SORBISTIN, A NEW AMINOGLYCOSIDE ANTIBIOTIC COMPLEX OF BACTERIAL ORIGIN

II. ISOLATION AND TAXONOMY OF SORBISTIN-PRODUCING ORGANISM

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The sorbistin-producing organism, *Pseudomonas sorbicinii* nov. sp., has been isolated from a soil sample by psychrophilic pre-incubation technique. The organism resembles *P. fluorescens* in many respects but differs in some of the important physiological characteristics such as oxidase production, media specificity for the production of fluorescent pigment, and carbohydrate utilization pattern. The type strain, No. D946-B83, has been deposited under the numbers ATCC 31086 and FERM-P 3328.

A new aminoglycoside antibiotic complex named sorbistin has been isolated from the fermentation broth of a bacterial strain, No. D946-B83, isolated from a soil sample collected in the eastern Himalaya district in Bhutan. With an intention to isolate psychrotrophic microorganisms, the soil was pre-incubated at low temperature and then subjected to the soil isolation procedure in the usual manner. The strain D946-B83 thus isolated belongs to the genus *Pseudomonas* and has been designated *Pseudomonas sorbicinii* nov. sp. This paper describes the morphological, cultural and physiological characteristics of strain D946-B83. The procedures described by STANIER PALLERONI & DOUDOROFF[1], RHODES[2], and IIZUKA & KOMAGATA[3] were generally followed for the characterization.

Isolation

The well-pulverized soil sample was nutritionally enriched with addition of a small amount of nutriment (*e.g.* nutrient broth) and incubated at 5~7°C for 7~10 days. A supplement of appropriate antibiotics (*e.g.* oxacillin and nystatin) to the soil sample was useful to inhibit common fast-growing bacteria and fungi. The pre-incubated soil sample was then treated in the usual manner to isolate microorganisms on an agar plate, and the plate was incubated at 7~12°C for 10 days. Strain D946-B83 was one of the psychrotrophic soil bacteria obtained by the above isolation procedure.

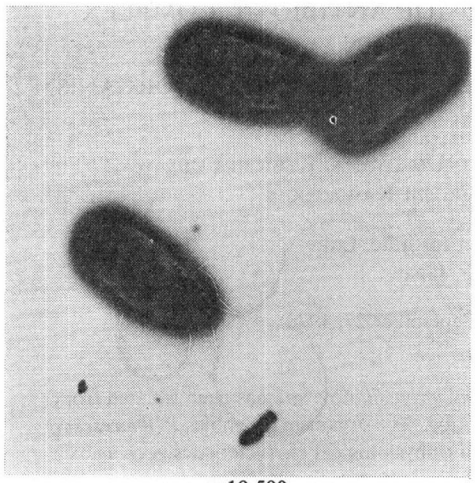
Morphology

Strain D946-B83 is characterized by its motile, non-sporulating and gram-negative cells. The cells are small, short rods in shape, and straight occasionally bent along the long axis. The cell measures 0.6~0.9 by 1.2~2.5 μm in size and produces uni-polar tuft flagella (Fig. 1). Poly-β-hydroxy butyrate is not contained as a cellular reserve. No sheath, stalk or slime is produced.

Growth Characteristics

Colonies on nutrient agar and yeast extract agar (at 28°C): Abundant growth. 0.5~1.5 mm diameter after 1 day. Diffused, circular and somewhat raised. Smooth and soft. Opaque, whitish cream,

Fig. 1. Electronmicrograph of *P. sorbicinii* strain D946-B83



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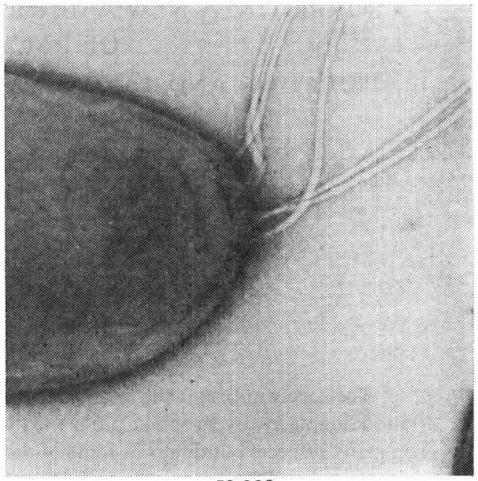

×50,000

later light buff-orange. Slightly viscous. No diffusible pigment.

Nutrient broth and yeast extract broth: Abundant growth. Turbid, later with sediment and occasionally pellicle.

Yeast extract agar stab: Growth only on surface. No growth in any anaerobic conditions.

