

An Agar Decomposing Organism Isolated from Soil

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A complex carbohydrate, agar is attacked by very few organisms and in text books published as recently as 1900 we find the statement that agar is not liquefied by any organism. Since that time a few agar decomposing organisms have been reported in the literature, the first being a species isolated from sea water and described by Gran in 1902. This organism, which has been named *Bacillus gelaticus*, (1) occurs in three varieties distinguished by chromogenesis and requires a high salt concentration when grown on artificial media. In 1905 Panek reported an acid producing rod which had the ability to liquefy agar and this is now known as *Bacterium betae viscosum*. Biernacki in 1911 isolated an agar decomposer from raisins and this organism, also an acid producing rod, was named *Bacterium nenckii*. (2) Perhaps the best known of the agar decomposers on record is that reported by Gray and Chalmers in 1924, a cellulose decomposer, this organism is known as *Microspira agar-liquefaciens* (3). The most recent report of such an organism is that of Stanier, (5) his description of *Actinomyces coelicolor* having appeared in 1942.

Attention was drawn to the organism here reported during experiments on cellulose digestion by soil organisms from the grounds at Purdue University. Active digestion of cellulose was observed in an aerated flask and plates were made in an attempt to isolate the cellulose-attacking organisms. The medium used was one reported by Dubos (4) as favorable for the growth of cellulose digesters and consisted of the following:

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| Na NO ₃ | 0.50 gms. |
| Ka HPO ₄ | 1.00 gms. |
| Mg SO ₄ . 7H ₂ O | 0.50 gms. |
| K Cl | 0.50 gms. |
| Fe SO ₄ . 7H ₂ O | 0.01 gms. |
| Distilled water to 1000 ml. | |

To the basal medium, agar was added to give a concentration of 1.5 per cent and filter-sterilized glucose was added directly to the plates in a concentration of 0.1 per cent. These plates were observed to contain many definite depressions with small yellowish deep colonies occupying the center of each and every depression. Since it was a mixed culture, there were many other organisms growing on the surface and isolation presented a problem. Various media were tried and while growth of the organism took place on many of these, the phenomenon of agar digestion was most satisfactorily observed on the original Dubos medium plus a 1.5 per cent concentration of agar. However a pure culture was obtained from surface colonies appearing on a medium en-

riched with a nutrient substantially the same as corn steep liquor, streaks from these colonies giving rise to the depressions on Dubos medium.

Platings of the organism give rise to yellow and white colonies with the white predominating. These colonies in turn are capable of giving rise to more yellow and white colonies both types resulting from either a yellow or white colony. Microscopically, stains of organisms from both colonies present a similar picture in that both are gram negative, non spore forming rods measuring 0.5μ by 2.0μ and possessing true motility. Stains from the yellow colonies, however, reveal some spindle-shape cells and it is thought possible that the difference in color is related to an age factor.

The organism fails to ferment carbohydrates with the production of acid and gas, nor does it attack cellulose. Mesophilic with an optimum temperature of 30°C , the organism is aerobic to microaerophilic and grows readily on potato with a characteristic yellow pigment. Litmus milk is reduced after two days, while indole, Voges-Proskauer, and methyl red tests are all negative and there is no reduction of nitrates.

There were no evidences of liquefaction around the depressions and a test devised by Gran making use of an iodine-potassium iodide solution failed to demonstrate liquefaction.

Varying the concentration of glucose disclosed that higher levels such as 2 per cent resulted in increasing amounts of growth, but the degree of decomposition is far less at this concentration than at the 0.1 per cent level.

It was found that growth of the organism depends upon the method of sterilizing the glucose, normal growth taking place when the glucose is filter sterilized and growth being inhibited to a very great degree with autoclaved glucose. Stanier has reported similar results with organisms of the cytophoga group and he explains it as a toxic effect of caramelization products even though the glucose is separately autoclaved in pure distilled water. This explanation seems the most satisfactory at this time.

Bibliography

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3. *Idem, ibid.*, 111-12.
4. Dubos, R. J. Influence of environmental factors on the activities of aerobic bacteria concerned in the decomposition of cellulose in soils. *J. Bact.* **13**:52, 1927.
5. Stanier, R. Y. Agar-decomposing strains of the actinomyces coelicolor species-group. *J. Bact.* **44**:555-70 1942.