AN ATYPICAL STRAIN OF PSEUDOMONAS AERUGINOSA

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Recently Kopper and Beard (1947) reported the isolation from human urine of a strain of *Pseudomonas* closely resembling *Pseudomonas aeruginosa* in cultural and biochemical characteristics, but differing from it in its ability to break down creatine and creatinine with the resultant formation of urea, ammonia, and carbon dioxide. The organism was grown on a medium containing 2 per cent creatinine, 2 per cent agar, and 5 per cent urine in distilled water.

Porter (1946), citing den Dooren de Jong, lists creatine and creatinine among a number of compounds which can serve as sources of nitrogen for *P. fluorescens* in a basal medium composed of 2 per cent agar, 0.1 per cent K_2HPO_4, 1.0 per cent glucose, and 1.0 per cent $CaCO_3$ in tap water. In this study the same medium was used without agar. Upon addition of 0.1 per cent creatinine it was found to support adequately the growth of *P. aeruginosa* and *Pseudomonas fluorescens*. Three strains of the former and two of the latter species were tested. The amounts of creatinine broken down were exceedingly small. The strain isolated from urine, on the other hand, proved to be a very active fermenter of creatinine.

EXPERIMENTAL PROCEDURE

An attempt to cultivate the variant strain in a 0.1 per cent solution of creatinine in tap water was unsuccessful. When 1.0 per cent autoclaved urine was added growth took place. A comparison of the reported analysis of Chicago tap water and the composition of urine revealed that the latter was rich in phosphates which the former lacked. A M/150 phosphate buffer solution of pH 7 prepared with KH_2PO_4 and Na_2HPO_4 in distilled water containing 0.1 per cent creatinine proved a suitable medium for the growth of the organism. For the buffer mixture, solutions of KH_2PO_4 of pH 4.5 or of Na_2HPO_4 of pH 9 could be substituted. Incubation at 37 C or at room temperature (20 to 24 C) was equally effective in promoting bacterial reproduction. In experiments reported in this study 0.1 per cent creatinine solutions in M/150 phosphate buffer of pH 7 were used and will be referred to as phosphate-buffered creatinine solutions.

Creatinine phosphate agar was prepared with 2 per cent agar and 2 per cent creatinine in a M/60 phosphate buffer solution of pH 7 in distilled water. Nutrient broth and nutrient agar were prepared with Difco neopeptone. All cultures were incubated at room temperature. Creatinine was determined with alkaline picrate. Color intensities were measured in a Cenco-Sheard photolometer.

RESULTS

The nature of the creatinine-decomposing enzyme (creatinase). To determine the constitutive or adaptive character of the creatinine-decomposing enzyme of

the variant strain, cultures were transferred in series into nutrient broth at 24-hour intervals. Twenty transfers were made. After each transfer the presence of creatinase was ascertained by inoculating a loopful of the nutrient broth culture into phosphate-buffered creatinine solution. The enzyme activity was not lost, but declined gradually as manifested in a delay of growth for 48 hours and in its limitation mainly to the surface of the medium.

Three stock culture strains of *P. aeruginosa* and two stock culture strains of *P. fluorescens* failed to grow in phosphate-buffered creatinine solution. An attempt was made to adapt one of the strains of *P. aeruginosa* to this medium. The organism was inoculated into nutrient broth containing 0.2 per cent of creatinine and was transferred in series at 24-hour intervals for 12 successive days. No measurable breakdown of creatinine took place, nor did growth occur in phosphate-buffered creatinine solution inoculated from the last one of the 12 serial transfers.

In another experiment successive transfers in decreasing amounts of nutrient broth, brought up to volume with distilled water, were made. The creatinine concentration was kept constant at 0.2 per cent. A method of bacterial adaptation described by Hegarty (1939) was used. Transfers were made in series every 90 minutes, at which time the end of the lag phase of *P. aeruginosa* was assumed to have been reached. All tubes were inoculated heavily. Six serial transfers were made. Tests for creatinase activity of the bacteria present in the last tube gave negative results.

Finally a heavy inoculum of the stock culture strain was streaked on several plates of creatinine phosphate agar medium and incubated at room temperature for 2 weeks. No growth occurred. It would seem that the strain used was not adaptable to creatinine under the experimental conditions outlined.

