

[54] LACTIC ACID BACTERIA WHICH DO NOT DECARBOXYLATE MALIC ACID AND FERMENTATION THEREWITH

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[21] Appl. No.: 539,028

[22] Filed: Oct. 4, 1983

[51] Int. Cl.⁴ C12N 1/20; C12N 15/00; C12Q 1/04; A23B 7/10

[52] U.S. Cl. 435/253; 435/172.1; 435/139; 435/34; 426/49; 426/52; 426/61

[58] Field of Search 435/34, 885, 253, 172.1, 435/139; 426/49, 52, 61

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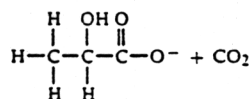
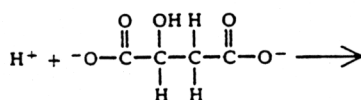
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[57] ABSTRACT

Bloating of brine fermented cucumbers can be greatly reduced by using lactic acid bacteria which do not decarboxylate malic acid and therefore do not produce carbon dioxide during fermentation. Also, certain high acid wines can be improved by fermenting fruit with bacteria which do decarboxylate malic acid.

A method has been discovered of differentiating between species of lactic acid bacteria which do and do not decarboxylate malic acid. This method comprises growing a lactic acid bacterium in a suitable malic acid-containing nutritive growth medium under conditions suitable for growth and monitoring the pH of the medium during growth. The pH will decrease only when a lactic acid bacterium is present which does not decarboxylate malic acid. When malic acid is decarboxylated, a proton is removed from the solution in accordance with the following equation:



Therefore, the pH of the medium either remains the same by neutralizing lactic acid produced by the fermentation of the carbohydrate source or increases when insufficient lactic acid is present.

The above method is particularly fast and easy for screening bacteria obtained from purposely mutated species of lactic acid bacteria. Many strains of bacteria can be tested together by streaking them on a single layer agar plate.

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LACTIC ACID BACTERIA WHICH DO NOT DECARBOXYLATE MALIC ACID AND FERMENTATION THEREWITH

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the detection and use of microbial cultures in the food processing industry.

2. Description of the Prior Art

Bloating of brine-fermented cucumbers has been attributed to the accumulation of CO₂ and other gases within the cucumbers [Fleming, H. P. and D. M. Pharr; "Mechanism for Bloat Formation in Brined Cucumbers"; *Journal of Food Science*; vol. 45, pp. 1595-1600; (1980)]. Fleming et al. ["Bloat Formation in Brined Cucumbers Fermented by *Lactobacillus plantarum*"; *Journal of Food Science*; vol. 38, pp. 499-503 (1973) and; "Carbon Dioxide Production in the Fermentation of Brined Cucumbers"; *Journal of Food Science*; vol. 38, pp. 504-506 (1973)] found that CO₂ was formed by brined cucumber tissue and by the homofermentative *Lactobacillus plantarum* during the fermentation of brined cucumbers. The combination of these two sources of CO₂ caused significant bloating even in controlled fermentations where other microorganisms were excluded. McFeeters et al. ["Malic acid as a Source of Carbon Dioxide in Cucumber Juice Fermentations"; *Journal of Food Science*; vol. 47, pp. 1862-1865 (1982) and; "Malic and Citric Acids in Pickling Cucumbers"; *Journal of Food Science*; vol. 47, pp. 1859-1861, 1865 (1982)] found that malate is the major organic acid in pickling cucumbers and that malate decarboxylation can account for most of the CO₂ produced during fermentation in cucumber juice by *L. plantarum*. Schultz and Radler ["Das 'Malatenzym' von *Lactobacillus plantarum* und *Leuconostoc mesenteroides*"; *Arch. Mikrobiol.*; vol. 91, pp. 183-202 (1973)] showed that *L. plantarum* has an active malolactic enzyme which decarboxylates malate to lactate and CO₂. Together, these results suggest that malate decarboxylation (MDC) is an important source of CO₂ in cucumber fermentations.

