NOTES

CITRATE METABOLISM AND CELL PERMEABILITY[1]

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Nonmetabolism of an added substrate by an intact microorganism, as opposed to its ready dissimilation by the same organism after, for instance, mechanical disintegration, is commonly explained by the presence of an impermeable cell wall. That such an assumption is not justified in at least one specific case is shown by the following experiments.

The distribution of citrate added to a suspension of fresh bakers' yeast was studied. One ml of 0.02 M sodium citrate was added to 20 ml of a 50 per cent (w/v) suspension of fresh or treated yeast. The suspension was shaken for 30 minutes at 0 C. The citrate content of both the supernatant and the cells was determined then after acidification and boiling. From the dilution of citrate the volume of water was calculated into which the citrate could freely permeate (citrate space). There was no evidence of citrate adsorption. A simultaneous experiment was carried out at 37 C to determine whether any citrate was metabolized. Some typical results are summarized in table 1.

The total extracellular water as determined by the distribution of chloride under identical conditions was 12.9 ml. It is clear from these results that in the fresh suspension citrate is restricted to the extracellular water. On freezing the cell becomes freely permeable to citrate but remains incapable of metabolizing it. The nonmetabolism of citrate cannot be attributed to enzyme inactivation as it is metabolized readily upon subsequent treatment of the cells with chloroform. The freezing neither alters the cell volume nor greatly affects the ability of the cell to maintain a high intracellular potassium and low sodium level against a concentration gradient. The endogenous citrate in both fresh and frozen cells is nondiffusible and is not metabolized.

TABLE 1

TREATMENT	CITRATE SPACE AT 0 C		CITRATE METABOLIZED AT 37 C
	ml	per cent of total H_2O	
			μM
Control	12.1	66	0
Cells frozen twice at −20 C	18.2	100	0.6
Cells frozen as above, then exposed to 0.1 ml $CHCl_3$	18.2	100	14.0

The conclusion can be drawn that impermeability of the cell wall is not sufficient explanation for the inability of whole yeast cells to metabolize citrate.

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THE GROWTH OF AN "OBLIGATE" THERMOPHILIC BACTERIUM AT 36 C

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Campbell and Williams (J. Bacteriol., **65,** 141, 1953; J. Bacteriol., **65**, 146, 1953) have reported on the effect of incubation temperatures on the nutritional requirements of a large number of facultative and obligate thermophilic bacteria. During the course of the investigation it was noted that one culture of *Bacillus stearothermophilus*, strain 2184-2, which was labeled as

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an obligate strain, indeed did not grow at 36 C in the usual bacteriological media such as nutrient broth (Difco), tryptose-phosphate broth (Difco), brain heart infusion (Difco), etc.; but to our surprise good growth was obtained at 36 C when the organism was inoculated into a medium of the following composition: tryptose, 2 per cent; basamin, 0.3 per cent; Na_2HPO_4, 0.25 per cent; KH_2PO_4, 0.1 per cent; NH_4Cl, 0.1 per cent, mineral supplement of Campbell and Williams, 0.1 ml per 100 ml.

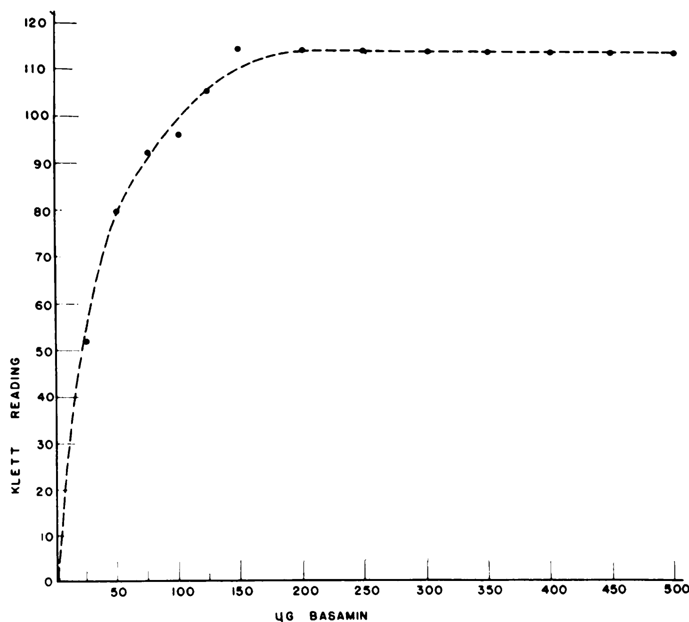

Figure 1. Assay curve for basamin factor(s) permitting growth of *Bacillus stearothermophilus*, strain 2184-2, at 36 C after 24 hr incubation.

