EFFECT OF URACIL ON THE PRODUCTION OF ACID BY *B. CIRCULANS*

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Summary

Effect of different carbohydrate sources on the growth of *B. circulans* has been studied. The influence of uracil on the production of acid by this organism from carbohydrates has been indicated. The observation that pyridoxin could enhance acid production by this organism from galactose prompted the use of other vitamins for studying their influcnce on the formation of acid. The results recorded are presented.

Introduction

Purines have already been established to play an important part in the nutrition of micro-organisms. Recently, however, pyrimidine compounds, notably uracil and its derivatives, have also been shown as vital in the carbohydrate metabolism of bacteria. In the light of the diverse observations made in the literature with regard to the role of pyrimidines in the nutrition and metabolism of bacteria, it was considered worthwhile to study the effect of uracil on the growth and metabolic activity of *B. circulans* since it offers a prompt response in growth to the addition of purines in the medium (Sharada and Bhat, 1955).

Materials, Methods and Results

The strain of *B. circulans* employed was the same whose nutritional requirements have already been indicated before (Sharada and Bhat, 1955). The medium employed was also the same as described before, but the inoculum used was a tenth of the dilution previously indicated (Sharada and Bhat, 1955). Growth responses were measured tubidimetrically (Klett-Summerson Photoelectric Colorimeter) and metabolic activity measured by titrating the fermented media for acid formation against N/20 NaOH.

The casein basal medium was fortified with only the essential vitamins, *viz.*, biotin and thiamine. Carbohydrate examined as sources of carbon and energy were glucose, galactose, maltose, sucrose, lactose and raffinose, the last two carbohydrates being those which have a galactose unit in their molecule. The carbohydrates were prepared in 20 per cent. solutions, sterilized separately and added into the basal medium so as to attain a final concentration of 2 per cent. In a preliminary experiment, the growth of the organism was studied in the carbohydrates with and without the presence individually of adenine, guanine,

xanthine and uracil, and it was observed that whereas the purines together promoted better growth of the organism the pyrimidine compound under test would neither increase nor decrease the growth when the carbohydrate sources used were glucose, maltose, sucrose and raffinose. In the subsequent experiments therefore the final basal medium was fortified with adenine, guanine and xanthine before use.

The observation that uracil promoted growth and helped to produce more acid from galactose prompted the employment of lactose and raffinose (two other sugars with galactose unit in their molecules) in a special study designed to observe the effect of uracil on the fermentation of these sugars in the presence of the purine bases in the basal medium. However, uracil was found to possess no effect on the growth of the organism when grown in a medium containing lactose and raffinose. What is more interesting is that lactose failed to support growth. Even after serial transfer of the culture into media containing lactose it failed to produce any response. Therefore, glucose to an extent of 0·5 per cent. had to be incorporated into media containing lactose to initiate growth of the organism before the substrate could even be studied.

The favourable influence uracil had indicated on the growth and metabolic activity of the organism called for another experiment designed to determine the optimal quantity of uracil in fermentation. For this experiment, galactose was the sugar of choice. Likewise, the observation that pyridoxin promoted better formation of acid from carbohydrates prompted an enquiry into the role of other vitamins of the B complex as riboflavin, nicotinic acid, pantothenic acid, folic acid, *para*-amino benzoic acid and B_{12} and accordingly each of these was put to test by studying its effect on acid production by the organism from galactose. The results of the various studies concluded are presented in Tables I, II, III and IV. The medium containing casein hydrolysate, thiamine and biotin is referred to as the basal medium. The values expressed for acid are in terms of N/20 NaOH.

