BACTERIOLOGY

A Non-Gummy Chromogenic Strain of *Azotobacter vinelandii*

BECAUSE of the widespread use of *Azotobacter vinelandii*, Wisconsin strain *O*, in biochemical studies, recent observations concerning colonial types obtained from transfers of this strain are worthy of more general knowledge. As many investigators have noted (private communications), cultures of this strain at times become more 'gummy' than usual, and their further use for physiological or biochemical studies is difficult. Although we have attempted in the past to isolate a non-gummy strain by selection of colonies, such efforts have been only temporarily successful.

A. vinelandii strain *O* was streaked on modified Burk's nitrogen-free agar plates[1]. Differences in colonial morphology were readily evident within 18 hr. of incubation at 30° C., when colonies were examined with the low power of a compound microscope, or within 48 hr., when colonies were examined with the unaided eye. Colonies were obtained which differed in size, gumminess or pigment production in proportions that depended on the origin of the culture. The stability of these colonial characteristics was checked by streaking the cells of a well-isolated colony on a fresh agar plate; two colony types were chosen for further study.

A gummy colony type that did not elaborate a pigment was easily recognized during microscopic examination of colonies, since at a magnification of 100 individual cells could be seen to be well separated by a clear slime. A non-gummy colony type was dense, yellow, and free of slime. Since a colony composed of both bacterial types was easily recognized, the selection of a pure culture of each strain was made only from colonies that were homogeneous by microscopic examination. After a limited number of streakings it was evident that a pure culture of each strain had been obtained. The non-gummy variety, henceforth to be designated strain *OP*, produced a yellowish-green fluorescent pigment that is characteristic of other strains of *A. vinelandii*. Each isolate grew readily in Burk's nitrogen-free liquid medium in shake flasks and fixed nitrogen as shown by total nitrogen analyses by the Kjeldahl method. Shake cultures of strain *OP* did not become gummy, whereas those of the other strain did. Even after numerous transfers in liquid or solid media strain *OP* remained non-gummy, and during frequent examination of isolated colonies no gummy colonies were observed. The two strains had cells with a similar size and form with peritrichous flagella. Both strains would be distinguished from members of the *Azotobacter agile* group on the basis of cell size and mannitol utilization[2]. *A. vinelandii* strain *OP*, which resembles very closely the first culture of *A. vinelandii* to be isolated[3], will be deposited with the American Type Culture Collection.

It is appropriate to mention again the frequent observations (private communications) that cultures of *Azotobacter* spp. may carry contaminants which are not detected unless special care is taken to search for them. Winogradsky[4] observed that cultures of *Azotobacter* spp. were impure even with primary isolation from natural material because of the limitations of the standard method of isolation by plating. In nitrogen-free media contaminants unable to fix nitrogen remain latent until nitrogenous products are released by the *Azotobacter*. Microscopic examination of colonies on solid nitrogen-free media may reveal contaminants as satellite colonies. The use of sugar-free peptone media recommended by Burk and Burris[5] is convenient to detect contaminants since these generally grow well in such media while the *Azotobacter* do not. Accordingly, the isolation of a pure culture of *Azotobacter* is best carried out when colonies are selected by microscopic examination.

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N,O-Diacetylneuraminic Acid and *N*-Acetylneuraminic Acid in *Escherichia coli*

DURING the course of an investigation of the biochemical and biological properties of endotoxins extracted from various Gram-negative bacteria, chiefly several *Escherichia coli* strains, we found and reported briefly[1] on the presence in some of these endotoxins of a material having the colour reactions of a sialic acid. The bacterial lipoproteins and lipopolysaccharides which yield this material were prepared by the phenol-water extraction method of Westphal[2], separated from accompanying nucleic acid and exhaustively dialysed. The sialic acid is released from this large molecule only upon mild acid hydrolysis, and we, therefore, proposed that it forms an integral part of the cell wall of these bacteria. Members of the sialic acid group had been found previously mainly in mammalian tissue. Barry and Goebel[3] had reported the elaboration of a sialic acid-like material, colominic acid, by a specific strain of *E. coli*. Barry[4] has since reported this to be a simple polymer of *N*-acetylneuraminic acid.

We now wish to report the isolation and identification of both *N*-acetylneuraminic acid and *N*,O-diacetylneuraminic acid from several strains of *E. coli*.

Washed living cells of *E. coli* $O_{111ab}B_4$:*HNM*[5] contain a minimum of 1 per cent neuraminic acid on a dry-weight basis, which is released optimally by hydrolysis in 0·1*N* sulphuric acid for 30 min. at 80° C. Such hydrolysates, neutralysed with barium hydroxide, were freed of cations by passage over a column of 'Dowex 50 *X*-8' resin in the *H*+ cycle. The neuraminic acids were adsorbed from this effluent by passage over a 'Dowex 2 *X*-8' acetate resin. After washing with water, the column was gradiently eluted with 2 *M* sodium acetate-acetic acid buffer at *p*H 4·8 and distilled water in equal volumes so as to yield a first-order relationship of volume to buffer concentration in the eluate[6]. The entire method is a modifi-

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