It would be desirable to obtain strains of lactic acid bacteria which lack the ability to produce CO₂ from malate, but which retain desirable characteristics for use in cucumber fermentations. However, a simple selection system is not available for rapid screening of strains or mutants of lactic acid bacteria which do (MDC+) or do not (MDC-) decarboxylate malic acid. Several methods have been used to test lactic acid bacteria for their ability to degrade malate. These entailed using tubed media with agar seals and observing CO₂ production from malate [Keddie, R. M.; "The Properties and Classification of Lactobacilli Isolated from Grass and Silage"; *Journal of Applied Bacteriology*; vol. 22, pp. 403-416 (1959), and; Whittenbury, R.; "The Differentiation of *Streptococcus faecalis* and *S. faecium*"; *Journal of General Microbiology*; vol. 38, pp. 279-287 (1965)], observing a rise in pH in tubed media containing malate [Whittenbury, R.; "The Differentiation of *Streptococcus faecalis* and *S. faecium*"; *Journal of General Microbiology*; vol. 38, pp. 279-287 (1965)], and assaying for the disappearance of malate and the production of lactic acid with paper chromatography [Chalfan et al.; "Isolation and Characterization of Malolactic Bacteria from Israeli Red Wines"; *Journal of Food Science*; vol. 42, pp. 939-943 (1977) and; Van de Westhuizen et al.; "Comparison of Procedures for Isolation of Malolactic Bac-

teria from Wine"; *American Journal of Enol. Vitic.*; vol. 32, pp. 168-170 (1981)]. None of the above methods would be practical for screening of large numbers of colonies for MDC+ or MDC- strains. Recently, Subden et al. ["An L-lactic Acid Dehydrogenase-Based Method for Detecting Microbial Colonies Performing a Malolactic Fermentation"; *Canadian Journal of Microbiology*; vol. 28, pp. 883-886 (1982)] introduced a plating medium for detecting microbial colonies performing a malolactic fermentation. The system is based upon the enzymatic detection of L-lactate, the decarboxylation product of L-malate. However, the method would only work with species that do not produce L-lactic acid from glucose, e.g., *Leuconostoc*. Glucose is necessary in the medium as an energy source since the malolactic reaction does not yield energy for growth [Kandler et al.; "Zur Frage de Beeinflussung der Glucosevergarung Durch L-Malat bei *Leuconostoc mesenteroides*"; *Arch. Mikrobiol.*; vol. 90, pp. 65 (1973)]. *Lactobacillus plantarum* produces DL-lactic acid from glucose [Buchanan et al. (eds.); *Bergey's Manual of Determinative Bacteriology*; 8th ed., p. 585, The Williams & Wilkins Co., Baltimore, MD (1974)], which would result in a false positive reaction on Subden's medium if the strain was MDC-.

Several other media for differential growth or selection of lactic acid bacteria have been developed. For example, LBS (Rogosa's) medium [Rogosa et al.; "A Selective Medium for the Isolation and Enumeration of Oral and Fecal Lactobacilli"; *Journal of Bacteriology*; vol. 62, pp. 132-133 (1951)] is used for the selective enumeration of lactobacilli. Kempler and McKay ["Improved Medium for Detection of Citrate-Fermenting *Streptococcus lactis* sub. sp. *diacetylactis*"; *Applied and Environmental Microbiology*; vol. 39, pp. 926-927 (1980)] developed a medium for detection of citrate-fermenting *Streptococcus lactis* sub. sp. *diacetylactis*. None of these media, however, are suitable for the differential selection of lactic acid bacteria that do not produce carbon dioxide from malic acid.

There are other areas in which a method of differentiating between MDC+ and MDC- strains of lactic acid bacteria would be useful. It is desirable to reduce acidity in certain high acid wines (Van de Westhuizen, supra). This could be accomplished by selecting only MDC+ lactic acid bacteria.

Also in the fermentation of silage for animal feed, pH should be reduced for purposes of preservation [*The Biochemistry of Silage*; P. McDonald (ed.); p. 131, John Wiley and Sons, New York (1981)]. For this, an MDC- strain of lactic acid bacteria could be selected.

SUMMARY OF THE INVENTION

We have discovered a method of differentiating between species of lactic acid bacteria which do and do not decarboxylate malic acid. This method comprises growing a lactic acid bacterium in a suitable malic acid-containing nutritive growth medium under conditions suitable for growth and monitoring the pH of the medium during growth. The pH will decrease only when a lactic acid bacterium is present which does not decarboxylate malic acid. When malic acid is decarboxylated, a proton is removed from the solution in accordance with the following equation:

