Physiological and Genetic Characterization of a Diazotrophic *Pseudomonad*

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A soil isolate, 4B, which had been previously assigned to the genus *Pseudomonas* and shown to be capable of reducing C_2H_2 with simple phenolic compounds as sole carbon source, was further characterized in comparison with two other diazotrophs which were identified as pseudomonads. The DNA base composition of 4B was 60.2 mol% G + C. Plasmid DNA was not detected in alkaline SDS lysates of 4B by agarose gel electrophoresis. Comparable maximum C_2H_2 reduction activities in 4B were observed under microaerobic conditions (pO_2 about 0.003 atm) with either 28 mM-glucose or 5 mM-protocatechuate as carbon source. N_2 fixation was confirmed by the cellular incorporation of $^{15}N_2$ in cultures of 4B grown in N-free medium. Extensive biochemical tests, including the carbon utilization pattern, demonstrated that 4B was closely related to *Pseudomonas delafieldii* (ATCC 17505) although the latter did not fix N_2 . 4B had metabolic patterns different from the two other strains reported to be diazotrophic pseudomonads; all three contained DNA homologous to the *nifHDK* genes of *Klebsiella pneumoniae* M5A1.

INTRODUCTION

Diazotrophy is widespread among prokaryotic micro-organisms (for review see Postgate, 1981, 1982). The diverse habitats of the N_2 -fixing bacteria include soil, aquatic, plant and animal ecosystems (Knowles, 1977, 1978). In such diverse environments different physiological requirements for optimal N_2 fixation, such as adjustment to energy source and oxygen concentration, have been evolved by these bacteria. Yet, all diazotrophs examined to date have been found to possess similar nitrogenase enzyme systems which are encoded by the highly conserved *nif* genes (Brill, 1980; Elmerich, 1984; Ruvkun & Ausubel, 1980).

Recently, two diazotrophic bacteria isolated from grass roots have been described (Barraquio *et al.*, 1983; Haahtela *et al.*, 1983). These have been identified as pseudomonads; yet there is no general agreement that the genus *Pseudomonas* includes diazotrophic species (Balandreau, 1983; Postgate, 1982; Palleroni, 1984). These isolates showed *Pseudomonas*-like properties upon gel immunodiffusion and in fluorescent antibody and lipopolysaccharide analyses, in addition to their morphological, cultural and biochemical characteristics. One of us (Chan, 1986) has also previously described a diazotrophic bacterium (designated 4B) which was isolated from a forest soil and tentatively identified as a *Pseudomonas* species. Its nitrogenase (C_2H_2 reduction) activity was supported by simple phenolic compounds as the sole carbon and energy source. We report here a more detailed physiological and genetic characterization of 4B which justifies its inclusion in the genus *Pseudomonas* and suggests that it is a new species. Proof of its diazotrophy is presented by $^{15}N_2$ incorporation as well as DNA homology with the *nif* structural genes of *Klebsiella pneumoniae*. A preliminary account of this investigation was presented at the Sixth International Symposium on Nitrogen Fixation, 4–10 August 1985, Corvallis, Oregon, USA.

METHODS

Bacterial strains, plasmids and DNA. Strain 4B, which has been tentatively assigned to the genus *Pseudomonas*, was originally isolated from a forest soil (Chan, 1986) and was recently deposited in the American Type Culture Collection (ATCC 43038). *P. delafieldii* (ATCC 17505) and *P. putida* (ATCC 33015) were obtained from the ATCC. *Pseudomonas* strain H8 (ATCC 35402) was a gift from W. L. Barraquio (Department of Microbiology, Macdonald Campus of McGill University, Canada). *Pseudomonas* strain DC, originally isolated from the roots of the grass *Deschampsia caespitosa* (Haahtela *et al.*, 1983), was kindly sent to us by K. Haahtela (University of Helsinki, Finland). *Klebsiella pneumoniae* M5A1 was obtained from V. N. Iyer, (Carleton University, Ottawa, Canada); *Escherichia coli* J53 (*proA metF* λ) carrying plasmid RP4 (Ap^rKm^rNm^rTc^r) was from the Plasmid Reference Center, Stanford University, USA; *E. coli* B was from the Department of Microbiology, University of Manitoba, Canada; and *E. coli* HB101(pSA30) was from D. R. Helinski, (University of California, USA).

Reference DNAs of *Clostridium perfringens*, *E. coli* B and *Micrococcus lysodeikticus* were purchased from Sigma.

