

TABLE 1-continued

Biochemical and Other Miscellaneous Tests Employed for the Strain S-60			
Ammonium from peptone	+	DNA	-
n-Galactosidase (ONPC)	+	Esculin	+
Arginine dihydrolase	-		
Lysine decarboxylase	-	Growth on various media:	
Ornithine decarboxylase	-	EMB agar	-
Tryptophan jeaminase	-	MacConkey agar	-
Phenylalanine deaminase	-	SS agar	-
Urease	+/-±	Mannitol salt agar	-
Indole	-	TCBS agar	-
MP test	-	Tinsdale tellurate	
VP test	-	blood agar	+
Nitrate reduction	-	Pseudoseal agar	-
Nitrate reduction	-		
Denitrification	-	Pigment production:	
N ₂ -fixation:		King A medium	-
Growth in Burk's medium	+	King B medium	-
Nitrogenase activity	-		
Malonate (oxidation)	-	Dye reaction:	
Phosphatase	+	Congo red	-
Haemolysis (sheep blood)	-	Nite blue	-
Litmus milk: acid, reduction only	-		
3-ketolactose production	-		
Survival at 60° C. for 30 min.	-		
TSI:			
Slant	color no change		
Butt	color no change		
Gas	-		
Egg Yolk Reaction	-		

FERMENTATION CONDITIONS

Heteropolysaccharide S-60 is produced during the aerobic fermentation of suitable aqueous nutrient media under controlled conditions via the inoculation with the organism of the *Pseudomonas elodea* species. The media are usual media, containing source of carbon, nitrogen and inorganic salts.

In general, carbohydrates (for example, glucose, fructose, maltose, sucrose, xylose, mannitol and the like) can be used either alone or in combination as sources of assimilable carbon in the nutrient medium. The exact quantity of the carbohydrate source of sources utilized in the medium depend in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 2% and 4% by weight of the medium. These carbon sources can be used individually, or several such carbon sources may be combined in the medium. In general, many proteinaceous materials may be used as nitrogen sources in the fermentation process. Suitable nitrogen sources include, for example, yeast hydrolysates, primary yeast, soybean meal, cottonseed flour, hydrolysates of casein, corn steep liquor, distiller's solubles or tomato paste and the like. The sources of nitrogen, either alone or in combination, are used in amounts ranging from about 0.05% to 0.2% by weight of the aqueous medium.

Among the nutrient inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, potassium, ammonium, calcium phosphate, sulfate, chloride, carbonate, and like ions. Also included are trace metals such as cobalt, manganese, iron and magnesium.

It should be noted that the media described in the examples are merely illustrative of the wide variety of media which may be employed, and are not intended to be limitative.

The fermentation is carried out at temperatures ranging from about 25° C. to 35° C.; however, for optimum results it is preferable to conduct the fermentation at temperatures of from about 28° C. to 32° C. The pH of

the nutrient media for growing the *Pseudomonas* culture and producing the polysaccharide S-60 can vary from about 6 to 8.

Although the novel polysaccharide S-60 is produced by both surface and submerged culture, it is preferred to carry out the fermentation in the submerged state.

A small scale fermentation is conveniently carried out by inoculating a suitable nutrient medium with the culture and, after transfer to a production medium, permitting the fermentation to proceed at a constant temperature of about 30° C. on a shaker for several days.

The fermentation is initiated in a sterilized flask of medium via one or more stages of seed development. The nutrient medium for the seed stage may be any suitable combination of carbon and nitrogen sources. The seed flask is shaken in a constant temperature chamber at about 30° C. for 1-2 days, or until growth is satisfactory, and some of the resulting growth is used to inoculate either a second stage seed or the production medium. Intermediate stage seed flasks, when used, are developed in essentially the same manner; that is, part of the contents of the flask from the last seed stage are used to inoculate the production medium. The inoculated flasks are shaken at a constant temperature for several days, and at the end of the incubation period the contents of the flasks are recovered by precipitation with a suitable alcohol such as isopropanol.

For large scale work, it is preferable to conduct the fermentation in suitable tanks provided with an agitator and a means of aerating the fermentation medium. According to this method, the nutrient medium is made up in the tank and sterilized by heating at temperatures of up to about 121° C. Upon cooling, the sterilized medium is inoculated with a previously grown seed of the producing culture, and the fermentation is permitted to proceed for a period of time as, for example, from 2 to 4 days while agitating and/or aerating the nutrient medium and maintaining the temperature at about 30° C.