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Histidine Decarboxylaseless Mutants of *Lactobacillus* 30a: Isolation and Growth Properties

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Mutants of *Lactobacillus* 30a deficient in their ability to form an inducible histidine decarboxylase (EC 4.1.1.22) were selected by plating nitrosoguanidine-treated cultures on a medium containing histidine and methyl red. Wild-type organisms produce histamine, thus raising the *p*H and forming yellow colonies; mutant colonies remain red. In the presence of added histidine, decarboxylase-producing cultures grow more heavily than mutant cultures when the initial *p*H of the growth medium is low or when the lactic acid produced lowers the *p*H to growth-limiting values. Addition of the decarboxylation products, histamine and carbon dioxide, did not favor growth in crude medium.

Many bacteria synthesize enzymes that catalyze the alpha decarboxylation of amino acids (1). Certain decarboxylases are constitutive biosynthetic enzymes that provide important intermediates for cell growth or function. Most of the known bacterial amino acid alpha decarboxylases are, however, substrate induced. Their function is unknown. In 1924 Hanke and Koessler (6) proposed that bacteria decarboxylate amino acids to prevent excessive intracellular acidity. Gale (4) suggested that the inducible decarboxylases could function by protecting the organism against an acidic environment or by producing carbon dioxide needed for carboxylation reactions, or both. These suggested functions apparently have not been tested experimentally. *Lactobacillus* 30a synthesizes inducible histidine and ornithine decarboxylases (8). In this report we describe a method for isolating mutants of *Lactobacillus* 30a which lack histidine decarboxylase and test the credibility of certain of the functions suggested for the enzyme by comparing growth habits of the mutant and wild-type strains.

Late log-phase cells (0.1 ml, 0.04-mg dry weight) grown on a single-strength crude medium (2), containing yeast extract and a casein hydrolysate as nitrogen sources, were diluted into 5 ml of Vogel-Bonner minimal medium (10). A few crystals (ca. 1 mg) of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co.) were added, and the culture was incubated at 37 C for 10 min. Then 0.1 ml of the mutagenized culture was diluted into 10 ml of crude medium, and the cells were grown overnight at 37 C. For plating, fully grown cultures ($A_{650}^{1\,cm} \sim 1.0$) were serially diluted 1 to 10^6, and 0.03 ml of the diluted suspension was spread on petri plates, each containing about 30 ml of solid medium. (Solid medium was similar to crude medium, but contained 3.8 g of casein hydrolysate, 20 g of agar, and 15 g of L-histidine per liter, and was adjusted to *p*H 5.0 with concentrated HCl.) Agar (2%) in 0.01 M ammonium acetate buffer, *p*H 5.0, containing 0.01% methyl red (Eastman Organic Chemicals) which had been liquefied in boiling water and cooled to about 45 C, was then layered over the cells. Within 1 week of plating, wild-type colonies raised the *p*H of their surroundings sufficiently to change the color of the indicator from red to yellow (Fig. 1). This *p*H change was dependent upon the presence of L-histidine in the medium and was coincident with the appearance of histamine in the agar adjacent to the colonies. Mutant colonies were unable to raise the *p*H of their surroundings and appeared red. Approximately one in a thousand of the original mutagenized cells was histidine decarboxylaseless by this criterion.

Seven mutants, whose histidine decarboxylase activities range from less than 0.5 to 5% of the wild-type activity, have been isolated by this procedure. Mutant 1 was used in the following growth experiments because it grows better than the other mutants on crude me-

FIG. 1. *Appearance of wild-type and histidine decarboxylaseless colonies of Lactobacillus 30a grown on methyl red plates. The wild-type colonies (on the right) raise the pH of their surroundings and appear yellow. The mutant colonies (on the left) cannot raise the pH of their surroundings and appear red.*

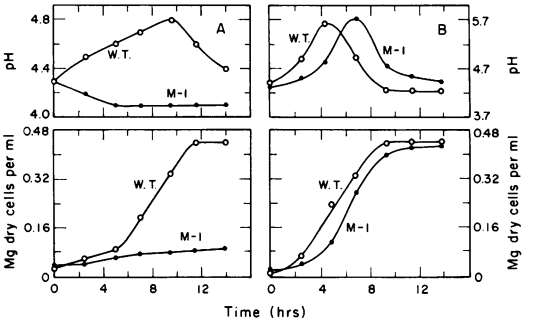

FIG. 2. *Growth and accompanying pH changes of cultures in crude medium adjusted to pH 4.3 and containing either 0.05 M L-histidine (A) or 0.05 M L-ornithine (B). W.T., Wild-type Lactobacillus 30a; M-1, histidine decarboxylaseless mutant 1. When these cultures were supplemented with 0.02 M histamine and 10% CO_2, no stimulation of growth was observed. (For growth in an atmosphere containing approximately 10% CO_2, culture flasks were placed in an 8-liter desiccator, 1.6 g of dry ice was added, the desiccator was immediately sealed, and 10% of the air was removed.) Growth in all cases was at 37 C. Wild-type cells grown in (A) had a histidine decarboxylase activity of 0.51 units per mg (dry weight), whereas cells of mutant 1 had no detectable activity. Both wild-type and mutant 1 cells grown in (B) had an ornithine decarboxylase activity of 1.0 unit per mg (dry weight).*

dium and shows no detectable histidine decarboxylase activity.