Culture media. The maintenance agar used for the diazotrophic species consisted of 0.1% (w/v) Difco tryptic soy broth solidified with 1.6% (w/v) Difco Bacto-agar; *P. delafieldii* was maintained on nutrient agar. Tests for autotrophic growth with H₂, O₂ and CO₂ were done in the defined medium described by De Bont & Leijten (1976). The modified Burk's N-free liquid medium (Dalton, 1980) with either 28 mM-glucose or 5 mM-protocatechuate as carbon source was used for the cultivation of N₂-fixing cells and in assays for their nitrogenase activity. Autoclaving was done at 121 °C and 103.4 kPa for 20 min. All media were prepared with distilled water.

Biochemical and cultural characterization. The comparative characterization of 4B, *P. delafieldii*, H8 and DC was done according to Stanier *et al.* (1966) with the following modifications. Growth temperature studies of 4B were done in duplicate screw-cap tubes containing 5 ml 0.1% tryptic soy broth (pH 6.8) inoculated with 0.1 ml of exponential-phase cells. OD₄₃₀ was determined after incubation at various temperatures for 7 d. Growth dependence on pH was similarly investigated but the broth was prepared in phosphate/borate buffer adjusted to various pH values and OD₄₃₀ was measured after 24 h incubation at 30 °C.

Autotrophic growth with H₂ was assessed in 100 ml of the medium of De Bont & Leijten (1976) contained in a 250 ml side-arm flask. An exponential-phase culture (2 ml) was added to the flask which was then closed with a Suba-Seal stopper, evacuated and flushed aseptically three times with Ar. The final gas phase was adjusted with filter-sterile gases (H₂, O₂ and CO₂ respectively at 10, 5 and 5%, by vol.) to a total pressure of 101.3 kPa. The flask was incubated at 28 °C for 7 d. Growth was verified by an increase in OD₄₃₀ compared to a control without the addition of H₂ and CO₂.

Heterotrophic denitrification was assayed in buffered peptone medium with 6 mM-NaNO₃ or NaNO₂ in the presence or absence of 10% (v/v) C₂H₂ as described by Chan (1985). Growth and nitrous oxide production were used as the criteria for positive denitrification.

Presumptive nitrogenase tests were done using the C₂H₂ reduction technique in 18 × 150 mm test-tubes containing 10 ml modified Burk's medium with 28 mM-glucose solidified with 0.2% (w/v) agar (Chan, 1986).

DNA base composition was determined by UV-absorption ratios (Ullitzur, 1972) with DNAs from *C. perfringens*, *E. coli* B and *M. lysodeikticus* as standards.

The carbon utilization pattern of 4B was independently confirmed by the Applied Sciences Department of the ATCC using the methods of Stanier *et al.* (1966).

DNA hybridization and plasmid techniques. Total DNA was isolated from 5 ml of bacterial culture by the method of Marmur (1961), modified to include a treatment with 0.2 mg proteinase K (Beckman) ml⁻¹, before the extractions with chloroform/isoamyl alcohol. DNA was treated with *Eco*RI (Boehringer-Mannheim) and the fragments were separated by electrophoresis in a 1.0% agarose gel. The DNA fragments were transferred onto nitrocellulose paper (Schleicher and Schuell, BA85) as described by Southern (1975). Nick translation of pSA30 was done as described by Rigby *et al.* (1977) using deoxycytidine 5'-[α -³²P]triphosphate [3000 Ci mmol⁻¹ (111 TBq mmol⁻¹), New England Nuclear] and DNA polymerase I (Boehringer-Mannheim). Hybridizations were done in 50% formamide at 42 °C but otherwise were as described by Botchan *et al.* (1976). After washing, the filters were dried and exposed using Kodak XAR-2 film.

Bacterial matings to transfer plasmid RP4 between J53(RP4) and 4B were done on nitrocellulose filter discs on nutrient agar at 34 °C. Kanamycin resistant transconjugants of 4B were selected by streaking the mating mixture after 24 h growth onto *Pseudomonas* minimal agar containing 10 mM-sodium succinate (Murray *et al.*, 1972) and 20 µg kanamycin sulphate ml⁻¹. Plasmid DNA was detected in agarose gels as described by Wheatcroft & Williams (1981) and Eckhardt (1978); pSA30 DNA was purified in a CsCl-ethidium bromide gradient (Guerrey *et al.*, 1973).

