

NOTES

NEW SALMONELLA TYPE: *SALMONELLA LANSING*

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A new serotype, *Salmonella lansing*, was isolated from sewage. The culture possessed biochemical characteristics typical of the Salmonella group. Acid production was observed in dulcitol, sorbitol, arabinose, xylose, rhamnose, maltose, mannitol, glucose (with gas), and, trehalose. Adonitol, salicin, inositol, lactose, and sucrose were not fermented. Indole was not produced and urea was not hydrolyzed. The culture was motile, produced hydrogen sulfide, reduced nitrate to nitrite, and utilized citrate. The methyl red test was positive and the Voges-Proskauer reaction was negative. The culture did not grow in KCN medium and failed to utilize malonate. Gelatin was not liquefied.

On serological examination *S. lansing* was strongly agglutinated by *Salmonella inverness* O (38) antiserum, and reciprocal adsorption tests indicated the somatic antigen to be the same.

Examination of the H antigen revealed that the organism was diphasic. Phase 1 was agglutinated to the titer of *Salmonella typhimurium* phase 1 antiserum (i). Reciprocal adsorption tests showed complete cross adsorption between *S. typhimurium* i and the new type phase 1. Phase 2 was agglutinated by antisera for all the nonspecific phases. When tested with antisera for serofactors 2, 5, 6, and 7, it was agglutinated only by antiserum for factor 5. By adsorption tests it was found that phase 2 (1, 5) of *S. lansing* was identical with phase 2 (1, 5) of *Salmonella thompson*. *S. lansing* has the antigenic formula 38:i-1, 5.

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OCCURRENCE OF POLY- β -HYDROXYBUTYRATE IN *PSEUDOMONAS PSEUDOMALLEI*¹

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The occurrence of poly- β -hydroxybutyric acid in a number of *Bacillus* species was shown by Lemoigne (Ann. inst. Pasteur, **41**, 148, 1927; Helv. Chem. Acta, **29**, 1303, 1946) and the polyester was also found among members of the genera *Azotobacter* (Lemoigne and Girard, Compt. rend., **217**, 557, 1943), *Chromobacterium*, *Rhizo-*

bium, and *Pseudomonas* (Forsyth *et al.*, Nature, **182**, 800, 1958), and, in all probability, *Actinobacillus (Malleomyces) mallei* (Umezū, Rept. Inst. Sci. Research, Manchoukuo, **4**, 273, 1940). McCrae and Wilkinson (J. Gen. Microbiol., **19**, 210, 1958) and others have ascribed a carbon storage function to this virtually insoluble polymer which, like starch and glycogen, is of high molecular weight and therefore exerts little or no osmotic pressure.

During studies on the pathogen *Pseudomonas pseudomallei (Malleomyces pseudomallei)*, chloroform extracts of the organism were found to

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contain very high concentrations of a compound which was poly- β -hydroxybutyric acid.

P. pseudomallei strain 118-5 (rough, yellow), grown at 37 C with aeration in 4 per cent glycerol-beef extract broth, was employed in most of the studies. Twenty-five g (dry weight) of 96-hr washed cells were autoclaved, rewashed by centrifugation, disrupted by sonic energy (10 kc/sec, 250 watts, 45 min, 10 C), and lyophilized. The dried product was extracted to exhaustion with chloroform in a Soxhlet apparatus. After concentration of the chloroform extract to a syrup, a precipitate equal to 48.4 per cent of the dry weight of the cells was obtained by the addition of 6 volumes of acetone. This precipitate was purified by treatment with activated charcoal and by several cycles of resolution in chloroform and precipitation by ether.

After this treatment the material was waxlike in appearance, white, and had the following properties. Its melting point was 171.5 C, but polymer preparations from different gram-negative species melt over a range of 164 to 178 C (Forsyth *et al.*, Nature, **182**, 800, 1958) presumably depending on chain length (Williamson and Wilkinson, J. Gen. Microbiol., **19**, 198, 1958). Its per cent carbon, hydrogen, nitrogen, and ash contents were: 55.6, 6.92, 0.0, and 0.0 (calculated for $[C_4H_6O_2]_n = 55.8, 6.96$ for per cent carbon and hydrogen, respectively). The solubility in various organic and inorganic solvents was as described for poly- β -hydroxybutyric acid by McCrae and Wilkinson (J. Gen. Microbiol., **19**, 210, 1958) and its infrared absorption spectrum was virtually identical with that of poly- β -hydroxybutyric acid from *Bacillus megaterium* (Haynes *et al.*, Applied Microbiol., **6**, 298, 1958).

As observed by Williamson and Wilkinson (J. Gen. Microbiol., **19**, 198, 1958) and others,

poly- β -hydroxybutyric acid can be converted to *trans*-crotonic acid by destructive distillation. The material isolated from *P. pseudomallei* was so treated and a crystalline product which formed was purified. It melted at 66 to 69 C and its anilide and amide derivatives melted, respectively, at 118 to 124 C and 155 to 156 C. These values are in close agreement with the melting points of *trans*-crotonic acid and its aforementioned derivatives. The neutralization equivalent of the crystalline compound was 86.6 (86.1 calculated for crotonic acid).

Two other strains of this pathogen (128, rough nonpigmented; 114-8, smooth nonpigmented) were also shown to contain poly- β -hydroxybutyric acid employing the criteria of solubility, odor of crotonic acid on heating, and melting point.

As shown by Forsyth *et al.* (Nature, **182**, 800, 1958) poly- β -hydroxybutyric acid is absent from most of their authentic species of *Pseudomonas*, being present only in *P. solanacearum*, and *P. antimycetica*. The present work demonstrates that poly- β -hydroxybutyric acid is present in yet another species recently assigned to the genus *Pseudomonas*. This species, *P. pseudomallei*, was incorrectly referred to by Morris and Roberts (Nature, **183**, 1538, 1959) as having been investigated by Umezū (Rept. Inst. Sci. Research, Manchoukuo, **4**, 273, 1940), who actually worked with *Actinobacillus (Malleomyces) mallei*.

Morris and Roberts (Nature, **183**, 1538, 1959) detected poly- β -hydroxybutyric acid in 18 water and soil strains considered by them to be in the genus *Pseudomonas* on biochemical and morphological bases as well as on the type of pigment produced. Since poly- β -hydroxybutyric acid is present in a number of diverse genera, it would seem that the demonstration of this compound in a bacteria strain is probably of taxonomic value only within the genus concerned.

COMPARATIVE EFFECTS OF VARIOUS ANTIMETABOLITES ON GROWTH AND SYNTHESIS OF CITROVORUM FACTOR BY *STREPTOCOCCUS FAECALIS*

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A number of compounds have been synthesized in this laboratory as possible inhibitors of the utilization of folic acid and its end products.

These have been tested in a model bacterial system against *Lactobacillus casei* (Hitchings *et al.*, J. Biol. Chem., **183**, 1, 1950). The probable