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A NEW DENITRIFYING SAPROPHYTE RELATED TO *PSEUDOMONAS PICKETTII*

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RÉSUMÉ

UNE NOUVELLE BACTÉRIE DÉNITRIFIANTE SAPROPHYTE APPARENTÉE À « PSEUDOMONAS PICKETTII »

Une bactérie dénitrifiante jusqu'ici non décrite a été isolée du sol d'une rizière du Sénégal par culture d'enrichissement dans un milieu minimal contenant du succinate placé sous une atmosphère de N_2O. Les cellules se présentent sous la forme de petits bâtonnets à Gram négatif, non sporulés, mobiles par les moyens d'un ou deux flagelles polaires. Elles produisent un bactériophage. Leur métabolisme est obligatoirement respiratoire et elles croissent seulement si l'un des accepteurs d'électrons suivants est présent : NO_3^-, NO_2^-, N_2O ou O_2. L'organisme donne une réaction positive au test à l'oxydase, a un cytochrome *c* et une catalase. Il n'exige aucun facteur de croissance ; il est chimio-organotrophe et peut utiliser dans les conditions aérobies, comme source de carbone et d'énergie, 80 substrats comprenant de nombreux hydrates de carbone, polyols, acides gras, acides dicarboxyliques, hydroxy-acides et amino-acides. Le poly-β-hydroxybutyrate est synthétisé. La température maximale de croissance se situe au voisinage de 44° C. La gélatine et le « Tween 80 » sont hydrolysés. La nitrate-réductase B, la nitrite-réductase respiratoire, l'uréase et la L-phénylalanine-désaminase sont présentes. La nitrate-réductase A, l'hydrogénase, la β-galactosidase, l'amylase exocellulaire, la lécithinase et l'arginine-dihydrolase constitutive sont absentes. L'assimilation du quinate a lieu par un clivage *ortho* du protocatéchuate. La teneur en guanine + cytosine de son ADN est de 64,3 %.

L'organisme s'apparente étroitement à *Pseudomonas pickettii* mais

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s'en différencie par 16 caractères. En effet, à la différence de notre souche, *P. pickettii* ne liquéfie pas la gélatine, peut utiliser comme source de carbone et d'énergie le caprate, la L-sérine, la L-thréonine, le L-aspartate et ne peut pas utiliser comme source de carbone et d'énergie l'éthanol, le D-mannitol, le D-ribose, le D-arabinose, le D-fucose, le D-mannose, le tréhalose, le pélargonate, la L-leucine, la L-ornithine, le pantothénate.

MOTS-CLÉS : *Pseudomonas*, Dénitrification ; Sol, Taxonomie.

INTRODUCTION

Previous articles [2, 3] have demonstrated the advantages of using nitrous oxide as a respiratory electron acceptor in the isolation, by enrichment culture, of denitrifying bacteria. In this manner we have succeeded in the isolation of numerous organisms which are clearly different from those usually isolated using nitrate as the electron acceptor. Among these encounters is a new denitrifying bacterium of the genus *Pseudomonas* which was isolated from the soil of a Senegalese rice paddy and which is characterized in this paper.

MATERIAL AND METHODS

1) The mineral basal medium used for the preparation of all media employed contained the following: $Na_2HPO_4.12\ H_2O$, 3.58 g; KH_2PO_4, 0.98 g; $MgSO_4.7H_2O$, 0.03 g; NH_4Cl, 0.5 g; $FeSO_4$, 0.6 mg; $CaCl_2$, 2 mg; distilled water, 1,000 ml; pH 7.

2) The inoculum was from the clay soil of a rice field located near the village of Boundoum Nord in the delta of the Senegal River. Samples were air dried soon after collection. Enrichment culture was in the liquid basal medium containing 0.5 percent sodium succinate in an atmosphere of pure nitrous oxide at 30° C. Following three successive passages, pure cultures were isolated on nutrient agar.

3) The methods are described or referred to in previous papers [2, 3, 7]. The nine strains of *P. pickettii* were isolated from various clinical specimens. They were sent to us by M. J. Pickett.

RESULTS

1) *Morphology.*

The organism is Gram-negative, motile, appearing as short rods with rounded extremities (1.2-3.0 μm × 0.6 μm) multiplying by binary fission. Neither endospores nor capsules have been observed. Electron microscopic examination reveals the presence of one or two polar flagella, some rare pili, and an occasional bacteriophage particle (fig. 1, 2 and 3).

