 was studied in detail. It is a P. stutzeri strain that is closely related to the genomovar 3 reference strain DSM50227.	[redacted]	

Related collection strains, including four known P. stutzeri strains, were not able to oxidize hypophosphite. However, strain DSM50227 (a clinical isolate) was able to oxidize phosphite. The genes required by strain WM88 have been cloned and studied, together with the respective enzymes. Two oper- ons in the Pho regulon (htx and ptx) are needed to use phosphite and hypophosphite as alternative P sources (395). An Alcaligenes faecalis strain was isolated recently. This strain has htx genes that are virtually identical to their homologs in P. stutzeri, indicating that horizontal gene transfer may have oc-curred. However, pdtx in A. faecalis is different from the homolog in P. stutzeri (400). Whether the htx genes are widely distributed in P. stutzeri or restricted to strain WM88 remains to be studied.	[redacted]	
Biodegradation and Useful Properties for Biotechnological Applications Pseudomonas stutzeri is a ubiquitous bacterium with a	[redacted]	

high degree of physiological and genetic adaptability. It is present in a large number of different natural environments (see "Habitats and ecological relevance," below). Like other Pseudomonas species (e.g., P. putida), P. stutzeri is involved in environmentally important metabolic activities. Some of its major tasks are metal cycling and degradation of biogenic and xeno- biotic compounds (oil derivatives—aromatic and nonaromatic hydrocarbons—and biocides).	[redacted]	
Metal cycling. Although metals are essential nutrients, they can be toxic in excess. Moreover, some metals are toxic and have no beneficial purposes. As a result, bacteria have developed systems to ensure the availability of essential metals and, simultaneously, to handle metal toxicity. P. stutzeri is no exception. Three distinct types of siderophores (nocardamine, an arginine conjugate of 2,3-dihydroxybenzoic acid, and pyridine- 2,6-dithiocarboxylic acid) have been described for this species.	[redacted]	

These siderophores ensure the availability of essential metals, such as cobalt, copper, iron, and nickel (63, 64, 224, 313, 345). Furthermore, several P. stutzeri strains have been described due to their high biosorption potential and resistance to metals such as aluminum (422), chromium (12, 172), cobalt (172), copper (69, 215), germanium (370), lead (215), manganese (172), nickel (272, 346), plutonium (255), selenium (157), silver (135), thallium (172), titanium (38), uranium (172), vanadium (172), and zinc (34, 172, 215). Nearly all metal resistance systems seem to be gene encoded, and they are found on plasmids in most cases (328). However, most reported P. stutzeri strains ' resistance mechanisms remain poorly understood. In fact, the only well-characterized systems are the mercury resistance mechanisms carried on plasmid pPB of "P. stutzeri" OX1 (16, 280, 281). As mentioned above, this strain does not belong to the P. stutzeri species (see "Definition of the species and differentiation from other Pseudomonas species," above). Two distinct P. stutzeri strains	[redacted]	

have generated enormous biological and biotechnological interest: strain AG259 and strain RS34.

P. stutzeri AG259 is a silver-resistant strain isolated from the soil of a silver mine in Utah (135). Its silver resistance mechanism is still poorly understood, but it seems to be gene encoded on the plasmid pKK1 (135, 362). Although plasmid pKK1 is still unsequenced, it has been demonstrated that the encoded silver resistance mechanism is energy dependent (329). This mechanism seems to produce intracellular silver- sulfide complexes (331). Interestingly, P. stutzeri AG259 is also able to accumulate large amounts of germanium, copper, lead, and zinc on the cells by energy-independent passive binding (215, 330). In contrast, a more recent report (183) shows that P. stutzeri AG259 accumulates silver-based single crystals in the periplasm. This suggests that silver resistance in P. stutzeri AG259 involves metal efflux and metal binding. This is also the case in other well-characterized bacterial silver resistance systems (134, 201). The size of the periplasmic silver-based crystals (up to 200 nm) and their well-defined compositions and shapes

(equilateral triangles and hexagons) suggest that they have great potential as organic-metal composites in thin-film and surface-coating technology (183).	[redacted]	
P. stutzeri RS34 is a zinc-resistant strain isolated from an industrially polluted soil in New Delhi, India (34). This strain efficiently accumulates large amounts of zinc on its outer membrane through morphological and ultrastructural changes (36). Its zinc resistance mechanism is still unknown and does not resemble any known mechanisms (70). However, its use in removing zinc from solutions, low-grade ores, and ore tailings has been demonstrated (35, 37).	[redacted]	
An exhaustive study of nickel-resistant bacteria from anthro-pogenically nickel-polluted and naturally nickel-percolated ecosystems has been undertaken (346). This study analyzed a P. stutzeri strain isolated from a soil sample from New Caledonia. The sample was taken from the rhizosphere of Sebertia	[redacted]	

acuminata, a plant that hyperaccumulates nickel in its latex (25%) and leaves (1%). After anaerobic enrichment, the strain was isolated as a denitrifier in the presence of 10 mM NiCl2. P. stutzeri was resistant to 3 mM Ni, as well as to Co, Zn, and Cu. Nickel resistance genes were detected by Southern blotting and DNA-DNA hybridization with DNA probes. These genes seem to be located in a plasmid detected by the Kado and Liu method.	[redacted]	
Crude oil, oil derivatives, and aliphatic hydrocarbons. Although P. stutzeri was one of the first alkane-degrading micro-organisms identified—it was identified as Bacterium stutzeri (333)—few reports of crude oil-, oil derivative-, and/or aliphatic hydrocarbon-degrading P. stutzeri strains have appeared in the literature (81, 95, 156, 163, 166, 268). In contrast, much information is available for other Pseudomonas species, such as P. aeruginosa, P. fluorescens, P. oleovorans, and P. putida (re-viewed in reference 365). Nevertheless, one study directly isolated (with no prior enrichment) and identified 297 gasoline- degrading bacteria	[redacted]	

