

DEACETYLATED POLYSACCHARIDE S-60

CROSS-REFERENCE

This is a divisional application of Ser. No. 178,053, Aug. 14, 1980, now U.S. Pat. No. 4,326,052, which is a continuation-in-part of Ser. No. 47,505, June 8, 1979, abandoned, which is a continuation-in-part of Ser. No. 966,538, Dec. 4, 1978, abandoned.

Heteropolysaccharide S-60 is disclosed and claimed in U.S. Pat. No. 4,326,053.

BACKGROUND OF THE INVENTION

Compound S-60 may be prepared by fermentation of a suitable nutrient medium with a hitherto undescribed organism. Based on extensive taxonomic studies, the organism has been designated to be a new species, *Pseudomonas elodea*. An unrestricted permanent deposit of an organism of this species employed in making our heteropolysaccharide was made with the American Type Culture Collection on Nov. 21, 1978, under Accession No. ATCC 31461.

Various classification keys for the genus *Pseudomonas* and the culture descriptions of *Pseudomonas* species are found in the 7th Edition of Bergey's Manual (Breed et al., (1957)) and the 8th Edition of Bergey's Manual (Doudoroff et al., (1974)), as well as by other schools in various publications; Hugh and Gilardi, 1974, *Pseudomonas*, *Manual of Clinical Microbiology*, 2nd ed., Lennette et al., Eds., pp. 250-269. American Society for Microbiology, Washington D.C.; Weaver et al., 1972, *Identification of Unusual Pathogenic Gram-Negative Bacteria*, E. O. King, Center for Disease Control, Atlanta; Iizuka et al., 1963, *Attempt at Grouping the Genus Pseudomonas*, *J. Gen. Appl. Microbiology* 9:73-82; and Hendric et al., 1966, *Identification of Certain Pseudomonas Species*, *Identification Methods for Microbiologists*, Part A, Gibbs et al., Eds., pp. 1-7, Academic Press, New York.

These keys and descriptions were searched for a *Pseudomonas* species having morphological and cultural characteristics similar to those of ATCC 31461. The following considerations make the assignment of a new *Pseudomonas* species justified and necessary.

DESCRIPTION OF THE STRAIN

1. Characteristics of Cell Morphology,

Single cells, straight or often curved, generally 0.6-0.8 by 2.0-3.0 μm , often with tapered end. The older cultures become larger and longer (0.8-1.0 by $>3 \mu\text{m}$), misshaped cells and pleomorphism appear, especially on media with limited amount of carbohydrates. On the contrary, cells keep rather consistent rod shapes when grown on media with carbohydrates, but again, most cells become large and pleomorphism develops during prolonged incubation. Gram-negative, non-capsulated, poly- β -hydroxybutyrate and polyphosphate

granules are seen especially in cultures of nitrogen-deficient media. Motile by polar multitrichous flagellation; one to four flagella are inserted at the polar end and occasionally subpolar insertion may be seen.

2. Characteristics of Colonial Morphology

On nutrient agar plates, small (0.8-1.1 mm in diameter) and large (3.2-3.5 mm in diameter) colonies appear. They are yellow carotenoid pigmented, smooth, round, and convex to pulvinate. Large colonies often have a concentric wrinkle. The surface of these colonies has a hard but not viscid texture and entire colonies are removed if pushed by a loop. On YM agar plates, only one type of relatively large (~6-7 mm in diameter) yellow, round, smooth, slimy and convex colonies appear. Slimy elastic membranes form on the surface of these colonies and whole surface membranes (of colonies) can be removed. The secondary growth may occur around the edge of the original colonies. The color of these colonies is darker yellow towards the center than the edge and concentric color formation appeared. In addition to the intracellular yellow carotenoid pigment(s), diffusible brown pigment developed as a result of autooxidation after prolonged incubation. This phenomenon is more easily recognized on Nutrient agar. No fluorescent pigment was produced.

3. Physiological and Biochemical Characteristics

The growth range of the strain S-60 is about 20° C. to 41° C. No growth occurs at 4° C. 3.0% NaCl is sufficient to inhibit the growth and the strain is capable of growth at pHs between 5 and 11.

Acid, but no gas is produced from almost all carbohydrates but not from polyalcohols. Urease may be produced. MR, VP, and indole tests were all negative. Arginine dihydrolase, lysine and ornithine decarboxylase are not produced. Acid and reduction occurs in litmus milk. Lipolytic egg yolk reaction is negative. Gelatin is weakly hydrolyzed but not casein, starch, alginate, pectin, cellulose, chitin and DNA.

4. Susceptibility to Antibiotics

The strain is very susceptible to Kanamycin, Neomycin, Chlortetracycline, Erythromycin, but not Streptomycin and Penicillin.

5. Nutritional Characteristics

Organic growth factors are not required and ammonium salts serve as sole nitrogen source. At least 35 organic compounds were utilized, those being most carbohydrates other than D-ribose, starch, 2-ketoglucuronate, and mucate. In addition, acetate, caproate, caprylate, pelargonate, succinate, azelate, L-malate, DL- β -hydroxybutyrate, pyruvate, ethanol, n-propanol, p-hydroxybenzoate, phenylacetate, L- α -alanine, L-threonine, L-leucine, DL-isoleucine, L-aspartate, L-glutamate, and L-tyrosine were utilized.

6. The G+C Content of the DNA

Evaluation of the DNA resulted in the mole % to be ~68 (by Tm).

TABLE 1

Biochemical and Other Miscellaneous Tests Employed for the Strain S-60

Oxidase: Kovac's Pathotech	+ (weak)	Hydrolysis of:	
	+ (weak)	Gelatin	+ (weak)
		Casein	-
Catalase	+	Starch	-
OT medium: Oxidative	+	Tween 80	+
Fermentative	-	Pactin	-
Gas from glucose	-	Alginate	-
H ₂ S production: TSI	-	Cellulose	-
from cystine	+	Chitin	-