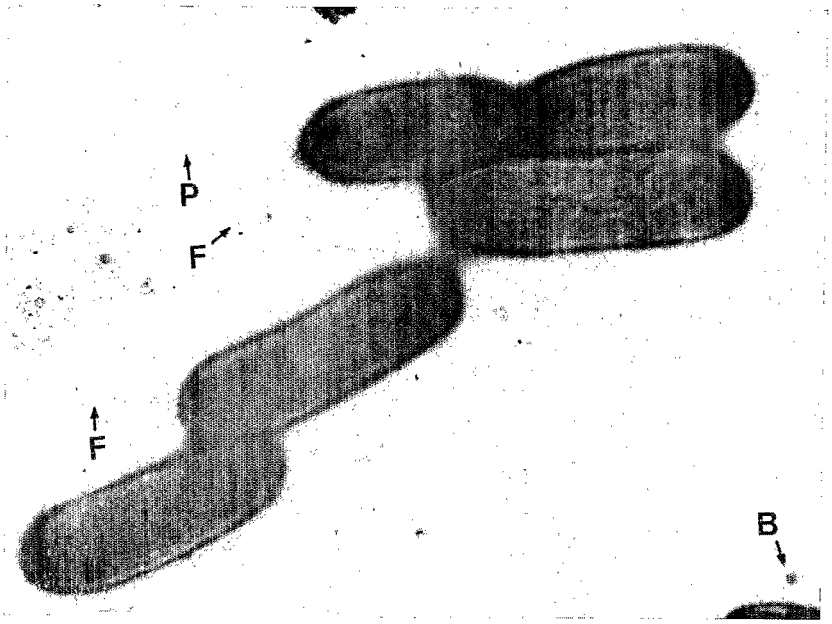

FIG. 1. — *Electron micrograph showing cells, the polar mode of insertion of the flagella (F), pili (P) and a bacteriophage particle (B).*
Negatively stained ; × 20,000.

Colonies are convex, circular, partly translucent, non-pigmented, and attain 5 mm diameter on nutrient agar.

2) *Cultural and physiological characters.*

The bacterium is a chemo-organotroph that does not grow in mineral media under atmospheres containing 65 % H_2, 6 % O_2 (or N_2O), 24 % N_2 and 5 % CO_2. It does not require any growth factors. Metabolism is respiratory, using O_2, NO_3^-, NO_2^- or N_2O as substrates ; by contrast, neither tetrathionate nor fumarate permit any growth in anaerobiosis. It grows in nutrient broth at pH 9.0, but not in peptone water containing 3 % NaCl. It does not grow at 4° nor above temperatures in the vicinity of 44°.

The bacterium synthesizes poly-β-hydroxybutyrate when grown in a medium containing DL-3-hydroxybutyrate deficient in nitrogen. Purified poly-β-hydroxybutyrate (from *Bacillus megaterium*) is neither hydrolyzed nor assimilated. Metachromatic granules are not observed within cells grown on nutrient agar.

In aerobiosis the following compounds are utilized as sources of carbon and energy: D-glucose, D-galactose, D-mannose, D-fucose, L-fucose, fructose, D-glucosamine, D-gluconate, D-glucuronate, D-saccharate, D-xylose, D-arabinose, L-arabinose, D-ribose, inosine, trehalose, 2-keto-D-

gluconate, D-mannitol, D-arabitol, glycerol, glycerate, 1,2-propanediol, ethanol, propanol, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, pelargonate, malonate, succinate, adipate, pimelate, suberate, sebacate, glycollate, citrate, DL-isocitrate, L-lactate, D-lactate, β-hydroxy-β-methylglutarate, L-malate, D-malate, DL-3-hydroxybutyrate, *meso*-tartrate, mucate, azelaidate, pyruvate, α-ketoglutarate, levulinate, fumarate, *trans*-aconitate, *cis*-aconitate, citraconate, crotonate, *para*-hydroxybenzoate, L-mandelate, quinate, hippurate, benzoylformate, kynurenate, *d*-pantothenate, glycine, α-L-alanine, α-D-alanine, β-alanine, DL-valine, L-leucine, asparagine, L-glutamate, DL-ornithine, L-histidine, L-phenylalanine, L-tyrosine, L-tryptophan, L-proline, acetamide, and 4-amino-*n*-butyrate.