from a contaminated aquifer (282). A strong predominance by Pseudomonas spp. was observed (86.9% of all strains). P. stutzeri was the third most frequently isolated Pseudomonas species in this study (7.4% of all strains, 10.2% of Pseudomonas strains). In addition, a cultivation-independent analysis (based on 16S rRNA amplification and sequence) of the dynamics of bacterial communities was carried out during a field-scale evaluation of bioremediation on a mudflat beach contaminated with buried oil (288). This study demonstrated that P. stutzeri and Alcanivorax borkumensis are key microorganisms in dissipating hydrocarbon pollution on maritime beaches. These results also show that enrichment methods may be biased toward the isolation of non-P. stutzeri Pseudomonas spp. In spite of the small number of aliphatic hydrocarbon-degrading P. stutzeri isolates, two distinct P. stutzeri strains are of biological and	[redacted]	

biotechnological interest: strain KC and strain JJ. As mentioned above, P. stutzeri strain KC is an aquifer isolate that transforms CT to carbon dioxide, formate, and other nonvolatile products. This process occurs only under anoxic conditions, and no chloroform is formed (81, 101, 198, 355). CT has been found as a pollutant in soils and groundwater (159). It can also be mineralized biotechnologically. The fast transformation of CT by strain KC has enabled it to be used in bioaugmentation strategies. In such strategies it forms a biocurtain for the in situ remediation of a CT-contaminated aquifer (102, 401, 402). P. stutzeri strain JJ was isolated from 1,2-dichloroethane-contaminated soil (95). It is the first microorganism known to grow anaerobically on 2-chloroethanol under denitrifying conditions. Strain JJ requires anoxic conditions for 2-chloro- ethanol degradation. However, it has	[redacted]	

been suggested that the 2-chlorocatechol degradation pathway in this denitrifying strain is the same as that found in aerobic bacteria (96). In industry, 2-chloroethanol is used mainly in the synthesis of insecticides and as a solvent (95). It is metabolized by mammalian alcohol dehydrogenase to 2-chloroacetaldehyde, which is considered to be mutagenic (217). Very little is known about the emission and fate of 2-chloroethanol in the environment, as this compound is not included in routine soil pollution analyses. However, the interest in strain JJ and its 2-chloro- ethanol anoxygenic degradation capability resides in the fact that (i) many soils contaminated with chlorinated aliphatics are anoxic and (ii) nitrate is often present in groundwater (79, 116). Therefore, strain JJ may be of use in 2-chloroethanol bioremediation (95). Aromatic hydrocarbons. The benzene ring is one of the most widely distributed chemical structures in nature, as it appears in the recycling process of plant-derived material (139). In addition, its	[redacted]	

persistence in the environment is induced by its thermodynamic stability (87). Aromatic compounds are considered to be major environmental pollutants. Benzene and four of its relatives [polychlorinated biphenyls (PCBs), pol- yaromatic hydrocarbons (PAHs), benzo(A)pyrene, and benzo- (B)fluoranthene] have been in the top 10 of the National Priority List of Hazardous Substances since 1997 (http://www .atsdr.cdc.gov/cercla/). This list was drawn up by the EPA and the Agency for Toxic Substances and Disease Registry. The ability of Pseudomonas species to aerobically degrade benzene and its relatives is well known (164, 175, 237, 264, 364, 398). As shown in Fig. 4, the aerobic catabolism of these compounds involves a wide variety of peripheral degradation pathways. These pathways channel substrates into a small number of common intermediates (catechol, methylcatechol, chlorocatechol, protocatechuate, and gentisate). The intermediates are further processed by a few central pathways to tricarboxylic acid intermediates. The literature

indicates that P. stutzeri strains are able to metabolize benzoate (2, 113, 311, 350); mono- and di -halogen Br, Cl, I, or F benzoates (188); 4-hydroxybenzoate (2, 311); benzenesulfonate and 4-methyl-benzenesulfonate (15); carbazole (151, 245); cresol (13); dibenzothiophene (150); fluoranthene (178); fluorene (349); indan (349); naphthalene (113,114,218, 296,311,349) and its methyl (113,114,349) and chloro (114) derivatives; PCBs (90, 107); phenanthrene (218, 348); phenol (2) and dimethylphenol (13); pyrene (177); quinoline (320); salicylate (113, 114, 311) and its methyl (113, 114) and chloro (114) derivatives; tetralin (311); toluate (113, 296, 311); toluene (13); and xylene (97, 113, 296). However, several environments, such as subsurface organic-impacted sediments, are commonly anaerobic. Thus, biodegradation in these anaerobic environments must occur in the absence of oxygen. Information on aerobic degradation of aromatic compounds is much more abundant than information on anaerobic degradation (reviews of anaerobic degradation of aromatic hydrocarbons can be	[redacted]	

found in references 142, 339, 396, and 219). However, several strains of P. stutzeri have been isolated under nitrate-reducing conditions as degraders of dibenzothiophene, pyrene, phenanthrene, naphthalene, benzoate, fluorobenzoate, and/or salicylate (150, 218, 286, 378). The most-studied "P. stutzeri" strain is the toluene/xylene-degradative strain OX1 (9-11, 13, 17, 32, 33, 40, 58, 97, 197, 302, 303, 306, 312, 334, 353, 372-377). However, as mentioned above, this strain does not belong to the P. stutzeri species (see "Definition of the species and differentiation from other Pseudomonas species," above). Two distinct P. stutzeri strains have been well studied due to their biological and biotechnological interest: strain P16 and strain AN10. P. stutzeri strain P16 is a PAH-degrading bacterium. It was isolated from a phenanthrene enrichment culture of a creosote-contaminated soil (348). Strain P16 is able to grow, via salicylate, using phenanthrene (three rings),	[redacted]	

