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Short communication

A *Photobacterium*-like bacterium able to fix nitrogen

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Abstract

A *Photobacterium*-like bacterium isolated from the roots of eelgrass (*Zostera marina*) was shown to fix nitrogen under anaerobic conditions. Nitrogen fixation by *Photobacterium* spp. has not been reported previous to this.

Abbreviation: PHB – Poly-β-hydroxybutyrate

Members of the halophilic, facultatively anaerobic bacteria are currently placed under either the genus *Vibrio* or *Photobacterium* of the family Vibrionaceae (Baumann & Schubert 1984). Although they are defined as being unable to fix molecular nitrogen (Baumann & Baumann 1984; Baumann et al. 1984), *Vibrio* spp. (Guerinot et al. 1982; West et al. 1985) and *Vibrio*-like organisms (Shieh et al. 1987; Shieh et al. 1988a) possessing nitrogenase (acetylene reduction) activity have been isolated from a diverse range of marine sources in the past several years. We report here that the genus *Photobacterium* may also include nitrogen-fixing members.

Roots of eelgrass (*Zostera marina*) were collected from an eelgrass bed in Aburatsubo Inlet, Kanagawa, Japan. Strain BDG1 was isolated from the homogenate solution of eelgrass roots using an anaerobic enrichment culture method (Shieh et al. 1988b). The methods and the compositions of the media used in this study have been described previously (Shieh et al. 1988b) with modifications as cited below. The glucose nitrogen-free liquid medium (glucose NFL medium) contained 20 g NaCl per liter instead of the original 28 g per liter. Poly-β-hydroxybutyrate (PHB) accumulation was tested in a medium containing the following two components:

- yeast extract (Difco), 0.5 g; Tris, 50 mmoles; dissolved in 900 ml of seawater and adjusted to pH 8.0;
- glucose, 2.0 g dissolved in 100 ml of distilled water.

The two components were mixed after autoclaving separately. Unless otherwise stated, incubation was carried out at 25° C without shaking.

Growth of strain BDG1 in the glucose NFL medium under various gas phase conditions is shown in Fig. 1. No significant growth occurred under air. However, under N_2, the optical density increased from 0.03 to 1.20 after incubation for 60 hours. The results indicate that strain BDG1 utilized N_2 as the sole nitrogen source under anaerobic conditions and also that no organic growth factors were required by this strain. The specific growth rate under N_2 was 0.15 h^{-1} (generation time, 4.6 h) during

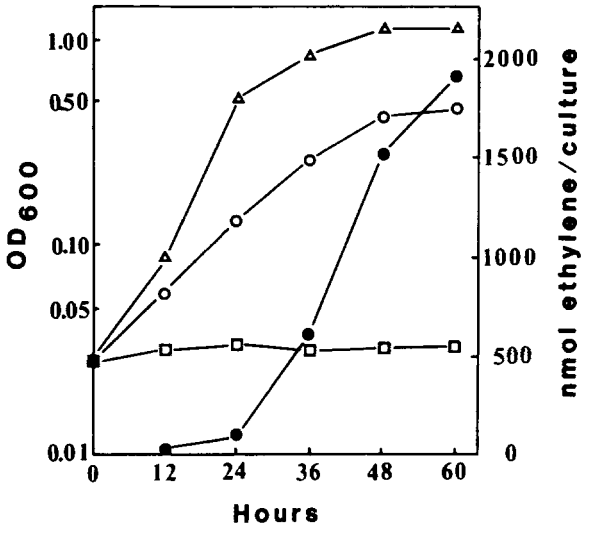

Fig. 1. Growth of strain BDG1 in glucose NFL medium under air (□) or under N_2 in the presence (○) or absence (△) of 0.05 atm C_2H_2. Nitrogenase activity of strain BDG1 under N_2 in the presence of 0.05 atm C_2H_2 (●).

the exponential phase of growth (12 to 24 h). The growth rate of a similar culture exposed to C_2H_2 (0.05 atm) was slow compared with that under N_2 (specific growth rate, 0.068 h^{-1} versus 0.15 h^{-1}). This can be explained by competitive inhibition of nitrogen fixation by acetylene which might occur in nitrogenase-containing microorganisms (Brouzes & Knowles 1971; Dilworth 1966). Nitrogenase (acetylene reduction) activity under N_2/C_2H_2 was also measured every 12 hours (Fig. 1). The most rapid production of C_2H_4 occurred during the late

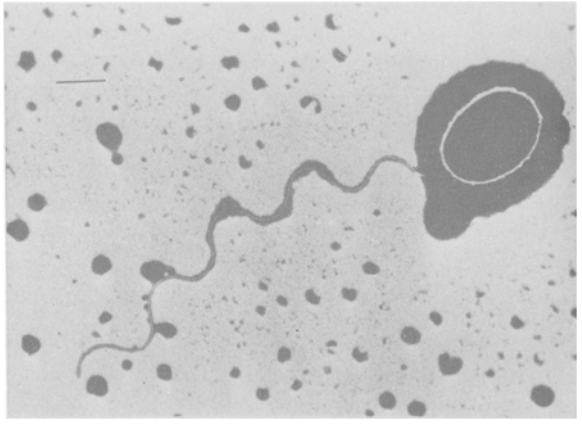

Fig. 2. Negatively stained cell of strain BDG1, showing a single, polar flagellum. Bar = 1 μm.