Further study of this strain employing the synthetic medium of Campbell and Williams revealed that good growth was obtained at 45 C and 55 C while no growth occurred at 36 C. Addition of 25 mg per cent basamin to the synthetic medium gave growth at 36 C comparable to that obtained in the unsupplemented synthetic medium at 45 C and 55 C. A survey of other natural materials showed that Armour's liver L fraction and Difco's yeast extract added at the same level also allowed good growth at 36 C. It was concluded that these complex natural materials must contain some factor(s) not present in the test medium which the organism could not synthesize at 36 C, thus requiring an exogenous source before growth could occur. On the other hand, the organism was capable of synthesizing the factor(s) at 45 C and 55 C since growth occurred in the synthetic medium in the absence of basamin.

Since the test medium employed is a rather simple one when compared to the numerous synthetic media described for lactic acid and other bacteria, a large number of metabolites were tested individually and collectively to determine if any one of these (or any combination) had growth promoting activity for the organism at 36 C. The following substances were checked for activity: L-alanine, β-alanine, aspartic acid, serine, threonine, phenylalanine, tyrosine, proline, hydroxyproline, cysteine, asparagine, glutamine, glycine, taurine, adenine, guanine, uracil, xanthine, hypoxanthine, adenosine triphosphate, adenosine-3-phosphoric acid, adenosine-5-phosphoric acid, cytidine, cytosine, cytidylic acid, desoxyribonucleic acid, guanosine, guanylic acid, uridine, uridylic acid, thymine, xanthosine, vitamin B_{12}, pantethine, inositol, diphosphopyridine nucleotide, thiamin pyrophosphate, glutathione, streptogenin, ribose-5-phosphate, orotic acid, oleic acid ("tween 80"), pyridoxamine, pimelic acid, desthiobiotin, α-lipoic acid, methyl β-lipoate, citrate, lactate, pyruvate, α-ketogluta-

rate, succinate, malate, fumarate, and oxalacetate. None of these substances allowed growth of the test strain at 36 C. The ash of basamin, liver L, and yeast extract was also inactive.

An assay has been worked out with this organism employing basamin as the test material. A typical growth response curve is shown in figure 1. It may be seen that as little as 25 μg basamin per ml gives a half maximum growth response after 24 hours of incubation at 36 C. Evidence from studies now in progress to determine the identity of the factor(s) required for growth at 36 C points to the involvement of a peptide factor(s).

The findings reported here are significant in that they may lead to the discovery of other strains heretofore thought to be obligate thermophiles which in reality are facultative in character when placed in the proper nutritional environment.

CHANGE IN BACTERIAL MORPHOLOGY AS A RESULT OF LOW CONCENTRATIONS OF STREPTOMYCIN

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During the screening of phytopathogens for antibiotic susceptibility it was observed that sublethal or noninhibitory concentrations of streptomycin caused marked change in the morphology of cells of 18 to 24 hour cultures of *Erwinia amylovora.*

A brief study of this phenomenon by electron microscopy showed that following the treatment of the cells with streptomycin a series of lumps appeared in the cytoplasm (figures 1a and 2a). Similar structures have been reported in spirochetes (Wykoff, *Electron microscopy*, p. 124, figures V11–13, p. 128, figures V11–18, Interscience Publishers, N. Y., 1949) and in *Mycobacterium tuberculosis* (Knaysi, *Elements of bacterial cytology*, 2nd ed., p. 116, figure 3, Comstock Publishing Co., Ithaca, N. Y., 1951) though not in conjunction with an antibiotic.

The observation in *Erwinia amylovora* may be similar to that occurring in *Mycobacterium tuberculosis* where shrinkage of the cell wall emphasizes whatever solid material remains within the cell. In a personal communication Dr. Georges Knaysi[1] has stated that the solid constituents or "lumps" are nuclei as previously reported by him and may very well be nuclei in the *Erwinia amylovora*. Actually the "lumps" can probably be almost any solid constituent of the cytoplasm including chromatin material, lipoids, or a combination of these.

[1] Grateful acknowledgment is accorded Dr. Georges Knaysi for his comments on these micrographs.