Nitrogenase assay. Cultures of 4B were grown at 22 °C in 1 litre Erlenmeyer flasks each containing 750 ml modified Burk's N-free medium with either 28 mM-glucose or 5 mM-protocatechuate as the carbon source. The cultures were continuously stirred and sparged with filter-sterilized 1% O₂ (v/v) in N₂ (Matheson Gas Products, Canada) flowing at 250 ml min⁻¹ until early stationary-phase was reached (about 24 h). Samples (9 ml) were dispensed into sterile serum bottles (60 ml capacity). In order to ensure there was sufficient carbon substrate at the

beginning of the assay, 1 ml sterile solution containing 0.28 mmol glucose was added to each glucose-grown culture sample while 50 µmol protocatechuate in 1 ml sterile solution was added to the protocatechuate-grown culture sample. The bottles were then closed with Suba-Seal stoppers, evacuated and back-filled aseptically four times with He.

To determine the optimum O₂ concentration for nitrogenase activity, the initial pO₂ in sets of triplicate bottles was adjusted to 0, 0.001, 0.003, 0.005, 0.0075 and 0.01 atm. The assay was started by introducing 5 ml C₂H₂ into each bottle. The bottles were incubated in a shaking water bath (28 °C, 150 r.p.m.) and ethylene production was monitored by gas chromatography up to 5 h.

For the ¹⁵N₂ incorporation experiment, after evacuation and back-flushing with He, 10 ml ¹⁵N₂ (99 atom%; Merck Sharp & Dohme Isotopes, Canada) was introduced into each bottle after the same volume of He was withdrawn from the head space. The pO₂ was adjusted to 0.003 and 0.005 atm respectively for the glucose and protocatechuate cultures. Triplicate bottles were similarly set up but with the addition of ¹⁴N₂ instead of ¹⁵N₂. Parallel C₂H₂ reduction assays were also done as described above. Ethylene production was found to be constant without lag up to 5.2 h.

Gas chromatography. Nitrous oxide was quantified by electron-capture detection (Chan, 1985). O₂ and ethylene were measured by thermal conductivity and flame-ionization detectors, respectively (Chan, 1986). All gas analyses were done on 0.1 ml samples.

¹⁵N analysis. The total N of cells and spent culture medium was determined as NH₄⁺ after Kjeldahl digestion by steam distillation and colour development with Nessler reagent (Keeney & Nelson, 1982). Undigested samples were converted to N₂ by a modified Dumas method and the ¹⁵N content was analysed using an optical emission spectrometer as described by Preston *et al.* (1981).

Protein determination. Washed cells were assayed for protein, after hydrolysis in 1% (w/v) Na₂CO₃ and 0.5% (w/v) NaOH by the Lowry method using bovine serum albumin (Sigma) as standard.

Chemicals. All chemicals used were of analytical grade. When in acid form they were neutralized with NaOH before use. Heat-labile compounds were filter-sterilized before adding to the culture medium.

RESULTS

Comparative characterization

4B has been tentatively identified as a *Pseudomonas* species based upon morphological and biochemical criteria (Chan, 1986); further biochemical characterization showed that this strain is closely related to *P. delafieldii*. Table 1 summarizes the features of 4B pertinent to its classification in comparison with *P. delafieldii* and two *Pseudomonas* strains, H8 and DC, which have been reported to be diazotrophs (Barraquio *et al.*, 1983; Haahtela *et al.*, 1983).

The DNA G + C content of 4B (60.2 mol%) and the reference pseudomonads were within the range 58–70 mol% characteristic for the genus *Pseudomonas* (Palleroni, 1984). The base compositions of DNA extracted from H8 and *E. coli* B were found to be within 4% of the reported values of 63.8 and 51.3 mol% G + C, respectively (Barraquio *et al.*, 1983; Marmur & Doty, 1962).

Table 1. *Characteristics differentiating Pseudomonas strains 4B, H8, DC and P. delafieldii**

Key character	4B	<i>P. delafieldii</i>	H8	DC
Mol% G + C of DNA	60.2 ± 1.2	65–66†	62.0	ND
Fluorescent pigment	—	—	—	+
Arginine dihydrolase	—	—	+	+
Denitrification	—	+	+	—
Autotrophic growth				
with H ₂ /O ₂ /CO ₂	—	—	+	+
C ₂ H ₂ reduction	+	—	+	+
Utilization of:				
Citrate	+	—	—	+
Poly-β-hydroxybutyrate	+	+	—	—
p-Hydroxybenzoate	+	—	—	+

ND, Not determined.