Growth on chemically defined inorganic salts medium: Moderate growth when added with glucose or lactate as a sole carbon source.

Requirement for growth factor: None.

Growth temperature: Restricted growth at 4°C, moderate to abundant growth at 10~32°C, scant growth at 37°C, and no growth at 41°C.

Effect of media pH: No growth at pH 4.0, restricted growth at pH 4.5 and pH 10.5~11.0, and moderate to good growth at pH 5.5~9.5.

NaCl effect: No growth at 12% NaCl, restricted growth at 6~9%, and moderate growth at 5%.

Physiological Characteristics

Strain D946-B83 produces diffusible fluorescent pigment in some specific media but not in yeast extract agar or KING's B medium[7] where *Pseudomonas fluorescens* showed abundant fluorescence production (Table 1). It does not produce phenazine pigments on KING's A medium[7]. Strain D946-B83 lacks the ability for denitrification, egg-yolk hydrolysis and starch hydrolysis, but gives positive reactions in the arginine dihydrolase and gelatin hydrolase systems. The mode of ring fission in catechols is *ortho*-cleavage. The strain utilizes glucose, trehalose, 2-ketogluconate, valine, β-alanine and arginine, but does not utilize inositol, geraniol and poly-β-hydroxybutyrate. Levan is not formed from sucrose. The oxidase tests, which were performed by three different methods described by STANIER *et al*[1], IIZUKA & KOMAGATA[4] and STEEL[8], were all negative or marginal (very weak coloration). The physiological and biochemical characteristics of strain D946-B83 are summarized and shown in Table 2. The results of carbon source utilization tests are shown in Table 3. The carbon source utilization pattern of strain D946-B83 was compared with *P. fluorescens* and *P. aeruginosa*, and Table 4 presents differences in the utilization pattern among the three strains.

Taxonomy

Strain D946-B83 appears to belong to the genus *Pseudomonas* in view of the morphological,

cultural and physiological characteristics described above. Additional three genera are described under Family *Pseudomonadaceae* in BERGEY's Mannual of Determinative Bacteriology (8th Ed., 1974), among which *Zoogloea* and *Gluconobacter* are clearly differentiated from strain D946-B83. Genus *Xanthomonas* resembles strain D946-B83 in the lack of oxidase activity. However, strain D946-B83 differs from genus *Xanthomonas* in its multiflagella, lack of yellow carotinoid pigment, positive nitrate reduction and no growth factor requirement[5].

Table 1. Production of fluorescent pigment

Medium	Sorbistin producer D946-B83	*Ps. fluorescens* NIHJ B254
Yeast extract agar	−	+
KING's B medium	−~±	++
20% Skimmed milk solution	++	++
Glutamate broth	++	++

According to the classification of genus *Pseudomonas* in the BERGEY's Manual, strain D946-B83 should be placed in Section I Group 1-a on the basis of its negative intracellular accumulation and negative extracellular hydrolysis of poly-β-hydroxybutyrate, its fluorescent pigment production, and

Table 2. Physiological and biological characteristics of Strain D946-B83

Test	Response	Method and medium employed
Arginine dihydrolase	Positive	NH_3 liberated was determined by NESSLER's reagent.
Catalase	Positive	Hydrogen peroxide on the colony.
Oxidase	Negative or marginal	Tested by three methods[1,4,8].
Extracellular hydrolases:		
Gelatin hydrolase	Positive	Method of STANIER *et al*[1].
Starch hydrolase	Negative	Method of STANIER *et al*[1].
VOGES-PROSKAUER reaction	Negative	Peptone broth plus 1% glucose.
Indole production	Negative	Peptone broth (KOVACS' reagent).
H_2S production from cysteine and thiosulfate	Negative	Ammonium-inorganic salts plus cysteine thiosulfate. Lead acetate paper for detection of H_2S.
Gelatin liquefaction	Positive (rapidly liquefied)	Peptone broth plus 25% gelatin[5].
Reactions in skimmed milk	Greenish yellow fluorescent pigment. Peptonized, pH alkalized.	20% Skimmed milk solution sterilized at 0.5 kg/cm^2 for 5 minutes.
Utilization of nitrate-N	Positive	RHODES' inorganic salts-glycerol-nitrate medium[2].
Nitrite production from nitrate	Positive	RHODES' inorganic salts-glycerol-nitrate medium[2].
Nitrite production from nitrate	Negative	Peptone broth plus 0.1% KNO_3.
Utilization of ammonium-N	Positive	KOSER's citrate medium.
Ammonia production from peptone	Positive	Peptone broth (NESSLER's reagent).
Denitrification	Negative	Method of STANIER *et al*[1].
Gas from carbohydrate	Negative	Nutrient broth plus 1% glucose.
Utilization of urea	Positive	CHRISTENSEN's urea medium.
Utilization of citrate	Positive	SIMMON's citrate medium and KOSER's citrate medium.
Utilization of oxalate	Negative	Modified SIMMON's and KOSER's media: oxalate instead of citrate was used.
Egg-yolk reaction	Negative	Method of STANIER *et al*[1].
Ring fission mechanism of catechols	*Ortho*-cleavage	Modified method of STANIER *et al*[1].
Production of phenazine pigment	Negative	KING's A medium
Levan formation from sucrose	Negative	