fluorene (two rings), naphthalene, and methylnaphthalenes (two rings) as the only carbon and energy sources (348, 349). It is also able to transform pyrene (four rings) to nonmineral products (177). Interestingly, the phenanthrene bacterial growth rate increased in the presence of Tergitol NP10, an anionic surfactant. The combination of strain P16, phenanthrene, and Tergitol has been proposed as a model for understanding the physical-chemical effects of surfactants on nonaqueous hydrocarbon bioavailability (130).	[redacted]	
P. stutzeri strain AN10 is a naphthalene-degrading bacterium isolated from polluted marine sediments in the western Med-iterranean Sea (113). Strain AN10 is able to dissimilate naph-thalene, 2-methylnaphthalene, and salicylate as sole carbon and energy sources (295). In contrast to the usual plasmid location of the naphthalene-catabolic pathway (397, 398), its dissimilatory genes are chromosomally encoded (296). Its entire naphthalene degradation pathway has been cloned and sequenced. It is	[redacted]	

organized into four operons: one coding for the enzymes involved in the conversion of naphthalene to salicylate (nahAAxAtA-tfiFCED) (42), two coding for the conversion of salicylate to pyruvate and acetyl-coenzyme A through the meta cleavage pathway enzymes (nahW and nahGTHINLOMKJ) (43, 44), and the last containing the regulatory gene nahR (44). Interestingly, two of these genes, nahG and nahW, encode two independent, inducible salicylate 1-hydroxylases (43). The gene nahW is unique to P. stutzeri. The two salicylate 1-hdyroxylases (NahG and NahW) from P. stutzeri AN10 were expressed upon incubation with salicylate. They are involved in naphthalene and salicylate metabolism (43). Both enzymes exhibited broad substrate specificities and metabolized salicylate, methylsalicylates, and chlorosalicylates. However, the relative rates at which the substituted analogs were transformed differed considerably. NahW was better at converting 3-chlorosalicylate, whereas NahG was more efficient at metabolizing methylsalicylates (43).	[redacted]	

Biocides. A biocide is a chemical agent that, under carefully controlled conditions, can kill organisms on objects and materials. Biocides are used extensively (in agricultural, clinical, and industrial fields, etc.). The amount of biocides released from human activities into the environment is extremely large (e.g., the release of cyanide from industry has been estimated to be above 14 million kg per year [103]). Biocides persist in nature and remain as a potential source of pollution. Bacterial populations could be useful in detoxifying these agents. Biocides that are degraded and/or resisted by P. stutzeri strains are tributyltin (167, 168, 299), an organostannic compound used industrially as a stabilizer in plastics and wood preservatives and as an antifouling agent in boat paints; nonoxidizing industrial water treatment bactericides (52-54, 194), used in industrial water cooling systems to avoid microbially induced corrosion of metal surfaces; fi-cyfluthrin (304), a pesticide used in agriculture to control lepidopteran pests	[redacted]	

affecting solanaceous crops; and cyanide and thiocyanates (129,174, 389-391), used in petro-chemical refining, the synthesis of organic chemicals and plastics, electroplating, aluminum works, and metal mining.	[redacted]	
of all P. stutzeri strains involved in biocide resistance and/or degradation, two are of biological and biotechnological interest: strain 5MP1 and strain AK61.	[redacted]	
P. stutzeri 5MP1 is a tributyltin-resistant strain (MIC > 1,000 mg ■ liter-1) isolated from the sediment of Arcachon Harbor (France) (167). Tributyltin resistance was found to be associated with the presence of the operon tbtABM. It is a member of the resistance-nodulation-cell division efflux pump family (168). Interestingly, TbtABM conferred a multidrug resistance phenotype to strain 5MP1, including resistance to n-hexane, nalidixic acid, chloramphenicol, and	[redacted]	

sulfamethoxazole (168). Many bacterial strains are capable of reducing the incidence of plant diseases caused by soilborne organisms such as bacteria, fungi, and nematodes. Such bacteria therefore act as biocontrol agents. Production of cyanide (HCN) by means of hydrogen cyanide synthetase has been studied intensively as one of the antibiosis mechanisms in the rhizosphere. Cyanide is highly toxic to living organisms because it inactivates the respiration system by tightly binding to cytochrome c oxidase (335). Some HCN-producing Pseudomonas species are plant beneficial, and others are plant deleterious. Such Pseudomonas species are common in soils. Thus, it is not surprising that bacteria have evolved with the capacity to degrade or detoxify HCN. P. stutzeri strain AK61 was isolated from wastewater at a metal-plating plant and was classified phenotypically and chemotaxonomically as a member of P. stutzeri (390). The aim of this study was to develop a biological treatment for cyanide. Such a treatment is needed, as cyanide is toxic and	[redacted]	

used in large amounts in the metal-plating, pharmaceutical, and agricultural-chemical industries. The biological treatment of cyanide may be cheaper and more environmentally acceptable than chemical methods such as alkaline chlorination, ozoniza-tion, and wet-air oxidation (100, 275). Whole cells of strain AK61 degraded cyanide rapidly in a 1 mM solution containing no organic substances. Induction of the cyanide-degrading activity was not dependent on the presence of cyanide. The cyanide-degrading enzyme was purified and characterized, and its encoding gene and potential active site were identified (389-391). Results indicate that the only enzyme responsible for the hydrolysis of cyanide to ammonia and formate was the cyanide-degrading nitrilase (cyanidase). More recently, the quaternary structure of the cyanide-degrading nitrilase from strain AK61 was determined. It is considered to be the model enzyme of the nitrilase superfamily (318). Enzymes from the nitrilase superfamily hydrolyze and	[redacted]	

condense a variety of non-peptide carbon-nitrogen bonds (49, 247). As a result, there is considerable interest in these enzymes as industrial catalysts. Therefore, uses include the production of nicotinic acid, R-(-)- mandelic acid, and S-(+)-ibuprofen and the detoxification of cyanide waste (78).

P. stutzeri is also involved in cometabolic degradative processes. A P. stutzeri strain was isolated from chemostat enrichment on bacteria that degrade the organophosphate insecticide parathion. This strain was able to cleave the substrate in p-nitrophenol and diethylthiophosphate but could not use either of the resulting molecules (234). Another P. aeruginosa strain in the consortium can mineralize p-nitrophenol but cannot attack intact parathion. The two-component enrichment degrades parathion synergistically with high efficiency. P. stutzeri apparently utilizes the products excreted by P. aeruginosa.