* Refer to Barraquio *et al.* (1983) and Haahtela *et al.* (1983) for the respective sources of strains H8 and DC.

† Unconfirmed results from literature (Palleroni, 1984).

Fig. 1. Effect of initial pO_2 on the C_2H_2 reduction activity of cultures of *Pseudomonas* 4B (about $2\ \mu g$ cell protein ml^{-1}) provided with (a) 28 mM-glucose or (b) 5 mM-protocatechuic acid as the sole carbon source in N-free medium. Data points and vertical bars represent means of triplicate cultures and SD values, respectively, determined in time-course experiments for up to 5 h incubation. Where not shown, bars are encompassed by symbols.

Pseudomonas 4B shared several metabolic properties with *P. delafieldii* (Table 1). Neither had arginine dihydrolase activity nor was able to grow autotrophically with exogenous H_2 , although *P. delafieldii* is phenotypically very similar to the autotrophic H_2 -utilizing pseudomonads (Davis *et al.*, 1970; Palleroni, 1984). Denitrification, a character which is considered useful for differentiating the H_2 -utilizing pseudomonads (Davis *et al.*, 1970), was not detected in 4B but, contrary to a previous report (Davis *et al.*, 1970), *P. delafieldii* was found to denitrify. Both H8 and DC utilized H_2 , and H8 was also capable of denitrification (Table 1; Chan, 1985).

Strain 4B had a more extensive carbon utilization profile than *P. delafieldii* when 52 organic compounds were individually tested as the sole carbon source for growth. The additional carbon compounds utilized were citrate, *p*-hydroxybenzoate, maltose, rhamnose, L-tryptophan and valerate. Tween 20 and 80 were hydrolysed by 4B but not by *P. delafieldii*. 4B also produced acid from the following carbohydrates in an O-F base (Hugh & Leifson, 1953): L-arabinose, D-fructose, D-glucose, glycerol, inositol, D-mannose, L-rhamnose, D-ribose and D-xylose. Thus strains 4B, H8 and DC have different metabolic patterns despite their common diazotrophic property.

Physiological characterization of 4B

The maximum growth rate of 4B in 0.1% tryptic soy broth (pH 6.8) was at $34\ ^\circ C$; however, the rate did not vary greatly over the temperature range $25\text{--}44\ ^\circ C$. At $30\ ^\circ C$ optimum growth took place over a narrow pH range with a maximum at pH 6.2.

4B was previously shown to reduce C_2H_2 using either a carbohydrate (glucose) or a phenolic compound (*p*-hydroxybenzoate or protocatechuic acid) as the sole carbon and energy source (Chan, 1986). Fig. 1 shows that maximum C_2H_2 reduction, whilst being comparable in rate, occurred at a higher initial pO_2 when protocatechuic acid was used as the substrate (0.005 atm) rather than glucose (0.001–0.003 atm). This indicates a different tolerance or requirement for O_2 by 4B growing with the different carbon sources under N_2 -fixing conditions.

N_2 fixation was confirmed by cellular incorporation of $^{15}N_2$ in cultures of 4B provided with their optimal initial pO_2 for C_2H_2 reduction determined above (Table 2). Diazotrophic activities were high in both protocatechuic- and glucose-supported cultures. Fixed N in the spent medium due to excretion and cytolysis was estimated to be 16–18% of the total $^{15}N_2$ fixed.

Table 2. Nitrogenase activity of *Pseudomonas* 4B supported by 5 mM-protocatechuate or 28 mM-glucose as carbon source

Triplicate cultures each containing about 20 µg protein in 10 ml Burk's N-free medium were incubated for 24 h at 28 °C with 0.2 atm ¹⁵N₂ (98.63 atom% excess). The initial pO₂ was 0.005 atm (for protocatechuate) or 0.003 atm (for glucose) with He as the balancing gas. Controls were similarly set up but with ¹⁴N₂ instead of ¹⁵N₂. C₂H₂ reduction was assayed in parallel experiments (10%, v/v, C₂H₂; see Methods for details).