TABLE I

Effect of different carbohydrate sources on the growth of B. circulans

	Media	K.S. readings
1.	Basal medium + adenine + guanine + xanthine	Control
2.	Control medium + glucose	170
3.	,, + galactose	181
4.	,, + maltose	190
5.	,, + sucrose	150
6.	,, + lactose	7·5
7.	,, + raffinose	134

TABLE II

Influence of uracil on acid production

	Media	Acid formed in ml./25 ml.	
		Without uracil	With uracil
1.	Basal medium + galactose	9·35	11·00
2.	,, + lactose + 0·5% glucose	14·65	14·68
3.	,, + raffinose	4·73	4·65

TABLE III

Variation of acid production from galactose with the concentration of uracil

	Media + μg. of uracil	Acid formed in ml./25 ml.
1.	Basal medium + 6·66 ..	14·00
2.	,, + 16·66 ..	14·50
3.	,, + 33·33 ..	15·25
4.	,, + 49·99 ..	15·75
5.	,, + 66·66 ..	16·25
6.	,, + 99·99 ..	16·00
7.	,, + 133·33 ..	13·00

TABLE IV

Effect of various vitamins and uracil on acid formation from galactose

	Media	Acid formed in ml./25 ml.		% increase in presence of uracil
		Without uracil	With uracil	
1.	Basal medium	3·70	4·23	14·3
2.	,, + riboflavin ..	3·70	4·30	16·2
3.	,, + nicotinic acid ..	3·60	4·20	16·6
4.	,, + folic acid ..	3·80	4·50	18·0
5.	,, + pyridoxin ..	4·10	5·13	25·1
6.	,, + pantothenic acid	3·85	4·50	16·8
7.	,, + *para*-amino benzoic acid	3·65	4·25	16·4
8.	,, + B_{12}	3·85	3·92	1·8
9.	,, + B_{12} + pyridoxin	3·85	3·90	1·3

Discussion

From Table I it is clear that maltose provides the most suitable source of carbohydrate for the organism the next in order being galactose, glucose, sucrose and raffinose respectively. Results recorded in Table II indicate that uracil exerts definite influence on the fermentation of galactose and the consequential formation of acid therefrom. This is not surprising since *L. bulgaricus* has been demonstrated to produce acid from galactose at a faster rate in the presence of uridine diphosphate glucose (a derivative of uracil) than in its absence (Rutter and Hansen, 1953). This derivative of uracil has also been recognised by some investigators (Rutter and Hansen, 1953; Caputto *et al.*, 1950; Leloir and Cabib, 1953) as a coenzyme capable of activating galactowaldenase and galactokinase in *L. bulgaricus* and certain yeasts. Moreover, uracil has been shown to induce the formation of β-galactosidase in a mutant of *E. coli* when melibiose is used as the source of energy and glycerol as the activator (Pardee, 1955). When the same enzyme system is induced in *Staphylococcus aureus* by the energy source galactose, uracil has been demonstrated to bring about an appreciable diminution in the lag period by the elaboration of the adaptive enzyme (Creaser, 1955). The above observations as also the fact that uridine complexes accumulate in *Staphylococcus aureus* treated with penicillin (Caputto, *et al.*, 1950) indicate that uracil plays an important part in the metabolism of carbohydrates in micro-organisms. In the case of *B. circulans* galactose is the only carbohydrate source whose fermentation is widely influenced by uracil, the optimal concentration of uracil being about the order of 66·66 μg/ml. Any further increase in its concentration results in a sudden decrease in acid production and in an unevenness in the growth of the organism, a phenomenon which deserves to be studied in this connection.

From Table IV it is evident that vitamins other than pyridoxin do not influence acid production. In presence of uracil, however, a marked increase in acid production is evidenced in all cases except that of B_{12}. Even the incorporation of pyridoxin into the medium containing B_{12} and uracil does not seem to influence acid production.

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References

1. Caputto, R., Luis, F., Leloir, C. E., Cardini and Paladini, A. C. — *J. Biol. Chem.*, 1950, **184**, 333.
2. Creaser, E. H. .. *J. Gen. Microbiol.*, 1955, **12**, 288.
3. Leloir, L. F. and Cabib, E. .. *J. Am. Chem. Soc.*, 1953, **75**, 5445.
4. Pardee, A. B. .. *J. Bacteriol.*, 1955, **69**, 233.
5. Rutter, W. J. and Hansen, R. G. .. *J. Biol. Chem.*, 1953, **202**, 311.
6. ———— .. *Ibid.*, 1953, **202**, 323.
7. Sharada, K. and Bhat, J. V. .. *J. Indian Inst. Sci.*, 1955, **37**, 84.