EFFECT OF PURINE BASES ON THE GROWTH OF *BACILLUS CIRCULANS*

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Summary

The stimulatory effect of the purine bases on the growth of *B. circulans* has been demonstrated. The replacement of its biotin requirement by pimelic acid, citrate, fumarate or succinate has also been shown.

Introduction

That the purine and the pyrimidine bases stimulate growth in micro-organisms has come to be observed since a long time ago. The growth of *B. anthracis*, for instance, is initiated by adenylic acid and adenosine (Brewer *et al.*, 1946). Likewise, the need for a purine base for the growth of all the strains of *B. larve* under test has been reported by Katznelson and Lochhead (1948). Grula *et al.* (1954) working with a strain of *Caulobacter* have reported a stimulatory response by this organism to adenine, guanine and uracil in the presence of ribo-flavine, an essential growth factor in its nutrition. Knight and Proom (1950) studied the nutritional requirements of *B. circulans* and though reported its ability to grow in a medium containing thiamine and biotin, its unsatisfactory growth in a casein hydrolysate medium fortified with vitamins suggested that supplementation with purine and pyrimidine bases perhaps might result in a luxuriant growth especially in view of what has come to be observed regarding the nutritional requirements of other sporeforming bacteria mentioned before. Also, in the light of the reports that amino acids can replace thiamine in certain organisms (Katznelson, 1947) and that the biotin requirements of some may be met with by pimelic acid, citrate, malate, oleate, pyruvate or succinate, it was considered worthwhile to see whether purines and the fatty acids can effectively replace thiamine and biotin in the growth requirements of *B. circulans*.

Materials, Methods and Results

Organism.—Two strains of *B. circulans* were chosen for study. Both these strains were isolated by Dr. V. Iyer from soil and by Dr. S. R. Khambata from the intestines of the earthworm and identified as *B. circulans* by resort to the methods recommended for the purpose in the *Bergey's Manual* (1948) and in the monograph by Smith *et al.* (1952). The organism was throughout maintained on nutrient agar slants and subcultured 72 hours before use.

Inoculum and the measurement of growth response.—For preparing the inocula, the organism was grown at 37° C. in a medium described by Cleverdon *et al.* (1949). After 48 hours of growth the cells were harvested by centrifugation, washed and redispersed in 10 ml. of physiological saline. Aliquots of 0·1 ml. of this suspension served as the inocula for the tubes. Growth responses were measured in Klett Summerson photoelectric colorimeter using 42 filter.

Nutritional studies.—The chemically defined basal medium employed in these studies was the same as that described by Knight and Proom (1950). Vitamin-free casein hydrolysate was dissolved in distilled water and devitaminised further by the method of Snell and Wright (1941) and its nitrogen content adjusted to 0·3 mg./ml. for all the experimentations.

All the vitamins listed by Knight and Proom, *viz.*, thiamine, riboflavine, nicotinic acid, pantothenate, pyridoxine HCl, biotin and folic acid, were incorporated into the basal solution in the same concentrations as suggested by the above investigators. This constituted the complete medium which could support moderate growth of the organism. Other vitamins added to the medium during the experimentations were B_{12}, inositol and *p*-aminobenzoic acid in the concentrations of 1 *m*μg./ml., 2 *m*μg./ml. and 0·04 μg./ml. respectively. Pimelic acid was added in a concentration of 0·05 mg./ml., and citrate, fumarate, oleate and succinate in a final concentration of 0·1 mg./ml. each.

For the sake of convenience, adenine, guanine, uracil and xanthine have been given the notations, A, G, U and X respectively, and these were added into the medium to give a final concentration of 15 μg./ml. each.

The results of the experiments are presented in Tables I, II and III. They represent the typicals recorded for a series of four experiments. Both the strains of *B. circulans* gave identical results.

TABLE I

Effect of vitamins and the purine and pyrimidine bases on the growth of B. circulans

No.	Composition of the medium	% light scattered
1	Complete medium with B_{12} and inositol	62
2	,, ,, with B_{12}	62
3	,, ,, with inositol	62
4	Complete medium	62
5	Basal medium (vitamin-free)	0
6	Complete medium with A, G, U, X, B_{12} and inositol ..	100
7	,, ,, with A, G, U and X	100
8	Basal medium with A. G. U. X and B_{12}	0
9	,, ,, with A, G, U, X and inositol	0
10	,, ,, with A, G, U and X	0
11	,, ,, with B_{12}	0
12	,, ,, with inositol	0

TABLE II

Effect of individual purine and pyrimidine bases on the growth of B. circulans

No.	Composition of the medium	% light scattered
1	Complete medium with A, G, U, X	100
2	,, ,, with A, G, U	99·5
3	,, ,, with A, G, X	104·5
4	,, ,, with A,U, X	105
5	,, ,, with G, U, X	101·3
6	,, ,, with A, G	103·3
7	,, ,, with A, U	87·5
8	,, ,, with A, X	108·6
9	,, ,, with G, U	92·8
10	,, ,, with G, X	92·8
11	,, ,, with U, X	98·5
12	,, ,, with A	86·3
13	,, ,, with G	92
14	,, ,, with U	65·8
15	,, ,, with X	94
16	Complete medium	68

TABLE III

Effect of substituents for thiamine and biotin on the growth of B. circulans

No.	Composition of the medium	% light scattered
1	Complete medium with A, G, X	100
2	,, ,, with A, G, X but without thiamine ..	0
3	,, ,, with A, G, X but without biotin	0
4	,, ,, with A, G, X and pimelic acid but without biotin	91·2
5	,, ,, with A, G, X and citrate but without biotin	94·7
6	,, ,, with A, G, X and oleate but without biotin	0
7	,, ,, with A, G, X and fumarate but without biotin	86
8	,, ,, with A, G, X and succinate but without biotin	92·3
9	Basal medium	0

Percentages have been calculated taking the scattering of light as 100 for the complete medium with A, G, U and X.

Discussion

From Table I it is clear that the purines are effective in promoting the growth of *B. circulans* in the presence of added vitamins only, but not otherwise. Even B_{12} and inositol supplementation to purines cannot enhance the growth of the organism in the absence of the other essential vitamins.

Table II indicates that the purines undoubtedly help promote growth when present in mixtures of two or three and that they are ineffective singly. Uracil does not seem to aid the growth at all and was as such eliminated during the subsequent work. The combination of adenine and xanthine appears to offer the best suited mixture for its growth to occur, but the medium containing all the three purine bases has been consistently observed to prompt early growth and yield better harvests. The observations made here have found support in what has been reported for *B. anthracis* as well as for a mutant strain of *E. coli* (Gots, 1950). A recent report indicates that the purine bases are even helpful for the elaboration of protective antigen by *B. anthracis* (Puziss and Wright, 1954).

Biotin, it appears, may be replaced by pimelic acid, citrate, fumarate and succinate in the growth requirements of *B. circulans.* This finding is again in conformity with what has been reported to be the case for some other organisms (Campbell and Williams, 1953). Oleate, however, does not seem to support the growth of this organism. But this is not surprising since oleic acid has been known to exert a toxic influence on many other micro-organisms (Hunter, 1942; Williams and Fieger, 1946; Hutchings and Boggiano, 1947; Williams *et al.*, 1947; Williams and Andrews, 1950; Thoma and Peterson, 1950). The thiamine requirement of *B. circulans*, however, cannot be met with by the purine bases.

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