NOTES

THE NUTRITIONAL REQUIREMENTS OF CLOSTRIDIUM ACIDI-URICI

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Since previous experiments (Barker and Beck, 1942) left some doubt as to whether growth factors and amino acids are required by *Clostridium acidi-urici*, its nutrition has been further studied. It now appears that this organism (strain 9a) is able to grow very well upon an entirely synthetic medium from which all growth factors and amino acids have been excluded.

The medium has the following composition in g. per 100 ml.: uric acid 0.3, sodium thioglycollate 0.05 or $Na_2S \cdot 9H_2O$ 0.01, K_2HPO_4 0.6, $MgSO_4 \cdot 7H_2O$ 0.02, Fe $SO_4 \cdot 7H_2O$ 0.001, $MnSO_4 \cdot 4H_2O$ 0.001, $CaSO_4 \cdot 2H_2O$ 0.001, NaOH to pH 8, distilled water. Oxygen was excluded by a pyrogallol-K_2CO_3 seal which evolved sufficient carbon dioxide to reduce the pH of the medium to about 7.4.

The only constituent of the above medium that might be contaminated with growth factors or amino acids is uric acid. To eliminate possible impurities, Merck's uric acid was repurified by being converted to the lithium salt, treated with Norite A, recrystallized and then reconverted back into the free acid. The acid was finally extracted with ether continuously for 36 hours. The purified material was as effective as the original commercial product in supporting the growth of *C. acidi-urici*.

When the growth-factor-free medium is inoculated with a spore suspension the initiation of growth is frequently delayed for 24–48 hours or even longer, as compared with growth on a similar medium containing 0.1% Difco yeast extract. Once growth starts, however, the rates and the ultimate cell yields in the two media are approximately the same through several successive transfers. The more rapid initiation of growth with yeast extract is probably due to a factor which stimulates spore germination.

During growth on the synthetic medium, at least three growth factors, riboflavin, vitamin Bc (folic acid, eluate factor) and biotin, are synthesized. 14.3 gamma of riboflavin and 0.45 gamma of biotin were found per gram of dry bacteria, while the yield of vitamin Bc was 0.03 gamma per ml. of fermented medium[1].

These results show that *C. acidi-urici* is a rather unusual organism from a nutritional point of view. It is one of very few anaerobic spore formers capable

[1] We are indebted to Dr. Regina Riker for the riboflavin assay by the *L. casei* method, to John Bowden for the vitamin Bc assay by the *S. lactis* method and to Miss Florence Fox for the biotin determination by the *L. casei* method.

of developing without growth factors or amino acids, and it is probably the only "putrefactive" *Clostridium* having such simple nutritional requirements. The simple requirements are a further indication of the remarkable synthetic powers of this organism which has already been shown to form acetic acid from carbon dioxide (Barker et al. 1940).

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A RAPID AND ACCURATE METHOD FOR TESTING PENICILLIN PRODUCTION BY DIFFERENT STRAINS OF P. NOTATUM

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Because of the growing interest in penicillin production and the desire for making rapid and accurate comparisons between large numbers of cultures, either isolated at random from the soil or from other natural substances or obtained from culture collections, a need for a convenient method arises. In order to meet this need, a simple method is proposed here. It consists in the preparation of a medium favorable for the growth of the penicillin-producing organisms, to which 2 per cent of agar has been added. Twenty ml. portions of this medium are placed in standard test tubes, having a diameter of 18 mm.; a column of agar to a depth of 77 mm. is obtained. The tubes are kept in a vertical position while the agar is solidifying so as to give an even surface. The tubes are inoculated on the surface with the spores of the test organisms and incubated at 20 to 30°C. At definite intervals, some of the tubes are removed, and the agar column in each is carefully shaken out in an unbroken state upon a plate (this can be facilitated by inserting a glass rod through the agar). Small plugs are now taken from the agar column by means of a glass tube, having an inside diameter of 7 mm., beginning just beneath the surface of the fungus (top layer) and at certain distances from the surface. The plugs of agar are then placed upon the surface of a nutrient agar plate previously inoculated with the test organism and are incubated for 20 hours at 28°C. The diameter of the clear zone around the agar is a measure of the rate of penicillin production and its diffusion through the agar medium. The results presented in table 1 amply illustrate the usefulness of this method for comparing not only the efficiency of