FORMATE AS A SUBSTRATE FOR THE ENRICHMENT OF *PSEUDOMONAS FLUORESCENS*

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SUMMARY

It has been shown that soil enrichments set up with formate as the sole source of carbon and energy result in the isolation of *Pseudomonas fluorescens*. A brief description of the organism as well as the list of substrates it can utilise is given.

INTRODUCTION

In a previous study on the oxalate-decomposing bacterium, *Vibrio oxaliticus* (Bhat and Barker, 1948), it was shown that the vibrio possesses constitutive enzymes for both oxalate and formate even though the organism was originally isolated by enrichment cultures set up with oxalate as the sole source of carbon and energy. It was then considered of interest to see whether or not the same vibrio would get isolated if formate be substituted for the oxalate in the enrichment culture medium. The purpose of the present communication is to report the results secured on setting up of the formate enrichments and to indicate that the enrichment of *V. oxaliticus* does not occur in the absence of oxalate and that it results in the isolation of pseudomonads instead.

EXPERIMENTAL PROCEDURE AND RESULTS

From a series of pilot studies it came to be observed that formate can support the growth of a Gram-negative bacterium in the enrichments even when the culture medium is free from yeast extract. Furthermore, it became clear that during the stages of enrichments, presence of yeast extract in the medium promotes the growth of other non-specific flora including a mesophilic sporeforming bacillus. The culture medium employed throughout the present study for isolation work was therefore made without the yeast extract and having the following ingredients in g./100 ml. of distilled water: sodium formate (hydrate), 0·1; $(NH_4)_2SO_4$, 0·05; K_2HPO_4, 0·05; $MgSO_4 \cdot 7\,H_2O$, 0·01; $FeSO_4 \cdot 7\,H_2O$, 0·002; $CaSO_4 \cdot 2\,H_2O$, 0·001; phenol red indicator, a few drops; pH 7·00.

The medium was dispensed in 25 ml. aliquots in 250 ml. Erlenmeyer flasks and after inoculation with different soils was incubated at the room temperature (26–29° C.) for growth to appear.

In every case within 6 days (usually after 4 days) the culture medium showed clear signs of turbidity accompanied invariably by the formation of a surface film.

The medium also turned alkaline owing to the utilisation by the growing [illegible] of the anions from the formate molecules. Subsequent transfers from the first enrichments usually resulted in the appearance of the growth within the course of 3 days. A loopful of the inoculum from the third or the fourth enrichment on streaking on the surface of the corresponding solid medium or nutrient agar generally resulted in the isolation of a large number of colonies, mostly made up of one type. On nutrient agar growth appeared within a day whereas on the formate agar the organism took 2 to 3 days to give visible colonies. Furthermore, the growth on nutrient agar may be characterized as vigorous and luxuriant, often pigmented greenish to olive green in comparison to that on the formate agar which may be described as slow-growing, pin-point colonies with only a faint [illegible] of fluorescence, if any. Some 20 strains isolated from 12 soils compare very closely to the species *Pseudomonas fluorescens* and the differences as have been noticed lie in a few biochemical activities.

Morphologically all cultures were Gram-negative and motile with a single polar flagellum and the cells did not resemble in any way those of the vibrios isolated from the oxalate enrichments (Bhat and Barker, 1948). In nutrient broth the growth was flocculer with general turbidity and greenish pellicle. In litmus milk no discernible action was observed besides reduction of the dye. In general, unlike the typical fluorescens strain, liquefaction of gelatin was absent and when observed the reaction was weak. Indole was never produced but nitrates were [illegible] reduced to nitrite; hydrogen sulphide was not formed by any one strain in peptone water medium. Most strains fermented glucose to form acid but lactose, sucrose, lævulose, arabinose, mannitol, glycerol, raffinose, xylose, galactose, starch, dextrin and salicin were not fermented to form acid. Only one strain fermented [illegible] lævulose and xylose but not the other carbohydrates or alcohols.

A number of carbon sources were tested as substrates of growth in a medium with no added yeast extract and utilisation was judged by increased turbidity over the control medium without the added carbon source. Almost all the cultures utilised well acetate, malate, citrate and succinate. Notable was also the appearance of the green pigment in the presence of acetate and succinate. Most of the strains also utilised butyrate, formate, propionate and lactate; tartrate and oxalate utilisation, when observed, was poor. Some strains could utilise benzoate.

Utilisation of formate though in general was poor in the absence of yeast extract, it was improved with the addition of increased amounts of yeast extract and this may be due to the presence of acetate in the yeast product. Growth enhancement was also observed with an increase in the formate concentration to a maximum of 0·8%, but this was possible only in the presence of 0·1% yeast extract in the medium. Oxalate utilisation was poor even in the presence of yeast extract.

DISCUSSION

It is clear from the description given of the organism isolated from the formate enrichments that it closely resembles *Pseudomonas fluorescens* except for the lack

of the enzyme gelatinase. The organism is unlike the species isolated from formate enrichments set up with leaf-galls derived from *Pongamia glabra* (Pavri and Bhat, 1953) and is also distinct from the pseudomonads and bacteria isolated by Khambata and Bhat (1953 *a* and 1953 *b*) from the intestines of the earthworm. The feeble growth of *P. fluorescens* observed by den Dooren de Jong (1926) in 0·5% of formic acid when examined together with its ability to utilise other acids in the light of the description given for the present species, one is led to the conclusion that the species, which gets isolated from the soil enrichments set with formate as the sole source of carbon and energy cannot be any other than *Pseudomonas fluorescens*. Attempts made by Lobo (1950) to isolate formate decomposing cultures from human fæces have also resulted in the isolation of this species on two occasions out of the nine enrichments set for the purpose.

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