AMINO ACID CONTROL OF RIBONUCLEIC ACID EXCRETION IN *BACILLUS SUBTILIS*

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When incubated in a medium composed of maltose, citrate, ammonium phosphate and other inorganic salts, *Bacillus subtilis* MB-1480 excretes large amounts of ribonucleic acid (RNA) during the growth phase (Demain *et al.*, 1965). The extracellular RNA is subsequently degraded to 5'-nucleotides and other derivatives which accumulate in the medium. This wasteful process has now been found to be inhibited by supplementation of the medium with amino acids.

While studying the effect of the nutritional environment on the excretion process, it was found that yeast extract markedly increased cell yield but inhibited extracellular accumulation of RNA. This is seen in Figure 1 where RNA excretion is estimated by determination of the concentration of guanine-containing compounds ("total guanines") in the centrifuged broth. This effect was also caused by enzyme-hydrolyzed casein, by acid-hydrolyzed casein and by a mixture of L-amino acids simulating the composition of casein hydrolysate.

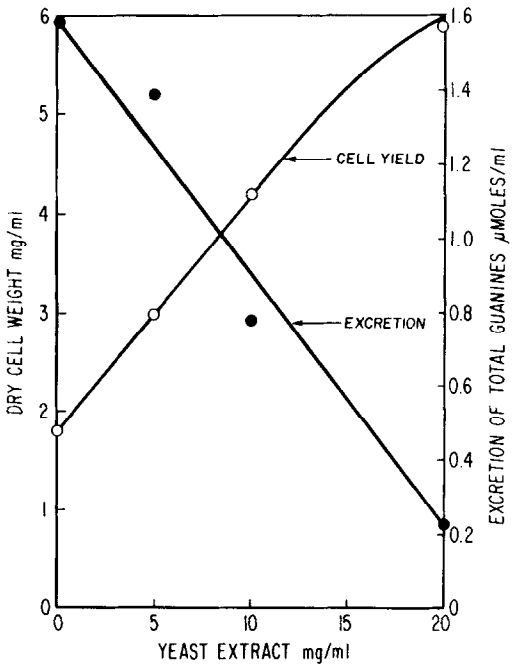

Figure 1. Effect of yeast extract on cell yield and excretion of RNA. Conditions of growth and assay of growth and excretion of guanine-containing compounds were as previously described (Demain et al., 1965).

The effects of twenty individual L-amino acids were tested at concentrations of 0.02 and 0.2 percent. Although no individual amino acid showed the same quantitative effect as 2 percent acid-hydrolyzed casein or amino acid mixture, especially on growth yield, a number of amino acids inhibited extracellular accumulation of RNA. Results with the more active amino acids are shown in Table I. Cystine and cysteine were more effective than the others. The amount of excretion per unit of cells ("specific accumulation") was reduced by as much as 80 percent by cystine and cysteine.

The greater activity of cyst(e)ine than that of

TABLE I

Effect of amino acids on growth and extracellular accumulation of RNA

Supplement	Concentration mg/ml	Growth O.D. 660	Accumulation O.D. 260	Specific Accumulation O.D. 260/O.D. 660
None	-	15	94	6.3
Acid-hydrolyzed casein	20.0	50	38	0.8
Amino Acid Mixture	20.0	40	53	1.3
L-cystine	0.2	22	68	3.1
	2.0	19	47	2.5
L-cysteine	0.2	23	92	4.0
	2.0	23	63	2.7
L-isoleucine	0.2	16	90	5.6
	2.0	15	52	3.5
L-threonine	0.2	18	89	5.0
	2.0	15	58	3.9
L-valine	0.2	20	92	4.6
	2.0	12	49	4.1
L-hydroxyproline	0.2	17	89	5.2
	2.0	12	56	4.7

Conditions of growth and assays were as previously described (Demain et al., 1965). Growth was determined by absorbancy of whole broth at 660 mμ in a Bausch & Lomb Spectronic-20 colorimeter in tubes with an outside diameter of 0.5 in. RNA excretion was estimated by absorbancy of centrifuged broth at 260 mμ in a Beckman model DB spectrophotometer with 1-cm light path.

the other amino acids suggested that the inhibition of extracellular RNA accumulation may be related to the reported abilities of cyst(e)ine (1) to inhibit formation of exoenzymes (Morihara, 1959; Tsuru, 1962; Oishi et al., 1963; and of extracellular glutamate (Tanaka et al., 1965); (2) to stimulate the formation of cell membrane and wall substance (Toennies, 1965); and (3) to repress the formation of cellular enzymes unrelated to cyst(e)ine metabolism (Tonomura and Novelli, 1960; Cox and MacLeod, 1963). The temptation to ascribe inhibition of RNA accumulation solely to cyst(e)ine was tempered by the observations that even a level of this amino acid as high as 0.2% failed to duplicate the effects of 2% amino acid mixtures which contain very little cyst(e)ine, and that other amino acids also were active to some degree. I am thus led to the possibility that the excretion phenomenon results from unbalanced growth characterized by a low rate of protein synthesis accompanied by normal RNA synthesis. Such unbalanced synthesis of RNA would resemble the intracellular accumulation of RNA by bacteria treated with chloramphenicol or deprived of potassium (Lubin and Ennis, 1964) or by "relaxed" mutants during amino acid starvation (Borek et al., 1955; Stent and Brenner, 1961). B. subtilis MB-1480 may represent a "relaxed" prototroph which synthesizes amino acids poorly in the chemically-defined medium and accumulates RNA faster than protein is synthesized. Since such RNA appears to be detrimental to the cell (Neidhardt, 1964), the ability to excrete it would be advantageous. Addition of amino acid mixtures would then be expected to correct this deficiency, to allow rapid synthesis of protein and

normal ribosomes, to stimulate growth, and to inhibit the wasteful process of RNA excretion.

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