Proteolytic activity: applications for biorestoration. P. stutzeri is not considered to be proteolytic, as discussed in "Phenotypic identification,"

above. Only 1% of the strains give a positive reaction in the gelatinase test. However, P. stutzeri strain A29 was selected from a group of other Pseudomonas strains, as it exhibited good proteolytic activity in culture supernatant (273).	[redacted]	
The aim of the study was to select the best proteolytic bacterium for hydrolyzing the insoluble animal glue on a fresco called Conversione di S. Efisio e Battaglia, painted by Spinello Aretino (1391 to 1392). The most abundant components in animal glue are collagen and casein. The fresco was removed from its wall by a technique that involved the use of animal glue as a consolidating agent and treatment with formaldehyde as an antimicrobial agent. After the fresco was removed, the front of it was treated with proteolytic enzymes to restore the painting to view. The usual proteolytic treatments did not work. However, spraying of the fresco with a high density of viable P. stutzeri cells resulted in a satisfactory biorestoration process in 10 to 12 h. The most abundant proteolytic enzyme was 120 kDa in size and	[redacted]	

showed collagenase and caseinolytic activities. This enzyme has been studied in detail (7). The current working hypothesis is that different proteases with unique activities may act cooperatively.

NATURAL TRANSFORMATION

Genome analysis and molecular microbial ecology studies have shown that horizontal gene transfer is a relevant force in bacteria for continuous adaptation to environmental changes. Three broad mechanisms mediate the efficient movement of DNA between cells: transduction, conjugation, and natural transformation. Natural transformation involves bacterial uptake of naked DNA from the surrounding environment and its integration into the genome. Natural transformation has been observed in the bacterial species of very different phylogenetic and trophic groups. Natural transformation is perhaps the most versatile mechanism of horizontal gene transfer (206). Pseudomonas

stutzeri can be considered a naturally transformable bacterium, as one-third of its members are naturally transformable (60, 207, 326). Its transformation capability has been extensively studied during the last two decades. Competence is not constitutive in most naturally transformable bacteria; it depends on physiological state. P. stutzeri competence occurs in broth-grown cultures during the transition from the log phase to the stationary phase (60, 205). P. stutzeri competence is also developed in media prepared from aqueous extracts of various soils (204, 205). It is further stimulated under carbon-, nitrogen-, and phosphorous-limited conditions (204, 205), such as those frequently encountered by bacteria in soil. It has been demonstrated that P. stutzeri can be transformed by mineral-associated DNA in laboratory-designed glass columns (203), DNA bound in autoclaved marine sediment (342), and DNA adsorbed in sterilized soil (250). P. stutzeri can also access and take up DNA bound	[redacted]	

to soil particles in the presence of indigenous DNases, in competition with native microorganisms (323). P. stutzeri can be transformed by chromosomal and plasmid DNA. However, initial studies considered transformation only in the presence of homologous DNA, speculating that recognition sequences were necessary for DNA uptake (60, 61). Later studies reported natural transformation by P. stutzeri with different broad-host-range plasmids formed only by heterologous DNA (207, 326). Thus, it can be concluded that competent cells of P. stutzeri take up foreign DNA as well as DNA from their own species. However, the frequency of foreign DNA acquisition events was only 0.0003% of the value observed for fully homologous DNA transformation (221). The presence of a short (311-bp) homologous sequence on one side of the foreign DNA increased this frequency by 200-fold. However, gene integration occurred mostly in the nonhomolo- gous region, with the help of an illegitimate	[redacted]	

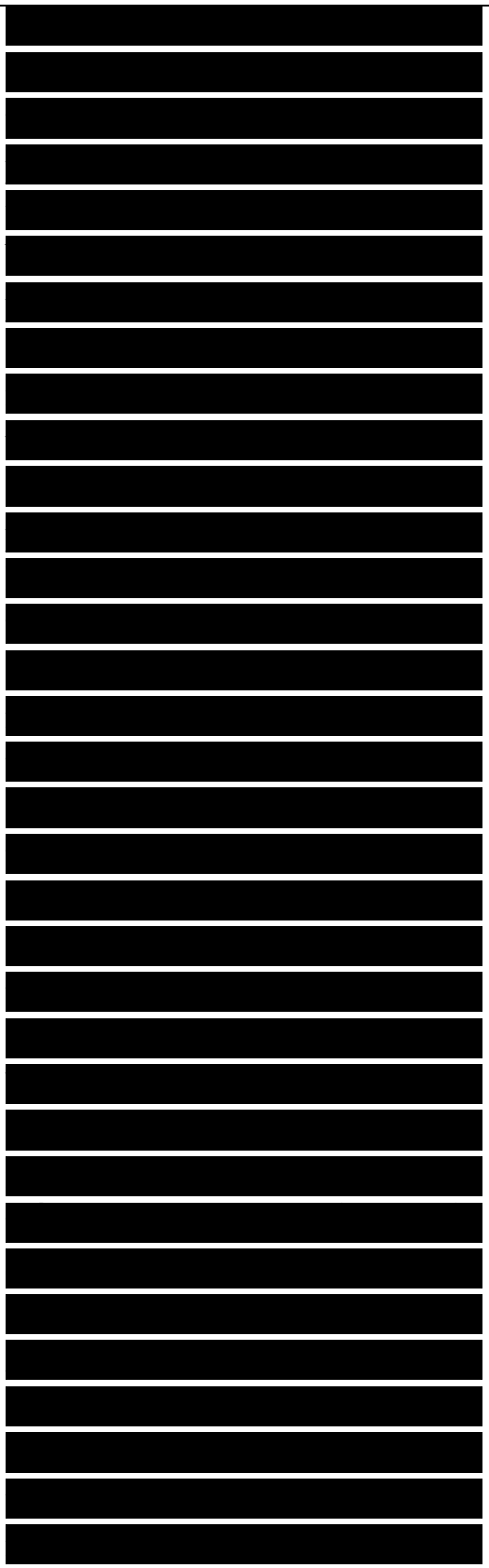
recombination event involving 3- to 6-bp G+C-rich microhomologies (221). In addition, a recA mutation decreased transformation with one-sided homologous DNA by at least 100-fold (221). These results suggest that genomic acquisition of foreign DNA by recA-dependent illegitimate recombination occurs in P. stutzeri. Transformability is widespread among environmental P. stutzeri strains. However, it has been shown that nontransform-ability and different levels of transformability are often associated with distinct genomic groups (326). This suggests that transformation capability may be associated with speciation in the highly diverse species P. stutzeri. In this respect, it has been shown that the presence of DNA restriction-modifica- tion systems and mismatch repair mechanisms in P. stutzeri act as barriers to the uptake of foreign DNA. These mechanisms may therefore contribute to sexual isolation and further speciation (31, 222). Natural transformation capability requires the presence of a considerable number of	[redacted]	

