THYMINE-REQUIRING VARIANT OF *LACTOBACILLUS CASEI*[1]

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During an investigation of characteristics associated with resistance to the folic acid antagonists in bacterial and leukemic cells (Anton and Nichol, Proc. Am. Assoc. Cancer Research, **2,** 91, 1956; Nichol, in *The Leukemias*, Academic Press, Inc., New York, p. 583, 1957), a strain of *Lactobacillus casei* was isolated which required thymine or thymidine in addition to folic acid. This variant was obtained during the daily transfer of the parent strain in synthetic medium (Flynn *et al.*, Anal. Chem., **23,** 180, 1951) to which was added pteroylglutamic acid (1 mμg per ml) and Amethopterin (Methotrexate, Lederle Laboratories, Pearl River, New York) in progressively increasing concentrations from 1 to 1000 mμg per ml of medium. After 20 transfers the resistant organism grew poorly and even in the absence of Amethopterin only slight turbidity was noted after incubation for 24 hr. Several attempts were made to stimulate the growth of the organism by alternating transfers in synthetic medium and in transfer broth containing liver extract. Good growth occurred in the crude medium. The unexpected lack of growth in the complete synthetic medium containing pteroylglutamic acid in the absence of Amethopterin led to an investigation of the nutritional requirements of this organism.

The variant, designated *L. casei*/T$^-$, did not grow despite the presence of 100 times the amount of pteroylglutamic acid or 5-formyltetrahydropteroylglutamic acid that resulted in maximal growth of the parent strain (adenine, guanine, xanthine, and uracil were present in the medium). Both strains grew readily on thymine or thymidine (5×10^{-6} M); the response of the variant was of a lower magnitude. Pteroylglutamic acid stimulated the growth of the variant only in the presence of thymine or thymidine, in contrast to the parent strain which grew maximally with pteroylglutamic acid alone (figure 1). The requirement of the variant for thymine could not be met by cytosine, deoxycytidine, 5-hydroxymethyl cytosine, uracil, or deoxyuridine in the presence or absence of folic acid. Various combinations of these compounds with vitamin B_{12} were also negative. On a modified medium lacking purines and pyrimidines, only the parent strain grew in the presence of added pteroylglutamic acid (1 mμg per ml); little or no growth was observed when thymine (5×10^{-6} M) alone was added; both strains grew well when thymine and pteroylglutamic acid were present. The normal growth of the variant in purine-free medium containing pteroylglutamic acid and thymine indicates that there is no defect in the folic-catalyzed reactions involved in the biosynthesis of purines. *L. casei*/T$^-$ has an evident requirement for thymine even in the presence of pteroylglutamic acid and purines.

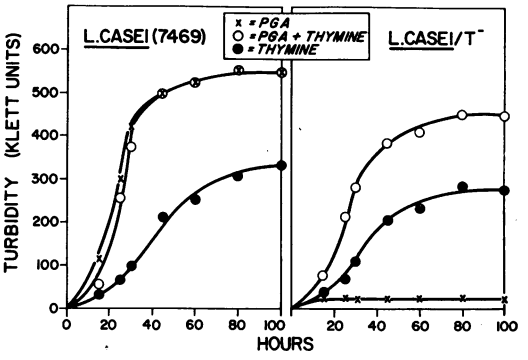

Figure 1. Response of *Lactobacillus casei* strain 7469 and a thymine-requiring variant, *L. casei*/T$^-$, to pteroylglutamic acid (PGA, 1 mμg per ml) and thymine (5×10^{-6} M). Turbidity was measured in a Klett-Summerson colorimeter using filter no. 66.

The lag in the evolution of Amethopterin-resistant cultures of *Streptococcus faecalis* in

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purine-free medium was shortened by the presence of thymine (Hakala, Prusoff, and Welch, Federation Proc., **13,** 223, 1954, and Hakala, Suomen Kemistilehti, **28,** 31, 1955). In medium containing thymine and purines sufficient for growth, *L. casei* and *S. faecalis* are insensitive to folic acid antagonists, and it is unlikely that any selection occurs unless such metabolites are limiting. It is noted that this strain, selected during the development of Amethopterin resistance, has a requirement for one important product of biosynthetic reactions involving folic acid coenzymes. A similar induction of a requirement for thymine occurred in *L. casei* during the development of resistance to pyrimethamine (Singer, Elion, and Hitchings, Bacteriol. Proc., **1958,** 127, 1958). The role of products of the inhibited reactions in the selection and metabolism of drug-resistant strains deserves detailed study.

NINHYDRIN POSITIVE SUBSTANCES IN CELL WALL HYDROLYZATES OF SOME ANAEROBIC COCCI[1]

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The isolation and chemical characterization of cell walls (Mitchell and Moyle, J. Gen. Microbiol., **5,** 981, 1951; Salton and Horne, J. Gen. Microbiol., **7,** 177, 1951; Salton, Biochem. et Biophys. Acta, **8,** 510, 1952) revealed differences sufficient for Cummins and Harris (J. Gen. Microbiol., **14,** 583, 1956) to postulate the taxonomic value of chemical analysis of cell walls of closely related bacteria. Because of equivocal taxonomy of some anaerobic micrococci and Veillonella (Langford, Faber, and Pelczar, J. Bacteriol., **59,** 349, 1950), a study was made of the content of amino acids and diaminopimelic acid in their cell walls.

The cultures studied were: a typical buccal isolate designated V-28, *Micrococcus lactilyticus* strain T12, obtained from Dr. E. L. Foubert, *Veillonella parvula* strain ATCC 10790, and *Veillonella alcalescens* strain ATCC 12641. The cultures were grown in a medium of the following composition in g per L: trypticase, 15; yeast extract, 10; NaCl, 5; K_2HPO_4, 2.5; sodium thioglycolate, 1.0; sodium lactate, final concentration 1 per cent; pH 7.2. After 16 hr at 37 C, the cells were harvested in a Sharples centrifuge, washed twice with distilled water, suspended in 0.066 M phosphate buffer pH 7.5, and disrupted in a Raytheon 10 kc magnetostrictor oscillator (0.2 g of cells per ml). The material was centrifuged 15 min at 2500 rpm to remove intact cells; the supernatant fluid was centrifuged 15 min at 10,000 rpm (Servall SS1). The sedimented cell wall fraction was successively washed, twice with distilled water, five times with buffer pH 7.5, and six times with distilled water. The sediment was suspended in distilled water and lyophilized.

TABLE 1

*Amino acids found in cell wall hydrolyzates (20 hr 6 N HCl at 105 C) of V-28, Micrococcus lactilyticus, Veillonella parvula, and Veillonella alcalescens**

Amino Acids	V-28	*M. lactilyticus*	*V. parvula*	*V. alcalescens*
Alanine	+++	+++	+++	+++
Arginine	++	++	+	++
Aspartic acid	++	++	++	++
Cysteic acid	+	0	+	+
Diaminopimelic acid	++	++	++	++
Glycine	++	+++	+++	++
Glutamic acid	+++	+++	+++	++
Lysine	+++	+++	+++	+++
Leucine + isoleucine	+++	+++	+++	+++
Phenylalanine	+	0	0	0
Proline	0	0	0	0
Serine	++	++	++	++
Threonine	++	0	0	++
Tyrosine	±	0	±	0
Valine	++	0	++	±
Hexosamine	++	0	++	±
Unidentified spot:				
No. 1	+	0	+	+
No. 2	+	+	+	0

* +++ = large, intensely colored spot on chromatogram; ++ = fairly large intense spot; + = small but definite; ± = faint spot; and 0 = no spot found.

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