

FERMENTATION PROCESS FOR ITS PREPARATION OF POLYSACCHARIDE S-60

CROSS-REFERENCE

This is a division of U.S. Ser. No. 178,054 filed Aug. 14, 1980, now U.S. Pat. No. 4,326,053, which is a continuation-in-part of U.S. Ser. No. 47,598 filed June 8, 1979, now abandoned, which is a continuation-in-part of U.S. Ser. No. 966,531 filed Dec. 4, 1978, now abandoned.

The deacylated and clarified deacylated S-60 described herein are claimed in U.S. Pat. No. 4,326,052.

BACKGROUND OF THE INVENTION

It is known that heteropolysaccharides can be produced by certain microorganisms. Some of such heteropolysaccharides function as hydrophilic colloids and because of their viscosity and rheology properties have been used as thickening agents for aqueous systems.

As with other fields of technology, research has continued with the objective of discovering new heteropolysaccharides having useful properties as thickening, suspending and/or stabilizing agents. It is an object of this invention to provide a new heteropolysaccharide having these desirable properties. It is another object to provide a method for making this new compound. A still further object is provision of formulations containing our new heteropolysaccharide as a thickening or suspending or stabilizing agent. Other objects of the invention will become evident from the ensuing description of this invention.

SUMMARY OF THE INVENTION

The present invention pertains to a novel heteropolysaccharide which is produced by the action of a bacterium on a selected carbon source. Further, the invention pertains to a novel process for producing a heteropolysaccharide by bacterial fermentation of a selected carbon source and fermentation medium ingredients under controlled conditions. The heteropolysaccharide of this invention is a high molecular weight polysaccharide containing primarily carbohydrate residues and a minor amount of protein. It is sometimes referred to as a "gum" but it is believed that the heteropolysaccharide terminology is more accurate and precise. In the following description of our invention, it will sometimes be referred to as Heteropolysaccharide 60, or S-60.

This novel compound 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 of 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 pigments(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.