gene products. Although much information has been obtained for Bacillus subtilis and Neisseria gonorrhoeae—see a review by Chen and Dubnau (67)—the transformation machinery of P. stutzeri has been studied only recently (125-128, 220). It has been demonstrated that P. stutzeri naturally transforms both duplex and single-stranded DNA using the same machinery. The levels of duplex DNA transformation are 20- to 60-fold higher than the levels of single-stranded DNA transformation (220).

It has been reported that type IV pili are essential to genetic transformation in P. stutzeri (125). In this study it was shown that insertional inactivation of two genes, pilAI and pilC, abolished pilus formation. In addition, mutants of both genes were not able to transform DNA. The pilAI gene showed high similarity to pilin genes of other species. Its product, PilAI, was defined as the structural protein of the P. stutzeri type IV pili.

PilAI was involved in the first step of transformation: the competence-specific binding of duplex DNA, its transport into the periplasm, and its transformation in a DNase-

resistant state (125). The pilC gene of P. stutzeri is transcribed with two other pil genes, pilB and pilD. Its product, PilC, was shown to be essential for DNA transformation. It seems to be a hydrophobic protein involved in the transport of processed PilAI protein (125). The pilB and pilD gene products, PilB and PilD, resemble accessory proteins in type IV pilus biogenesis. They are probably located in the cytoplasm and in the inner membrane, respectively (125). Interestingly, a new gene, pilAII, was identified downstream from the pilAI gene. Its product, PilAII, is 55% identical in amino acid sequence to that of PilAI (127). Although both genes were cotranscribed, the expression of pilAII was only 10% of that observed forpilAI (127). Secondary pilin-coding genes have been found in other well-studied trans-formable bacteria, such as Neisseria gonorrhoeae, Acinetobacter sp. strain BD4, Bacillus subtilis, Streptococcus pneumoniae, and S. gordonii. Their inactivation results in a loss of transformation capability (56, 71, 210, 262, 403). Surprisingly, the genetic inactivation of P. stutzeri pilAII produced a



hypertransformation phenotype (127). It has been suggested that the role of PilAII is to interfere with DNA transport within the cell following DNA uptake. PilAII therefore acts as a factor that is antagonistic to genetic transformation. Its controlled expression defines the level of transformability shown by naturally competent P. stutzeri cells (127). The second step of transformation consists of the translocation of DNA from the periplasm to the cytoplasm. In P. stutzeri, this step is totally dependent on the comA gene product (126). ComA is a polytopic integral membrane protein that is thought to form the pore through which single-stranded DNA reaches the cytoplasm (126). The nuclease involved in the transformation of duplex DNA into a single-stranded molecule remains unknown (67). No ATP-binding site has been found in the ComA amino acid sequence. This suggests that ComA is not the driving force behind DNA translocation. Instead, ComA may act in a		

protein complex with an energy-supplying enzyme (126). Inactivation in P. stutzeri of the exbB gene led to a reduction in its natural transformation rate (126). The product of exbB has been described as a member of the TonB-ExbB- ExbD complex (126). In E. coli, this complex is thought to mediate energy transfer of the electrochemical potential from the cytoplasm to the periplasm (193). Thus, it has been suggested that ExbB interacts with ComA in P. stutzeri to supply the energy needed for DNA translocation (126).

Finally, two other cotranscribed genes, pilT and pilU, have been identified and shown to be required for full transform- ability of P. stutzeri (128). In fact, pilT inactivation produces a transformation-deficient strain that is unable to take up DNA. A pilU mutant was only 10% naturally transformable compared with the wild-type strain (128). Both gene products, PilT and PilU, are homologous to components of a specialized protein assembly system—competence traffic NTPases—that is widely found in bacteria. This system is responsible for depo-lymerizing the pilus into pilin

monomers. Consequently, it is also responsible for pilus retraction (387). Thus, it has been suggested that pilus retraction pulls DNA into the periplasm from the bacterial surface. Subsequently, DNA is somehow moved to the ComA complex, where one strand is degraded. The resulting single-stranded DNA is finally translocated into the cytoplasm (128).

PATHOGENICITY AND ANTIBIOTIC RESISTANCE

For a 15-year period after 1956, several reports described the isolation of P. stutzeri from clinical and pathological materials. However, there was no clear association of this species with an infectious process (117,118,182,191, 260, 340,394). In fact, 15 of the 17 strains studied in 1966 by Stanier et al. (340) were of clinical origin. In 1973, the first well-documented case of P. stutzeri infection appeared in the literature. It involved a nonunion fracture of a tibia (119). Since then, a few cases of P. stutzeri infection have been reported in association with bacteremia/septicemia (124, 180, 266, 267, 379); bone infection, i.e., fracture infection, joint infection,

osteomyelitis, and arthritis (119, 211, 279, 298, 361); endocarditis (290); eye infection, i.e., endophthalmitis and panophthalmitis (165, 195); meningitis (287, 354); pneumonia and/or empyema (59, 62, 187, 244, 266, 317, 407); skin infection, i.e., ecthyma gangrenosum (269); urinary tract infection (352); and ventriculitis (381). Only two of the above cases resulted in death (62, 180). This reflects P. stutzeri's relatively low degree of virulence. In fact, it is doubtful whether death was due to P. stutzeri infection in these two cases, as both patients had severe malfunctions caused by underlying conditions: chronic renal failure (180) and chronic liver disease (62). Interestingly, almost all patients with the aforementioned P. stutzeri infections had one or more of the following predisposing risk factors: (i) underlying illness, (ii) previous surgery (implying probable nosocomial acquisition),	[redacted]	