To test whether decarboxylase activity can protect *Lactobacillus* 30a against excessively acidic media, cells were grown in crude medium containing 0.05 M L-histidine overnight; 1 ml was then transferred to 30 ml of the same medium adjusted to *p*H 4.3. The wild-type organism raised the *p*H of the medium and grew well, whereas the mutant was unable to raise the *p*H of the medium and grew poorly (Fig. 2A). Addition of CO_2 and histamine (the products of the decarboxylation reaction) did not stimulate growth of the mutant. *Lactobacillus* 30a can also synthesize a substrate-induced L-ornithine decarboxylase (8). When L-ornithine was substituted for L-histidine in the media, both the mutant and the wild-type strains raised the *p*H of the media and both grew well (Fig. 2B).

Lactobacillus 30a is homofermentative and produces large amounts of lactic acid during growth (8). The optimal initial *p*H for growth on crude medium is between 5.0 and 6.5 (5); the *p*H of the culture at the end of exponential growth falls to about 4.0. Under conditions where fall in *p*H limits growth, presence of the decarboxylase with its substrate should favor growth. To test this supposition, cells were grown on double-strength crude medium initially adjusted to *p*H 5.2 and containing 0.05 M L-histidine. Double-strength medium was used so that growth would be limited by acid production and not by a requirement for nutrients. The *p*H of the mutant culture fell steadily until cell growth stopped around *p*H 4.0 (Fig. 3). The wild-type culture, however, maintained a more favorable *p*H during the

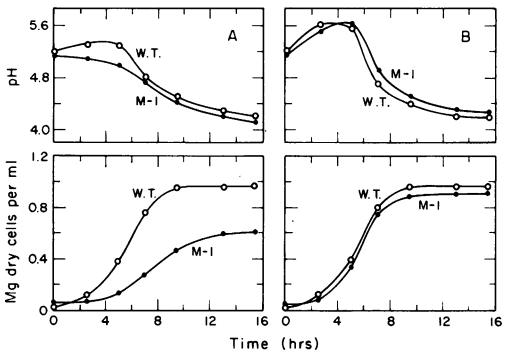

FIG. 3. *Growth and accompanying pH changes of wild-type (W.T.) and histidine decarboxylaseless mutant 1 (M-1) in double-strength crude medium (initial pH 5.2) containing either 0.05 M L-histidine (A) or 0.05 M L-ornithine (B). When these cultures were supplemented with 0.02 M histamine and 10% CO_2, no stimulation of growth was observed. Decarboxylase activities induced on the double-strength medium were the same as those in Fig. 2. Growth in all cases was at 37 C.*

greater part of its growth cycle and grew faster and to a higher, final cell density. When L-ornithine was substituted for L-histidine in the media, both mutant and wild-type cultures maintained a favorable *p*H during growth, and

the mutant grew as well as the wild-type strain. Histamine and CO_2 did not stimulate growth of the mutant on double-strength medium containing either histidine or ornithine (Fig. 3).

Under the in vitro growth conditions described here, either the extracellular or intracellular *p*H becomes growth-limiting and, in the presence of the appropriate substrate, either histidine or ornithine decarboxylase activity favored growth by restoring the *p*H to levels which permit growth. Bacteria that synthesize inducible amino acid decarboxylases have been isolated most commonly from the digestive tract of animals. The substrates of these enzymes become available mainly in the small intestine of nonruminants (3) and in the rumen of ruminants (7). These environments are relatively anaerobic and rich in CO_2 (7, 9) but are not particularly acidic (3, 7). Therefore, it appears likely from our experimental results that the inducible bacterial amino acid decarboxylases function in these environments by neutralizing intracellularly the acidic products of anaerobic carbohydrate fermentation. The possibility that these enzymes might favor growth in additional ways in special environments is, of course, not eliminated by these results.

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LITERATURE CITED

1. Boeker, E. A., and E. E. Snell. 1972. Amino acid decarboxylases, p. 217–253. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 6. Academic Press Inc., New York.
2. Chang, G. W., and E. E. Snell. 1968. Histidine decarboxylase of *Lactobacillus* 30a. II. Purification, substrate specificity, and stereospecificity. Biochemistry **7**:2005–2012.
3. Davenport, H. W. 1961. Physiology of the digestive tract. Year Book Medical Publishers, Chicago.
4. Gale, E. F. 1946. The bacterial amino acid decarboxylases. Advan. Enzymol. **6**:1–32.
5. Guirard, B. M., and E. E. Snell. 1964. Nutritional requirements of *Lactobacillus* 30a for growth and histidine decarboxylase production. J. Bacteriol. **87**:370–376.
6. Hanke, M., and K. Koessler. 1924. Studies on proteinogenous amines. J. Biol. Chem. **59**:855–866.
7. Hungate, R. E. 1966. The rumen and its microbes. Academic Press Inc., New York.
8. Rodwell, A. W. 1953. The occurrence and distribution of amino acid decarboxylases within the genus *Lactobacillus*. J. Gen. Microbiol. **8**:224–232.
9. Spencer, R. P. 1960. The intestinal tract. Charles C Thomas, Publisher, Springfield, Ill.
10. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.