Enzyme specificity and growth requirements. Kopper and Beard (1947) observed that the creatinine-decomposing enzyme of the atypical strain acted on creatinine, creatine, and glycocyamidine, but failed to attack hydantoin. Phosphate-buffered solutions containing 0.1 per cent creatine, glycocyamidine, and hydantoin, respectively, were prepared and inoculated with the organism. Growth developed in creatine but not in glycocyamidine or hydantoin. In order to determine a possible cause for the discrepancy between the action of the enzyme on glycocyamidine and the failure of the strain to reproduce on this substrate, an attempt was made to grow the organism on the hydrolytic products of creatinine and glycocyamidine. These two chemical compounds are internal anhydrides of creatine ($NH_2 \cdot C(=NH) \cdot N(CH_3) \cdot CH_2 \cdot COOH$) and glycocyamine ($NH_2 \cdot C(=NH) \cdot NH \cdot CH_2 \cdot COOH$), respectively. The products of the hydrolysis of creatine would be urea and sarcosine, of glycocyamine urea and glycine. Phosphate-buffered solutions of 0.1 per cent urea, sarcosine, and glycine, respectively, were prepared and inoculated with cultures of the atypical strain and of three strains of *P. aeruginosa* and two strains of *P. fluorescens*. No growth took place in urea. Sarcosine supported adequately the growth of all

strains. Glycine proved to be a poor medium. The organisms either failed to multiply in it or did so only slightly after prolonged incubation. This would seem to present additional evidence for the hydrolytic action of the creatinine-decomposing enzyme of the atypical strain. The enzyme may effect the splitting of C · N and C · N linkages, but growth can only be supported by the

resulting split products. On a good nutrient such as sarcosine the organisms multiply readily, which leads to the production of more enzyme, which in its turn causes a further breakdown of creatinine or creatine, whichever is the substrate, and a greater accumulation of sarcosine. On a poor nutrient such as glycine, on the other hand, reproduction is so slow that no proper chain reaction can develop, which may account for the inadequacy of glycocyamidine as the sole source of carbon and nitrogen in a culture medium for the growth of the atypical strain.

Preservation of cultures of the atypical strain. Cultures of the atypical strain were kept on creatinine phosphate agar slants at room temperature. When bacteria were transferred from such slants to phosphate-buffered creatinine solution or nutrient broth after 8 to 10 days, they failed to grow. The organisms lost their viability also on nutrient agar, to which 2 per cent creatinine had been added, within the same length of time. Their creatinase activity, however, was unaffected, as shown by the disappearance of creatinine from solutions incubated with suspensions of the dead organisms. Both viability and creatinase activity could be preserved by keeping cultures on nutrient agar slants aerobically or on creatinine phosphate agar slants under oil. This was proved by transfers from such slants after 45 and 60 days, respectively. Work is now in progress to investigate the cause of the delayed lethal effect of creatinine agar media on cultures of the atypical strain under aerobic conditions.

DISCUSSION

Karstrŏm's differentiation of bacterial enzymes into "constitutive" and "adaptive" enzymes was enlarged upon by Krebs and Eggleston (1939), who subdivided the latter into "partially adaptive" enzymes, which are formed in the absence of the specific substrate but increased in its presence, and "totally adaptive" enzymes, which are formed only in the presence of the specific substrate. The creatinine-decomposing enzyme described by Dubos and Miller (1937) was shown to be "totally adaptive." Evidence presented in this study would favor the classification of the creatinase of the atypical strain of *P. aeruginosa* as a "partially adaptive" enzyme. One can only speculate on the mode of origin of such enzymes. They may arise from mutations of the parent strain, which are of a more fundamental character than those producing "totally adaptive" enzymes. This would explain the greater difficulties encountered in inducing them.

As pointed out by Luria (1947), most bacterial classifications are only determinative keys, which cannot be compared with the well-defined systems of zoological and botanical taxonomy. Many bacterial species and even genera

are separated on the basis of character differences that may be brought about by a single mutational step. The organism described here may have arisen in such a way, since aside from its creatinase activity it is indistinguishable from the species *P. aeruginosa*.

SUMMARY

A strain of *Pseudomonas aeruginosa*, first isolated from urine, was cultivated in a phosphate-buffered solution containing creatinine as the sole source of carbon and nitrogen.

The strain possesses a specific creatinine-decomposing enzyme, creatinase, which is not lost after 20 successive transfers through nutrient broth without creatinine.

Attempts to adapt a stock culture strain of *P. aeruginosa* to grow in a phosphate-buffered creatinine solution were unsuccessful.

The creatinase acts on glycocyamidine, but this compound is inadequate for serving as the sole source of carbon and nitrogen for the growth of the atypical strain.

Sarcosine, a hydrolytic product of creatinine and creatine, represents a good culture medium for the atypical strain and five other strains of *P. aeruginosa* and *Pseudomonas fluorescens* tested. Glycine, a hydrolytic product of glycocyamidine, is a poor nutrient for all strains.

Cultures of the strain could be preserved on nutrient agar aerobically or on 2 per cent creatinine phosphate agar under oil for 45 and 60 days, respectively.

Cultures kept aerobically on creatinine phosphate agar or on 2 per cent creatinine nutrient agar lost their viability but not their creatinase activity within 8 to 10 days.

The nature of the enzyme and its possible mode of origin are discussed.

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