The following compounds are not utilized as sources of carbon and energy: L-glucose, L-sorbose, α-methyl-D-glucoside, β-methyl-D-glucoside, α-methyl-D-galactoside, β-methyl-D-galactoside, α-methyl-D-mannoside, salicin, L-rhamnose, α-methyl-D-xyloside, β-methyl-D-xyloside, D-erythrose, lactose, maltose, sucrose, cellobiose, melibiose, arbutin, D-melezitose, raffinose, starch, inulin, D-sorbitol, D-dulcitol, *meso*-erythritol, ribitol, L-arabitol, 2,3-butanediol, 1,2-ethanediol, butanol, isobutanol, méthanol, 2-phenylethanol, caproate, caprate, oxalate, glutarate, tartronate, *d*-tartrate, *l*-tartrate, maleate, itaconate, mesaconate, benzoate, *ortho*-hydroxybenzoate, *meta*-hydroxybenzoate, phthalate, D-mandelate, terephthalate, nicotinate, cinnamate, anthranilate, *para*-aminobenzoate, phenylacetate, naphthalene, testosterone, dodecane, hexadecane, sarcosine, creatine, betaine, DL-norvaline, L-norleucine, L-isoleucine, DL-serine, DL-threonine, DL-methionine, DL-aspartate, L-lysine, L-arginine, L-citrulline, histamine, D-trytophan, ethanolamine, tryptamine, methylamine, *n*-butylamine, *n*-amylamine, benzylamine, DL-2-amino-*n*-butyrate, putrescine, spermine and geraniol.

The organism is oxidase-positive, produces catalase and a cytochrome *c* which in the reduced form has major absorption peaks at 551.5 nm (α) and at 522 nm (β). Neither pyocyanin nor fluorescent pigments are produced in King A or King B media, respectively. Gelatin is rapidly liquefied. Tween 80 is hydrolyzed. Nitrate reductase B, respiratory nitrite reductase, urease, and L-phenylalanine deaminase are present. In contrast, nitrate reductase A, hydrogenase, β-galactosidase, extracellular amylase, lecithinase, and constitutive arginine dihydrolase are absent. An acidification of glucose minimal medium is observed under aerobic conditions. Dinitrogen is not fixed. Nitrate is assimilated.

Cells grown aerobically with quinate as sole carbon and energy source produce protocatechuate-3,4-oxygenase and accomplish an *ortho* cleavage of protocatechuate [1, 6]. This enzyme is inducible and is not synthesized

FIG. 2. — *Electron micrograph showing the mode of insertion of flagella and pili (P) and a bacteriophage particle (B) with contracted tail and sheath attached to a cell wall fragment (Pa).* Negatively stained ; × 82,000.