Determination	Experiment		Control	
	Protocatechuate	Glucose	Protocatechuate	Glucose
Cellular ¹⁵ N (atom% ± SD)	29.85 ± 3.16	18.31 ± 2.75	0.54 ± 0.09	0.56 ± 0.03
Excess ¹⁵ N (atom%)	29.31	17.75	0	0
Total cell N (µmol ± SD)	1.32 ± 0.15	1.74 ± 0.15		
¹⁵ N ₂ incorporated in 24 h (nmol)	196.3	156.8		
	231.7*	181.9*		
Specific ¹⁵ N ₂ fixation activity [nmol N ₂ fixed h ⁻¹ (mg protein) ⁻¹]	442.1	267.8		
	521.8*	310.6*		
C ₂ H ₄ produced in 5.2 h (nmol ± SD)	142.7 ± 3.3	202.3 ± 7.4		
Specific C ₂ H ₂ reduction activity [nmol C ₂ H ₄ h ⁻¹ (mg protein) ⁻¹]	1483.4	1594.4		

* Values corrected for N lost to the medium.

Increase in cellular ¹⁵N enrichment was also observed in cultures incubated up to 72 h with a high initial pO₂ of 0.05 atm. However, these diazotrophic activities were two orders of magnitude lower than those attained with the optimal initial pO₂ (data not shown) which further demonstrated that microaerobic conditions were required for optimal N₂ fixation by 4B.

To determine the relationship between C₂H₂ reduction and N₂ fixation, parallel experiments were set up for the C₂H₂ reduction assay (Table 2). Ethylene was produced without any lag at a constant rate up to 5.2 h when protocatechuate or glucose was present. The molar ratio of ethylene formed to N₂ fixed (corrected for N lost to the medium) ranged from 2.8 to 5.1 for the respective carbon substrates, assuming that the rate of ¹⁵N₂ fixation was constant during the 24 h incubation period. The apparent discrepancy in the relative protocatechuate- and glucose-supported nitrogenase activities between ¹⁵N₂ fixation and C₂H₂ reduction is not clearly understood (see Discussion).

Genetic characterization of 4B

The structural genes for nitrogenase are highly conserved among N₂-fixing organisms (Ruvkun & Ausubel, 1980). To determine whether the genomes of 4B, H8 and DC contained DNA sequences homologous to the known structural genes of *Klebsiella pneumoniae*, the plasmid pSA30 containing the *nifHDK* genes of *K. pneumoniae* M5A1 cloned in pACYC184 was used as a hybridization probe (Cannon *et al.*, 1979). An autoradiogram of *Eco*RI digests of total DNA from *K. pneumoniae* hybridized with the probe showed a 6.9 kb band (Fig. 2). No bands were visible in the digested DNA of *P. putida* (ATCC 33015), a control organism which does not reduce C₂H₂. However, DNA from 4B, H8 and DC all showed hybridization bands with pSA30 (Fig. 2). When pACYC184 was used as a probe no homology was observed, demonstrating that the hybridization was attributable to the *K. pneumoniae* DNA of the probe. Hence 4B, H8 and DC all contain DNA homologous to the *nifHDK* structural genes of *K. pneumoniae* nitrogenase.

No plasmid DNA was detected in strain 4B either by the gentle lysis technique of Eckhardt (1978) or by the alkaline-SDS lysis technique devised for the visualization of *Pseudomonas* plasmids (Wheatcroft & Williams, 1981). As it proved possible to detect plasmid RP4 in a transconjugant of 4B, which was still capable of C₂H₂ reduction, it is likely that the nitrogenase genes of 4B are not carried on a plasmid.

Fig. 2. Autoradiogram showing hybridization of pSA30 to *Eco*RI digests of total DNA of the following strains (the sizes of the hybridization bands are shown in parentheses): A, *P. putida*; B, *K. pneumoniae* M5A1 (6.9 kb); C, *Pseudomonas* 4B (2.0, 1.7 kb); D, *Pseudomonas* DC (2.7, 1.5 kb); E, *Pseudomonas* H8 (5.4 kb).