(iii) previous trauma or skin infection, and (iv) immunocom-promise. only two cases lacked any of these known risk factors: a man with vertebral osteomyelitis (279) and a 4-year-old boy with pneumonia and empyema (187). Studies to determine the distribution rates of P. stutzeri in hospitals have also been carried out. Two different studies were undertaken with all of the bacterial isolates obtained in university hospitals during a defined period from samples of wound pus, blood, urine, tracheal aspirates, and sputum. Both studies concluded that 1 to 2% of all the Pseudomonas spp. isolated were P. stutzeri (104, 238). Similar isolation rates (1.8%) were obtained in a study of Pseudomonas sp. infections in patients with human immunodeficiency virus disease (213). Interestingly, the highest rate of P. stutzeri isolation was reported by Tan et al. (352), who showed that 3% of all urine- isolated bacteria were P. stutzeri. Thus, it can be concluded that P. stutzeri is also ubiquitous in hospital environments and that this species could be	[redacted]	

considered an opportunistic but rare pathogen. Sensitivity tests for several antibiotics were performed in nearly all of the epidemiological and case reports mentioned above. There is a summary of these studies in Table 2. Nearly all studies involving several antibiotics and bacterial species showed that P. stutzeri was sensitive to many more antibiotics than P. aeruginosa, its most closely related species and a well-known human pathogen (238, 352, 356). Its higher sensitivity was explained by its reduced occurrence in clinical environments and, consequently, its lower exposure to antibiotics. In spite of these results, when bacterial isolates were obtained from immunosuppressed patients (i.e., patients with human immunodeficiency virus disease) no significant differences in antibiotic susceptibility between P. aeruginosa and other Pseudomonas spp., including P. stutzeri, were detected (213). Immunosuppressed patients are normally hospitalized for long	[redacted]	

periods. They are generally in contact with more types of antibiotics at higher doses. This extensive use of antibiotics could be responsible for the higher rate of isolation of antibiotic-resistant P. stutzeri strains. Interestingly, with the exception of fluoroquinolones, resistant P. stutzeri strains have been isolated for almost all antibiotic families (Table 2). This suggests that P. stutzeri has a wide range of antibiotic resistance mechanisms. At least two such antibiotic resistance mechanisms in

TABLE 2. Antibiotic sensitivities of P. stutzeri strains

Test results by yr (no. of isolates analyzed)'

a S, all strains analyzed were sensitive; R, one or more strains were resistant; —, not tested. References to studies from each year are as follows: 1970 (260), 1972 (118), 1974 (211, 301), 1977 (82, 352), 1983 (180, 317), 1987 (266, 290), 1994 (238, 257), 1997 (59, 110, 256, 383), 1998 (165), 1999 (356, 357), 2000 (111, 112), 2004 (187).

P. stutzeri have been described: (i) alterations in outer membrane proteins and lipopolysaccharide profiles (357-359) and
(ii) the presence of p-lactamases that hydrolyze natural and semisynthetic penicillins, broad-spectrum “p-lactamase-stable” cephalosporins, and monobactams with similar rates (108).

HABITATS AND ECOLOGICAL RELEVANCE

The remarkable physiological and biochemical diversity and flexibility of P. stutzeri is shown by its capacity to grow or- ganotrophically through mineralizing or degrading a wide range of organic substrates; its ability to grow anaerobically, using different terminal electron acceptors in a strictly oxidative metabolism; its oxidation of inorganic substrates, as a chemolithotrophic way to gain accessory energy; its resistance to heavy metals; and the variety of nitrogen sources it can use. We have discussed how P. stutzeri participates in key processes of element cycling, including C, N, S, and

P. In addition, a wide range of temperatures support P. stutzeri growth. This is an important physiological characteristic when the habitats that can be colonized by this species are considered. Phenotypic heterogeneity may be explained by P. stutzeri's huge range of habitats and growth conditions, including the human body. Spiers et al. classified ecological opportunity and competition as the main ecological causes of diversity (338). They emphasized that the underlying cause of diversity is genetic and that diversification occurs through mutation and recombination. The natural competence demonstrated by many P. stutzeri strains can help to increase genetic diversity. It provides new genetic combinations for colonizing new habitats or for occupying new ecological niches, even when the population is essentially clonal. It has insertion sequences, and mosaic gene structures have also been reported. There is considerable variation in the length of its genome (121). All of these factors suggest that different events may contribute	[redacted]	

to overall species diversity. The presence of P. stutzeri is almost universal. It has been detected through specific DNA sequences extracted directly from environmental samples (nirS, nosZ, nifH, 16S rRNA). It has also been isolated intentionally or accidentally from many habitats. Some of these, including extreme habitats, are considered below.

Soil, Rhizosphere, and Groundwater

The composition of the bacterial rhizosphere population, and in particular that of the diazotrophic bacteria, is of major interest. New isolation media and enrichment conditions have been developed with low oxygen tensions simulating rhizo- sphere conditions. This has led to the conclusion that the genus Pseudomonas is dominant or predominant in association with wheat, barley, and wetland rice (66, 93, 208). The role of diazotrophic P. stutzeri strains in soils might be more relevant than previously considered. A recent study involving PCR and denaturing gradient gel electrophoresis analysis of the N2- fixing

bacterial diversity in soil revealed a high percentage of nifH genes identical to those of P. stutzeri (93). Molecular analysis of diazotroph diversity in the rhizosphere of smooth cordgrass (Spartina alterniflora) suggests that P. stutzeri-related strains are present in the Spartina rhizosphere. Recently, analysis of bacterial populations in the rhizosphere of cordgrass, based on PCR amplification of nifH sequences and separation of the amplicons by denaturing gel electrophoresis, revealed nifH sequences highly similar to those of strains A1501 (a derivative of strain A15) and CMT.9.A (208, 209). The activity of an aromatic amino acid aminotransferase and the production of indole-3-acetic acid in P. stutzeri A15 have also been reported. This may be involved in the production of growth- regulating substances in plants in addition to their nitrogen- fixing ability (261). As mentioned above, many P. stutzeri strains have been isolated from contaminated soil sites, where degradative and contaminant-resistant strains have to develop relevant ecological activities. Some strains, such as KC, and several methyl-naphthalene-	[redacted]	