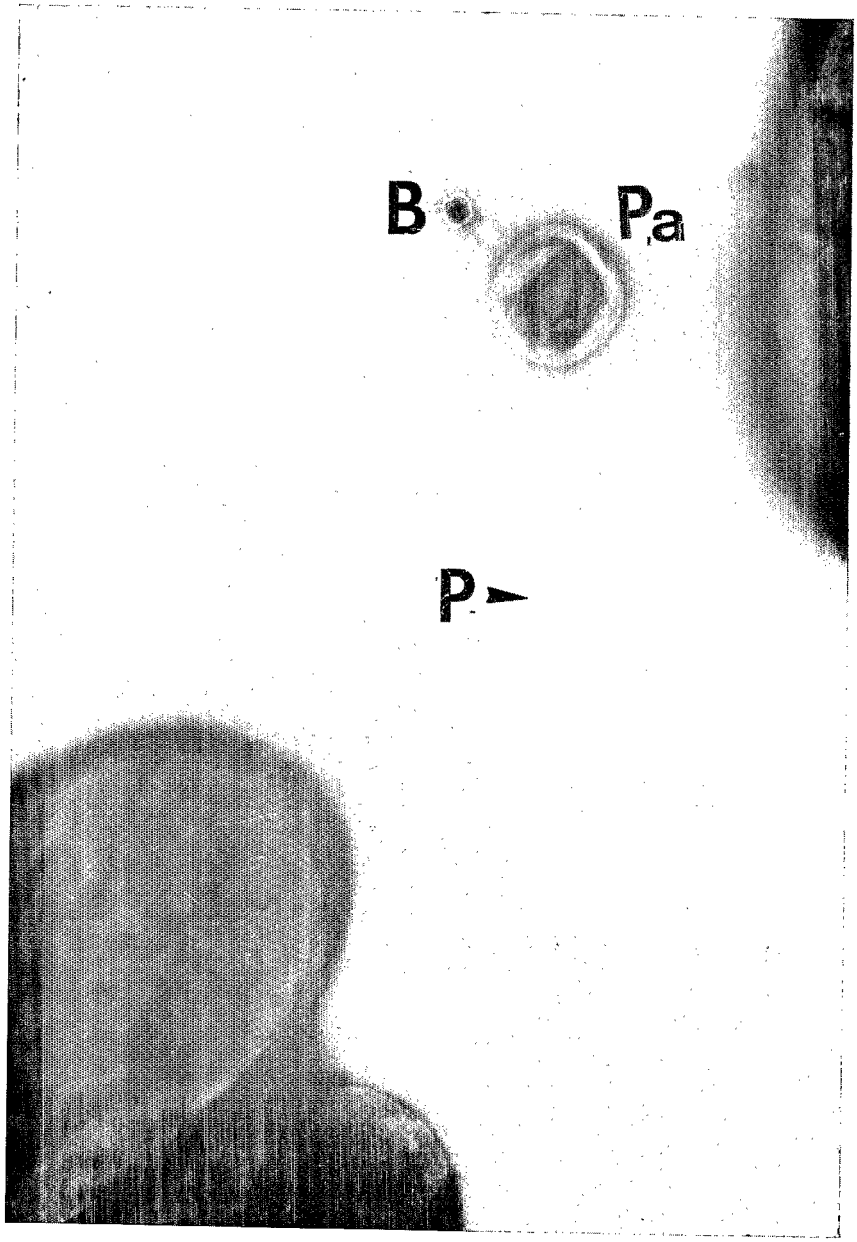

FIG. 2

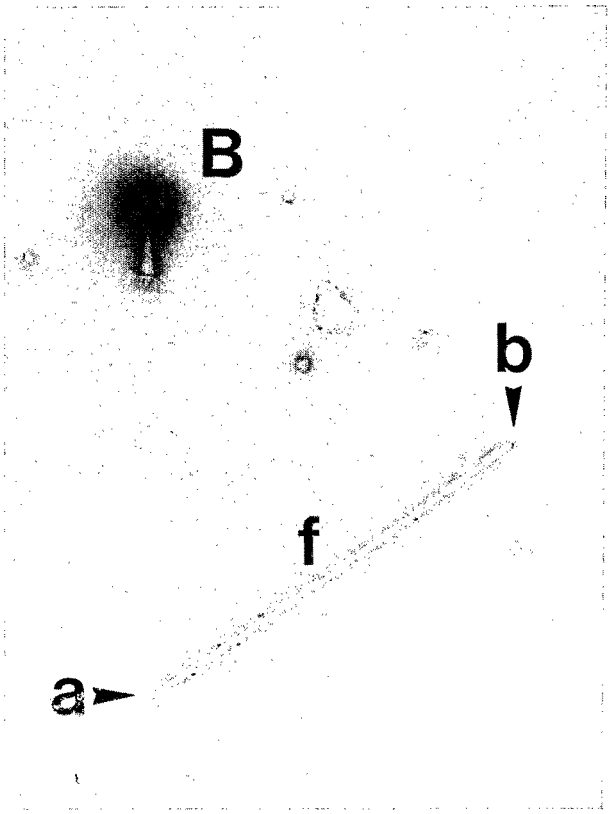

FIG. 3. — *Electron micrograph showing a bacteriophage particle (B) which has not contracted and a flagellar filament (f) with a fracture (at a) in the form of a swallow tail indicative of a conical arrangement of the flagellin. The crochet or hook is evident at b.* Negatively stained ; × 81,500.

by cells grown in a medium containing only yeast extract. Indole is not produced.

