PROBLEMS IN THERMOPHILY

IV. The Nutritional Requirements of Some Thermophilic Bacilli

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Summary

Screening of 80 isolates of the various species of bacilli growing at 55° C. revealed that none of them could grow at that temperature in a medium containing only an inorganic source of nitrogen. Twenty-seven isolates were able to grow in the same medium at 37° C. Addition of biotin did not promote growth of those that failed to grow at either temperatures. A closer examination of two strains of *B. coagulans* and one of *B. brevis* indicated that the sulphur-containing amino acids constituted essential amino acids for them to grow.

Introduction

Available information on the nutritional requirements of thermophilic bacilli may be admitted to be scanty and conflicting and as such a probe into this aspect of thermophilic life was considered to be a step which would help furnish a clue to the secret underlying the capacity of these micro-organisms to live at high temperatures. An examination of 80 isolates in our collection revealed that those of the cultures which could grow at the elevated temperature of 55° C. would do so only if the synthetic medium (devised after that of Knight and Proom[2]) containing an inorganic source of nitrogen is supplemented with external sources of organic nitrogen. However, several of the isolates—27 to be exact—did give evidence of good growth at 37° C. even in the absence of organic nitrogen. This led to the assumption that probably those of the thermophiles which failed to grow at 55° C. with inorganic nitrogen would develop if the medium be fortified with biotin, a growth factor whose role in nutrition indicates this possibility.[3,4,5] Accordingly, 22 isolates which typify all those in our collection and representing *B. subtilis*, *B. brevis*, *B. megaterium*, *B. coagulans*, *B. firmus*, *B. sphæricus* and some unidentified species were tested for their ability to grow in an inorganic basal solution with 2 per cent glucose for the carbon and the energy source and in the presence of 9 *mμg./ml.* of biotin. The results recorded are presented in Table I.

It is clear from the table that the supplementation of biotin did not favour in any way the growth of the bacteria at 55° C. The two strains of *B. subtilis* that gave growth at 37° C. under the conditions of the test were the ones capable of

TABLE I

Growth response of thermophilic bacilli in a basal medium supplemented with biotin

Isolate Nos.	Species	Growth at ° C.	
		37	55
E15A	*B. subtilis*	+	—
Chap	,, ...	+	—
Jog 3	*B. coagulans*	—	—
Jog 4	,, ...	—	—
Nain	,, ...	—	—
Bom 1*a*	,, ...	—	—
Bom 1*b*	,, ...	—	—
Bang 1	,, ...	—	—
Ag 1*d*	,, ...	—	—
C 3*e*	,, ...	—	—
Bom 1*c*	*B. megaterium*	—	—
L_1	*B. firmus*	*	—
L_6	,, ...	*	—
L_4	*B. brevis*	—	—
Centre	,, ...	—	—
II	*B. sphæricus*	—	—
C_3c	,, ...	—	—
Air 4	Unidentified	*	—
GS	,, ...	*	—
N_2	,, ...	*	—
N_4	,, ...	*	—
RHS	,, ...	*	—

Legend: + = growth
— = no growth
* = Isolates which grow at 55° C. only.

growing at that temperature even in the absence of biotin. This unexpected results left us with no other alternative but to examine more closely and in greater details one or two selected species for their nutritional requirements. After critical survey of the literature, it was considered worthwhile to pursue the study relating to the nutritional requirements of two strains of *B. coagulans* and a strain of *B. brevis* and for this purpose *Bang* 1 and *Jog* 3 strains of the former species and the *Centre* strain of the latter bacillus were selected for further work. All these strains were inoculated into a medium similar in composition to that devised by Campbell and Williams[1] and incubated in duplicate sets at 37 and 55° C. respectively and, after a series of experimentations, it was possible to conclude that all the three isolates under test demanded sulphur-containing amino acids for their growth at 55° C. It is interesting to record here that whereas the *Bang* 1 strain of *B. coagulans* required cysteine for its growth, *Jog* 3 together with the

B. brevis strain, showed their dependence for methionine. The first mentioned strain of *B. coagulans* also showed its incapacity to grow at 55° C. in the absence of thiamine and histidine. At 37° C. however, both the strains of *B. coagulans* grew well in the medium in the absence of these supplementations. The strain of *B. brevis* at the lower temperature gave inconsistent and inconclusive results.

In the course of several repetitions of these experiments with suitable changes in the methods adopted, it came to be realised that occasionally growth would appear even in a deficient medium. This indicated that probably variants with synthetic abilities were easily being selected[2] by the growing cells in the absence of essential nutrilites, the unavoidable introduction of heavy inoculum in the tubes providing better opportunities for this selection to occur. It has already been stated in a previous paper (this series) that the use of heavy inocula could not be avoided in the study of these bacteria. However, the results recorded here are interesting and are indicative of the usefulness of nutritional data in understanding the problems underlying thermophily.

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