Table 3. Utilization of carbon sources by strain D946-B83

	Utilization		Utilization
Glycerol	+	D-Sorbitol	−
L-Arabinose	+	Dulcitol	−
D-Xylose	+	Geraniol	−
L-Rhamnose	−	Starch	−
D-Fructose	+	Cellulose	−
D-Galactose	+	Saccharate	−
D-Glucose	+	2-Ketogluconate	+
D-Mannose	+	Adipate	−
D-Fucose	−	Methanol	−
Trehalose	+	Ethanol	−
Cellobiose	−	*n*-Propanol	−
Maltose	−	Propyleneglycol	−
Sucrose	−	Acetamide	−
Lactose	−	L-Arginine	+
Raffinose	−	L-Valine	+
Adonitol	−	δ-Aminovalerate	+
Inositol	−	β-Alanine	+
D-Mannitol	+	Poly-β-hydroxybutyrate	−

Table 4. Differences of carbon source utilization among three species of *Pseudomonas*

Substrate	*P. sorbicinii* D946-B83	*P. fluorescens* NIHJ B254	*P. aeruginosa* ATCC 19660
L-Arabinose	+	+	−
D-Xylose	+	+	−
D-Galactose	+	+	−
D-Mannose	+	+	−
Trehalose	+	+	−
Adonitol	−	+	+
Inositol	−	+	−
D-Sorbitol	−	+	−
Geraniol	−	−	+
Saccharate	−	−	+
Adipate	−	−	+
Methanol	−	−	+
Ethanol	−	−	+
n-Propanol	−	−	+
Propyleneglycol	−	−	+
Acetamide	−	−	+
δ-Aminovalerate	+	−	+

the presence of arginine dihydrolase. Five *Pseudomonas* species are described under Section I Group 1-a, among which *Pseudomonas fluorescens* is the closest to strain D946-B83 in such characteristics as formation of plural flagella, lack of phenazine pigment, no growth factor requirement, no growth at 41°C, positive gelatin hydrolysis and negative starch hydrolysis. However, strain D946-B83 is differentiated from *P. fluorescens* in its negative utilization of inositol and sorbitol, and its negative or very weak oxidase production. Furthermore strain D946-B83 is different from any one of the established biotypes I, II, III and IV of *P. fluorescens* described in the BERGEY's Manual in view of its lack of denitrifying ability and negative levan formation.

Strain D946-B83 resembles *P. syringae* (Section I, Group 1-b) in the negative oxidase production, no growth at 41°C and negative denitrification. However, they are differentiated from each other by the production of arginine dihydrolase and utilization of trehalose, 2-ketogluconate, inositol, valine and β-alanine.

Thus it was concluded that strain D946-B83 is a new species in Section I Group 1-a of Genus *Pseudomonas*, for which the name *Pseudomonas sorbicinii* sp. nov. is proposed. The epithet *sorbicinii* is derived from the name of the antibiotic produced by the organism. The type strain is No. D946-B83 and has been deposited in the American Type Culture Collection and the Fermentation Research Institute of Japan, where it has been assigned the designations ATCC 31086 and FERM-P No. 3328, respectively.

Discussion

Soil microorganisms are generally mesophilic and their optimal temperature range for growth is 25~50°C. Cold-loving microorganisms, such as psychrophiles or psychrotrophs defined by MORITA[6], should naturally be rich in soils collected from cold environments, and attempts were made in our laboratories to isolate such microorganisms for new antibiotic screening. The psychrophilic pre-

incubation technique described in this paper seems to have advantages in the selective isolation of cold-loving microorganisms: that is, to restrict the growth of predominant mesophilic organisms and multiply the population of psychrotrophs which would be a minor and inferior group of microorganisms in the original soil sample. The sorbistin-producing organism, *Pseudomonas sorbicinii* nov. sp. strain D946-B83, was first isolated from a soil sample of cold zone in the east Himalaya, but it is interesting to note that several strains identified as *P. sorbicinii* have subsequently been isolated by a similar isolation procedure from soil samples collected in the usual temperature environment.

Acknowledgement

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