Table 1. Characteristics of strain BDG1.

Characteristic	Reaction
Gram stain	−*
Glucose fermentation	+
Gas from glucose	−
PHB accumulation	+
Oxidase	+
Catalase	+
Nitrogenase	+
Arginine dihydrolase	+
Lysine decarboxylase	−
Ornithine decarboxylase	−
Amylase	−
Chitinase	w
DNAase	+
Lipase	+
Gelatinase	+
Sensitivity to:	
O/129 (150 μg)	−
O/129 (750 μg)	+
Growth at:	
4° C	+
20° C	+
40° C	−
Growth in:	
0% NaCl	−
3% NaCl	+
6% NaCl	+
8% NaCl	−
Luminescence	−
Acid from:	
d-Arabinose	−
Cellobiose	+
Galactose	+
Inositol	−
Lactose	−
Mannitol	+
Mannose	+
Melibiose	+
l-Rhamnose	−
Sucrose	+
Xylose	−
Utilization as sole carbon source	
d-Arabinose	−
Cellobiose	+
Citrate	+
Dulcitol	−
Galactose	+
Glucose	+
Glycerol	+
β-Hydroxybutyrate	−
Mannitol	+
Mannose	+
Melibiose	+
l-Rhamnose	−
Acetate	+

* + positive
− negative
w weakly positive

exponential phase of growth (36 to 48 h; 45–67 nmol C_2H_4/mg dry wt cells/h). Only a trace of C_2H_4 was produced within the first 12 hours, which might be partially attributed to the low culture turbidity during this period. The exposure of the preculture to air during the transfer process might have inactivated the oxygen-labile nitrogenase system of the cells, and this might have also delayed the appearance of nitrogenase activity. Strain BDG1 failed to grow in glucose NFL medium under N_2 at 35° C or when Na^+ was excluded from the medium (data not shown). Yeast extract (Difco, 2 g/l), NH_4Cl (2 mM) and KNO_3 (2 mM) added to the glucose NFL medium could serve as N sources for cells growing both aerobically and anaerobically under Ar (data not shown). The above results indicate that strain BDG1 is a halophilic, facultatively anaerobic, nitrogen-fixing bacterium.

Strain BDG1 produced round and non-pigmented colonies on PY agar plates. Cells grown in PY broth medium were straight rods which were motile by means of a single, polar flagellum (Fig. 2). The strain was Gram-negative. It fermented glucose with the production of acid but no gas and required NaCl for growth. The DNA base composition was 46.5 ± 0.3 mol % G + C. These characteristics clearly indicate that strain BDG1 belongs to either the genus *Photobacterium* or *Vibrio* of the family Vibrionaceae (Baumann & Schubert 1984). Additional characteristics of the strain are summarized in Table 1. Strain BDG1 might belong to the genus *Photobacterium* and not *Vibrio* because it accumulated PHB as the intracellular product and failed to utilize β-hydroxybutyrate as the sole carbon source (Baumann & Baumann 1984). The ability to utilize glucose, mannose and glycerol as sole carbon sources and the inability to produce amylase are all typical of *Photobacterium* spp. Morphologically, strain BDG1 being a plump, straight rod (Fig. 2) also suggests that it might belong to *Photobacterium*. Strain BDG1 is not luminescent, however, this does not exclude it from the genus because bioluminescence is no longer treated as a generic-key characteristic of *Photobacterium*. The strain was distinct from all the recognized species of *Photobacterium* by its ability to utilize mannitol and to fix molecular nitrogen (Baumann & Baumann 1984). Its guanine-plus-cytosine content (46.5 mol%) was also slightly higher than all the recognized *Photobacterium* spp. However, until more species of *Photobacterium* have been described, these differences are not sufficient to exclude the strain from the genus. In view of the numerous similar characteristics as shown above, we conclude that strain BDG1 is best considered as a *Photobacterium* sp.

Photobacterium spp. are widely distributed in many different marine habitats but most of the interest in the genus has been focused on the luminous organisms found as symbionts in specialized luminous organs of marine fishes (Reichelt et al. 1977; Ruby & Morin 1978). The present study suggests that some of them may also play a role in providing fixed nitrogen into coastal marine environments.

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