Accumulation of 5(4)-Amino-4(5)-imidazolecarboxamide Riboside by Base Auxotroph of *Bacillus subtilis*

Sir:

This communication presents observation on the accumulation of 5(4)-amino-4(5)-imidazolecarboxamide riboside (AICA-R), which is known to be a precursor of purines, in the growing culture filtrate by a base auxotroph of *Bacillus subtilis* with a amount twenty times of those reported in the published literatures¹⁻⁴. The experiments were made as follows: the strain used was D-421, a purineless mutant which was obtained from *Bacillus subtilis* No. 2093 (AICA-R non-

producing strain) after X-ray irradiation; the media employed contained glucose 5%, KH_2PO_4 0.5%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.04%, Fe^{++} 2 p.p.m., Mn^{++} 2 p.p.m., NH_4Cl 1.5%, purine base 5~80 mg%, vitamin-free casamino acids (Difco) 0.2%, and CaCO_3 2% (as a neutralizing agent); 20 ml of these media in 500 ml flask, after sterilization and inoculation of cells, was incubated at 31°C for seventy to ninety hours under aeration with reciprocal shaking; the quantitative determination of AICA-R was performed by the colorimetric

FIG. 1. The Time Course of Accumulation of AICA-R

The medium used contained 20 mg% of adenine. Growth was measured by turbidity readings at 562 mμ by the Hiraama photoelectric colorimeter after CaCO_3 present in the medium was dissolved with a small amount of dil. HCl and diluted up to 26 times by volume with distilled water.

- 1) J.S. Gots, *Nature*, **172**, 256 (1953).
- 2) J.S. Gots and S.H. Love, *J. Biol. Chem.*, **210**, 395 (1954).
- 3) D.P. Lones, C. Rainbow and J.D. Woodward, *J. Gen. Microbiol.*, **19**, 146 (1958).
- 4) T. Shiro, *This journal*, **25**, 350 (1961); **26**, 75, 269 (1962).

method modified by Bratton and Marshall⁵).

Fig. 1 illustrates an example of the time

- 5) A.C. Bratton and E.K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).

course studies of this fermentation. It may be seen that, although the growth of cells attains about one half of its maximum value within the first thirty hours, little AICA-R appears in the medium by then; from which time, however, the amount increases sharply to reach a maximum level about seventy to eighty hours after incubation, as much as 10 mM (2.6 mg/ml) of the amine being accumulated in the extracellular fluid. The identification of AICA-R thus produced was carried out with paper chromatography using

effect on the accumulation of AICA-R, though the amount required for a maximum production may be varied according to the kind of purines supplemented.

Although the higher concentration of purines is favorable for the abundant growth of the cells, nevertheless the accumulation of AICA-R is extremely depressed in these circumstances presumably due to the negative feed-back inhibition by these purine bases.

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TABLE I. EFFECTS OF CONCENTRATION OF PURINE BASES ON THE ACCUMULATION OF AICA-R

Concentration of purine bases supplemented mg %	Adenine		Hypoxanthine		Guanine		Xanthine	
	Growth O. D.	AICA-R mM	Growth O. D.	AICA-R mM	Growth O. D.	AICA-R mM	Growth O. D.	AICA-R mM
0	0.01	> 0						
5	0.127	4.53	0.137	3.54	0.110	2.29	0.030	0
10	0.311	4.83	0.275	3.60	0.163	3.69	0.109	0.96
15	0.407	6.00	0.335	4.95	0.210	4.52	0.179	1.49
20	0.535	7.26	0.448	6.99	0.290	5.24	0.335	3.84
30	0.503	6.65	0.425	3.81	0.415	6.78	0.415	4.62
40	0.454	4.01						
50			0.425	0.85	0.593	5.97	0.463	5.88
60	0.450	0.95						
80	0.381	0.90	0.425	0.72	0.460	0.96	0.495	1.70

All media were the same as described in Fig. 1, except the amounts of purine bases supplemented, and were cultured for seventy-two hours.

several kinds of solvents, paper electrophoresis with 10 percent acetic acid, ultra violet absorption spectrum, orcinol reaction^{6,7)}, and the Bratton and Marshall test⁵⁾; the results of which were all in good agreement with the authentic one.

As can be seen in Table I, the concentration of purines in the medium have a marked

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6) S.E. Kerr and K. Sereidarian, *J. Biol. Chem.*, **159**, 211 (1945).

7) H.F. Drury, *Arch. Biochem.*, **19**, 455 (1948).