degradative strains have been isolated in our laboratory from groundwaters contaminated with aircraft fuel (JetA1). The efficacy of strain KC in detoxifying groundwaters has been shown through bioaugmentation.	[redacted]	
Marine Water and Sediment and Salt Marshes	[redacted]	
Most strains isolated from marine environments and initially classified in the genus Pseudomonas have been transferred to other genera after an analysis of their phylogenies. These transfers include P. doudoroffii to Oceanimonas doudoroffii, P. nautica to Marinobacter hydrocarbonoclasticus, P. stanieri to Marinobacterium stanieri, P. elongata to Microbulbifer hydrolyti- cus, and P. marina to Cobetia marina. Not many species within the genus Pseudomonas sensu stricto have been detected in marine waters. For a strain to be considered of marine origin, it must have the physiological characteristic of requiring, or at least tolerating, NaCl. P. stutzeri (including strain ZoBell, formerly P. perfectomarina), P. balearica,	[redacted]	

and P. xanthomarina (isolated from ascidian specimens in the Sea of Japan [289]) seem to be true marine Pseudomonas species. In addition, P. alcaliphila and P. aeruginosa (181) have been isolated from marine waters. Further research is required to define whether the latter pseudomonads might be considered marine bacteria or allochthonous to the ecosystem. Marine strains of P. stutzeri are located in the water column and in sediment. The most relevant strains studied in detail are ZoBell (isolated from the water column in the Pacific ocean and studied as a model denitrifier in marine environments), AN10 (isolated from polluted Mediterranean marine sediment and studied as a naphthalene degrader), NF13 (isolated from a sample taken at 2,500- to 2,600-m depth in the Galapagos rift from near a hydrothermal vent and studied as a strain that oxidizes sulfur chemolithotrophically), and strains MT-1 and HTA208	[redacted]	

(isolated from deep-sea samples taken at the Mariana Trench at 10,897-m depth). The main ecological role of these strains seems to be denitrification, besides their specific physiological properties.	[redacted]	
The study by Sikorski et al. (325) is the only one in which a large number of P. stutzeri strains have been isolated from the same sample, in this case marine sediment from the shore of the North Sea. This enabled a genetic study of the populations present in a single habitat to be undertaken.	[redacted]	
The ability of P. stutzeri to oxidize thiosulfate to tetrathionate both aerobically and anaerobically was not known before the work of Sorokin et al. (337). Several strains were isolated from the Black Sea at more than 100 m in depth. It was suggested that this widespread bacterium could be important in the turnover of thiosulfate in marine environments and that it may compete with thiosulfate	[redacted]	

disproportionation and reduction by thiosulfate-reducing bacteria.		
Spartina marshes support high rates of macrophyte primary production and microbially mediated nutrient cycling. The possible ecological role of P. stutzeri in such marshes seems to be its contribution to global carbon and nitrogen budgets. Primary production and decomposition in Spartina marshes are nitrogen limited (208). In these systems, diazotrophy is a key source of new nitrogen, and denitrification completes the nitrogen cycle. P. stutzeri participates in both processes. Direct molecular analysis of diazotrophic diversity in the rhizosphere of Spartina alterniflora demonstrates that gene sequences of nifH are highly similar to those of P. stutzeri. In addition, they are located in the same phylogenetic branch as many other sequences of nif genes obtained from marine microorganisms.	sơ cấp vi khuẩn (do vi khuẩn làm trung gian) khả dĩ vào sơ cấp (xảy ra trong điều kiện ít ni tơ) nitơ tương đồng rất nhiều với	
Wastewater Treatment Plants To screen bacteria with unusual metabolic properties, such as the degradation of anthropogenic compounds for bioremediation purposes, it is common to examine samples	sự chuyển hóa	

taken from wastewater treatment plants or to design bioreactors imitating the conditions of a treatment plant. Naphthalene degraders, thiosulfate oxidizers, chlorobenzoate degraders, and cyanide oxidizers have been isolated in this way. It has been demonstrated that P. stutzeri is also distributed in wastewater. However, no attempt has been made to quantify P. stutzeri in such habitats or to determine its relevance. CONCLUSIONS P. stutzeri genomovars can be considered genomospecies, as defined by J. P. Euzeby. According to his recommendations, if a genomospecies has been identified it is possible to look for phenotypic traits that differentiate it from the other genomo- species. If the genomospecies can be identified phenotypically, it must receive a name and be converted into a new species. If no phenotypic characteristic can be used to identify the genomospecies easily, it is left without a name. We prefer to maintain the genomovar concept for the genomic groups in P. stutzeri, because all of them share the basic phenotypic traits of the species. If DNA-DNA similarity	Vi khuẩn phân hủy vi khuẩn vi khuẩn phân hủy vi khuẩn phân lập KẾT LUẬN Các genomovar P. stutzeri có thể được coi là genomospecies, theo cách định nghĩa của J.P. Euzeby. Theo kiến nghị của ông, nếu một genomospecies đã được xác định thì có khả năng cao để tìm kiếm những đặc điểm kiểu hình để phân biệt với các genomo-species khác. Nếu genomospecies có thể xác định được kiểu hình, nó phải có một tên gọi và được chuyển sang một loài mới. Nếu không có đặc điểm kiểu hình nào sử dụng được để xác định các genomospecies dễ dàng, nó sẽ không có tên gọi. Chúng tôi muốn duy trì (giữ lại) khái niệm genomovar cho các nhóm gene của P. stutzeri, bởi vì tất cả chúng đều có những đặc điểm kiểu hình cơ bản của loài. Nếu chỉ có một tiêu chí duy nhất để phân định loài, là dựa trên sự giống nhau của kết quả DNA -DNA, hoặc	