DISCUSSION

The biochemical and physiological characterization of 4B presented above confirms its initial phenotypic assignment to the genus *Pseudomonas* (Chan, 1986). It could also be designated as a new diazotrophic species since it differs metabolically from the other two diazotrophic *Pseudomonas* species H8 and DC (Table 1), reported previously (Barraquio *et al.*, 1983; Haahtela *et al.*, 1983). Although *P. delafieldii* showed a similar, but more restricted, carbon utilization pattern than that of 4B, it was shown not to reduce C_2H_2 (Table 1). Recently, *P. saccharophila* Doudoroff (ATCC 15946) was reported to be the first authenticated *Pseudomonas* species capable of N_2 fixation (Barraquio *et al.*, 1986). It is also known to be able to grow chemolithotrophically with H_2 . Thus, it is similar to H8 and DC (but different from 4B and *P. delafieldii*) in possessing both H_2 -utilizing and N_2 -fixing abilities. However, both *P. delafieldii* and *P. saccharophila* are presently classified in rRNA group III of the genus *Pseudomonas* and each respectively belongs to the non- H_2 -utilizing and H_2 -utilizing subgroups (Palleroni, 1984). Since most N_2 -fixing bacteria may possess hydrogenase (Robson & Postgate, 1980), a conventional reversible hydrogenase may be present in 4B. However, nucleic acid hybridization experiments are required to determine the relationship of 4B with these pseudomonads.

The genus *Pseudomonas* has not generally been considered to contain natural diazotrophic species (Palleroni, 1984). This is probably due to a threefold problem in recognizing

diazotrophic pseudomonads: (i) mistaken or uncertain identification of the diazotroph (De Ley & Park, 1966; De Smedt *et al.*, 1980); (ii) the lack of rigorous tests for diazotrophy in pseudomonads (Hill & Postgate, 1969); and (iii) the accepted multigeneric nature of the genus *Pseudomonas* (De Ley & De Vos, 1984; De Vos & De Ley, 1983). Nevertheless, there is no known reason why diazotrophy should not exist in the genus *Pseudomonas* (Postgate, 1982). In the case of 4B, the first two problems have been adequately dealt with by our extensive physiological and genetic characterization. The third problem is, however, inherent in the current taxonomy of the pseudomonads whose phylogeny may have to be more precisely defined (De Vos & De Ley, 1983). Hence, by the present definition of *Pseudomonas*, 4B should be recognized as a genuine diazotrophic *Pseudomonas* species.

The nitrogenase activity (assayed by C₂H₂ reduction) of *Pseudomonas* 4B with protocatechuate as the sole carbon source was previously shown to be highest among the other monomeric phenols, and was about 50% of that supported by glucose (Chan, 1986). In the present study, where 4B was found to be a microaerophilic diazotroph (Fig. 1), this activity was similar with either carbon substrate when the optimal initial pO₂ was used (Table 2). However, the ¹⁵N₂ fixation activity assayed in parallel showed that the protocatechuate-supported activity was 68% higher than the glucose-supported activity. This apparent difference may have resulted from lags in ¹⁵N₂ reduction or unknown factors differentially affecting N₂ and C₂H₂ reduction. Nevertheless, N₂ fixation was proven to have occurred in cultures of 4B provided with an aromatic compound (protocatechuate) as the sole carbon and energy source. This supports the suggestion that simple aromatic substrates can serve as important energy sources for significant N₂ fixation in plant and soil environments devoid of sugars and organic acids (Chan, 1986).

Pseudomonas species 4B, H8 and DC were all found to contain DNA homologous to the *nifHDK* genes of *K. pneumoniae*, as determined by probing with plasmid pSA30. No plasmids were detected in 4B by using either of the two techniques known to be capable of detecting large plasmids in *Pseudomonas* and other bacteria. We infer that the nitrogenase structural genes of this strain are located on the chromosome. This has generally been found to be the case amongst free-living diazotrophic bacteria (Robson *et al.*, 1983), though exceptions are known (Derylo *et al.*, 1981; Singh *et al.*, 1983).

In conclusion, a description of *Pseudomonas* species strain 4B is summarized as follows.

The organism is Gram-negative, non-pigmented, nonspore-forming, rod-shaped (about 0.4 × 1.5 µm) and motile with polar flagellation. Cells are frequently present in pairs. The optimum growth temperature and pH are 34 °C and 6.2, respectively. Exopolysaccharide is produced in glucose medium. Cells accumulate poly-β-hydroxybutyrate.

Metabolism is oxidative. Oxidase and catalase are produced. Many organic compounds are utilized for growth. Gelatinase and arginine dihydrolase activities are absent. N₂ is fixed without supplements under microaerobic conditions and fixation can be supported by certain aromatic compounds. The organism does not grow autotrophically with H₂. Denitrification does not occur but nitrate is reduced to nitrite. The DNA mol% G + C is 60.2 ± 1.2 SD (*n* = 4; UV absorption ratio).

The bacterium was isolated from the litter layer of a forest soil (Chan, 1986) and has been deposited with the American Type Culture Collection as ATCC 43038.

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