Denitrification has been studied anaerobically in the presence of succinate as electron donor. The gases produced have been identified and measured by gas chromatography. Suspensions of cells grown anaerobically in the presence of nitrate reduce NO_3^-, NO_2^- and NO to N_2O and N_2. Cells grown anaerobically in an atmosphere of N_2O reduce this gas to N_2.

3) *Base composition of the deoxyribonucleic acid.*

The buoyant density of the DNA in neutral CsCl was determined to be 1,723 gcm^{-3}, equivalent to 64.3 moles percent guanine+cytosine.

DISCUSSION

The bacterium described in this paper uses 80 different carbon substrates amongst which are numerous carbohydrates, polyols, fatty acids, dicar-

boxylic acids, hydroxy acids and amino acids. It is clearly a member of the genus *Pseudomonas* [7] and appears to be quite similar to *P. pickettii.* The latter species, also an omnivorous denitrifier capable of growth at 41° and of the production of poly-β-hydroxybutyrate has, however, not been isolated from environments other than clinical specimens [4].

We have verified that nine cultures of *P. pickettii* (K-300, K-214, K-230, K-288, K-286, K-336, K-298, K-279 and K-468) synthesize nitrate reductase B anaerobically in the presence of NO_3^-. Of these, only K-300 was found to be able to denitrify nitrous oxide.

There are sixteen distinctive characters between *P. pickettii* and the new culture. The 20 strains of *P. pickettii* are distinguished from this organism by their inability to liquefy gelatin, their ability to use caprate, L-serine, L-threonine, L-aspartate and their inability to use ethanol, D-mannitol, D-ribose, D-arabinose, D-fucose, D-mannose, trehalose, pelargonate, L-leucine, L-ornithine, pantothenate. The molar percentage of guanine plus cytosine in the DNA of *P. pickettii* is 64 % [4], a value not significantly different from that found for the rice paddy isolate.

The holotype has been deposited in the collection of the Pasteur Institute (n° CIP 291-75).

The majority of the pseudomonads do not grow at 44°. Nevertheless another omnivorous denitrifying organism capable of accumulating poly-β-hydroxybutyrate, the agent of melioidosis —*P. pseudomallei*— can grow at 42°. And it too has not been isolated from soils other than in the tropical regions [5]. It would be of interest, from the ecological standpoint, to know whether our bacterium is similarly distributed. *P. aeruginosa* is a denitrifying bacterium that grows at 43°. This survey would require the definition of greater selectivity in the enrichment procedure than we have thus far attained. We have not yet disclosed suitable sensitive indicator strains for attack by the bacteriophage which is present in the culture we have studied.

SUMMARY

A denitrifying bacterium, hitherto undescribed, was isolated from the soil of a Senegalese rice paddy by enrichment culture in minimal-succinate medium under an N_2O atmosphere. The cells are small, Gram-negative rods, non-sporulating, and motile with one or two polar flagella. They produce bacteriophage. Their metabolism is obligatory respiratory and they grow only if one of the following electron acceptors is present: NO_3^-, NO_2^-, N_2O or O_2. The organism gives a positive reaction to the oxidase test and contains cytochrome *c* and catalase. It requires no growth factor, is chemo-organotrophic, and under aerobic condition can use as carbon and energy source 80 substrates including many carbohydrates, polyols, fatty acids, dicarboxylic acids, hydroxy acids and amino acids. Poly-β-hydroxybutyrate is synthesized. The maximal growth temperature is about 44°. Gelatin and Tween 80 are hydrolyzed. Nitrate reductase B,

respiratory nitrite reductase, urease and L-phenylalanine deaminase are present. Nitrate reductase A, hydrogenase, β-galactosidase, exocellular amylase, lecithinase and constitutive arginine dihydrolase are absent. The assimilation of quinate proceeds by an *ortho* cleavage of protocatechuate The percentage of guanine+cytosine in its DNA is 64.3 %.

The organism is closely related to *Pseudomonas pickettii* but can be distinguished from it by 16 different characters. Unlike our strain, *P. pickettii* does not liquefy gelatin, can use caprate, L-serine, L-threonine, L-aspartate as carbon and energy source but cannot use ethanol, D-mannitol, D-ribose, D-arabinose, D-fucose, D-mannose, trehalose, pelargonate, L-leucine, L-ornithine, pantothenate.

KEY-WORDS: *Pseudomonas*, Denitrification; Soil, Taxonomy.

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