results, or a multigenic sequencing approach, are accepted as the only criteria for species delineation, then P. stutzeri should be split into 17 different species. However, in our opinion, this situation would not help to clarify the taxonomic position of a phylogenetic and phenotypically coherent group of strains, as is the case for members of P. stutzeri.	dựa trên thứ tự sắp xếp chuỗi đa gene, thì P. stutzeri nên được chia thành 17 loài khác nhau . Tuy nhiên, theo ý kiến của chúng tôi, việc này sẽ không giúp làm sáng tỏ vị trí phân loại của một loài mới phát sinh và nhóm kiểu hình thống nhất của các chủng, như với trường hợp ở các phân loài của P. stutzeri.	
As demonstrated, genomovars are monophyletic biological and evolutionary units in which different ecotypes may be differentiated by their adaptation to new environmental conditions. P. stutzeri is widely distributed in natural environments and shows great metabolic versatility, which is consistent with a large effective population size. This species shows very low recombination rates. When there is a large population size and no assortive recombination, bacterial clones diverge freely by accumulating neutral mutations. The occurrence in a particular population of adaptive mutations conferring selective advantages in specific ecological situations leads to the elimination of genetic diversity within the population. However, in the presence of	Như đã chứng minh, genomovars là những đơn vị sinh học và tiến hóa có cùng nguồn gốc, trong đó các kiểu sinh thái khác nhau có thể được phân biệt bằng khả năng thích ứng của chúng với các điều kiện môi trường mới. P. stutzeri phân phối rộng rãi trong môi trường tự nhiên và có đặc điểm linh hoạt chuyển hóa lớn, phù hợp với kích cỡ loài lớn và hiệu quả. Loài này có tỷ lệ tái tổ hợp rất thấp. Khi kích cỡ loài lớn và không xảy ra tái tổ hợp assortive (có chọn lọc), các vi khuẩn nhân bản phân ra một cách tự do bằng cách tích lũy các đột biến trung tính. Sự xuất hiện khả năng biến đổi có chọn lọc trong một quần thể cụ thể ở các môi trường sinh thái cụ thể dẫn đến việc loại bỏ sự đa dạng di truyền trong quần thể. Tuy nhiên, khi tỷ lệ tái tổ hợp cực kỳ thấp, những đột biến như vậy không ngăn chặn phân tách di truyền giữa các quần thể. Vì vậy, sự đa dạng di truyền đặc biệt cao	

very low recombination rates, such mutations do not prevent genetic divergence between populations. Thus, the exceptionally high genetic diversity of P. stutzeri may be the result of niche-specific selection that occurs during colonization and adaptation to a wide range of microenvironments. Horizontal gene transfer seems to be an efficient mechanism for introducing new phenotypes into the genomes of P. stutzeri, without affecting the housekeeping genes. Integrons may play an important role in the acquisition of these new properties.	của P. stutzeri có thể là kết quả của việc lựa chọn tùy theo từng hốc sinh thái trong quá trình xâm chiếm và thích ứng với một loạt các môi trường vi sinh. Chuyển gene ngang có vẻ là một cơ chế hiệu quả để đưa các kiểu hình mới vào bộ gene của P.stutzeri, mà không ảnh hưởng đến các gene housekeeping (giữ nhà). Các Integron có thể đóng vai trò quan trọng trong việc tiếp nhận các đặc tính mới này.	
In conclusion, P. stutzeri exhibits exceptionally high diversity within a clonal population structure. In such cases, the existence of a strong linkage disequilibrium can be explained by considering that P. stutzeri forms a metapopulation made up of multiple ecological populations. These populations occupy different ecological niches. Although recombination is possible within populations, it is rare or absent between different populations (216, 278, 410). More-extensive studies are required to assess the population structure of these	Nói tóm lại, P. stutzeri thể hiện tính đa dạng rất cao trong cấu trúc quần thể vô tính. Trong những trường hợp như vậy, sự tồn tại của một sự mất cân bằng liên kết mạnh mẽ có thể được giải thích bằng việc P. stutzeri tạo thành một quần thể lớn (siêu quần thể) bao gồm nhiều quần thể sinh thái. Các quần thể này chiếm các hốc sinh thái khác nhau. Mặc dù hiện tượng tái tổ hợp có thể xảy ra trong các quần thể, nhưng việc này hiếm hoặc không xảy ra giữa các quần thể khác nhau (216, 278, 410). Các nghiên cứu chuyên sâu hơn sẽ cần thiết để tiếp cận được cấu trúc quần thể sinh thái của P. stutzeri. Tuy nhiên, các kết quả cho đến thời điểm này phù hợp	

ecological populations of P. stutzeri. However, the results reported to date are consistent with the conclusion that this bacterial species represents a good example of a phenotypically cosmopolitan ecological species sensu Istock (160), i.e., a species characterized by limited phenotypic variation, restricted local sets of genetic clones, and no or rare recombination. The clonal sets are genetically diverse, but phenotypic resemblance is sufficient to make phen- etic classification and identification possible. P. stutzeri is the species with the highest genetic diversity described to date. MLEE and MLST data confirm the results obtained by other techniques that have shown that some clones of P. stutzeri are distinct enough to warrant taxonomic differentiation (23).	với kết luận rằng loài vi khuẩn này là điển hình cho một loài sinh thái kiểu hình phổ biến sensu iStock (160), cụ thể hơn là, một loài đặc trưng bởi sự hạn chế biến đổi kiểu hình, hạn chế di truyền vô tính cục bộ, và không có hoặc hiếm khi diễn ra tái tổ hợp. Các dòng vô tính thường đa dạng di truyền, nhưng việc giống nhau về kiểu hình ~~là~~ đủ để thực hiện phân loại theo ngoại hình và định danh. P.stutzeri là các loài có sự đa dạng di truyền cao nhất tính đến nay. Các dữ liệu MLEE và MLST xác nhận các kết quả thu được bằng các kỹ thuật khác đã chỉ ra rằng một số dòng vô tính của P. stutzeri đủ khác biệt để đảm bảo sự phân biệt phân loài (23).	