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CHAPTER 22

Modification of Lactic Acid Bacteria for Cucumber Fermentations: Elimination of Carbon Dioxide Production from Malate[a]

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Malic acid, a natural constituent of cucumbers, undergoes decarboxylation (malate → lactate + CO_2) by naturally occurring lactic acid bacteria and commercial lactic starter cultures. The CO_2 produced by this reaction is of sufficient quantity to cause the fermenting cucumbers to bloat (hollow fruit), resulting in an economic loss. Research is under way to develop lactic starter cultures for cucumber fermentations that lack the ability to decarboxylate malic acid but retain desirable fermentation traits. A selection system has been developed to isolate *Lactobacillus plantarum* mutants that have lost the ability to decarboxylate malic acid. Mutant cultures obtained by this system are presently being evaluated in experimental cucumber fermentations for use as starter cultures.

INTRODUCTION

Malic acid, a dicarboxylic organic acid, is present in many types of plant material, including apples, grapes, cereal grains, legumes, and grasses. Malolactic enzyme, demonstrated to be present in most lactic acid bacteria (Caspritz and Radler 1983) but not in other bacteria, catalyzes the reaction: 1 malate + H^+ → 1 lactate + 1 CO_2. The importance of this reaction during the processing of wine has been well documented (Kunkee 1967). Decarboxylation of malate in wines of high acidity is generally desirable in order to reduce the acidity. In cucumber fermentations, decarboxylation of malate is not desirable because the carbon dioxide produced can cause cucumber bloater damage.

Bloating of cucumbers was a major defect in commercially fermented cucumbers for many years (Jones et al. 1941). Studies of the bloating process pointed to the fact that it was associated with the production of CO_2 gas by microorganisms in the fermentation brines. Early work pointed to yeasts as the

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major source of CO_2 production (Etchells and Bell 1950). Later evidence showed that in low-salt conditions the heterofermentative lactic acid bacterium, *Lactobacillus brevis,* could also produce sufficient CO_2 to cause bloating (Etchells et al. 1968).

The results of those studies suggested that bloating could be prevented by limiting the microflora in cucumber fermentations to homofermentative lactic acid bacteria, such as *Lactobacillus plantarum* and *Pediococcus pentosaceus,* which were thought to be unable to produce significant amounts of CO_2. However, Fleming et al. (1973a; 1973b) showed that CO_2 was produced by both the brined cucumbers themselves and by the homofermentative lactic acid bacteria used in controlled cucumber fermentations. The combination of these two sources of CO_2 was sufficient to cause significant cucumber bloating damage. These results led to successful efforts to control the CO_2 concentrations in cucumber fermentation by gas purging the brines to remove enough of the CO_2 produced by the fermentation to prevent cucumber bloating. Nitrogen purging of fermentation tanks (Etchells et al. 1973; Fleming 1979) has been widely adopted in the pickling industry and has greatly reduced the incidence of commercially significant bloating.

Recent work in this laboratory has led to the recognition that the major mechanism for CO_2 production by homofermentative lactic acid bacteria in cucumber fermentation is the decarboxylation of malic acid to lactic acid and CO_2. The intent of this paper is to review recent research conducted by our laboratory as it relates to the significance of malic acid in cucumber fermentations and current efforts to develop starter cultures that lack the ability to decarboxylate malic acid.

Discussion

Malic acid and CO_2 production. McFeeters et al. (1982a) found that malic acid is the major organic acid present in the immature cucumber fruit used for commercial processing. The concentration of malic acid in six different cultivars ranged from 14.2 to 23.4 m*M*. In cucumber juice fermentations with *L. plantarum* (McFeeters et al. 1982b), the CO_2 production was directly proportional to the amount of malic acid that was added to the juice (Fig. 1). The relationship between malic acid decarboxylation, CO_2 production, and bloating in fermented cucumbers was demonstrated using strains that do and do not decarboxylate malate (McFeeters et al. 1984). Fig. 2 illustrates this relationship. CO_2 production by the brined cucumbers, not related to malate decarboxylation, was 12.5 m*M* when fermented by *L. plantarum* 965, a nonmalolactic strain. Fermentations conducted with *L. plantarum* WSO, a decarboxylating strain, developed increasing concentrations of CO_2 when additional amounts of malate were added to the fermentations. Fig. 3 shows the relationship of CO_2 production to bloating. Bloater index values indicate the percentage of product damage that renders the pickle unusable for processed dill slices (Fleming et al. 1977). The cucumbers fermented with strain 965 showed only slight bloating. The data indicated that if malic acid decarboxylation could be prevented, bloating could almost be eliminated in pure culture fermentations using homolactic starter cultures. This

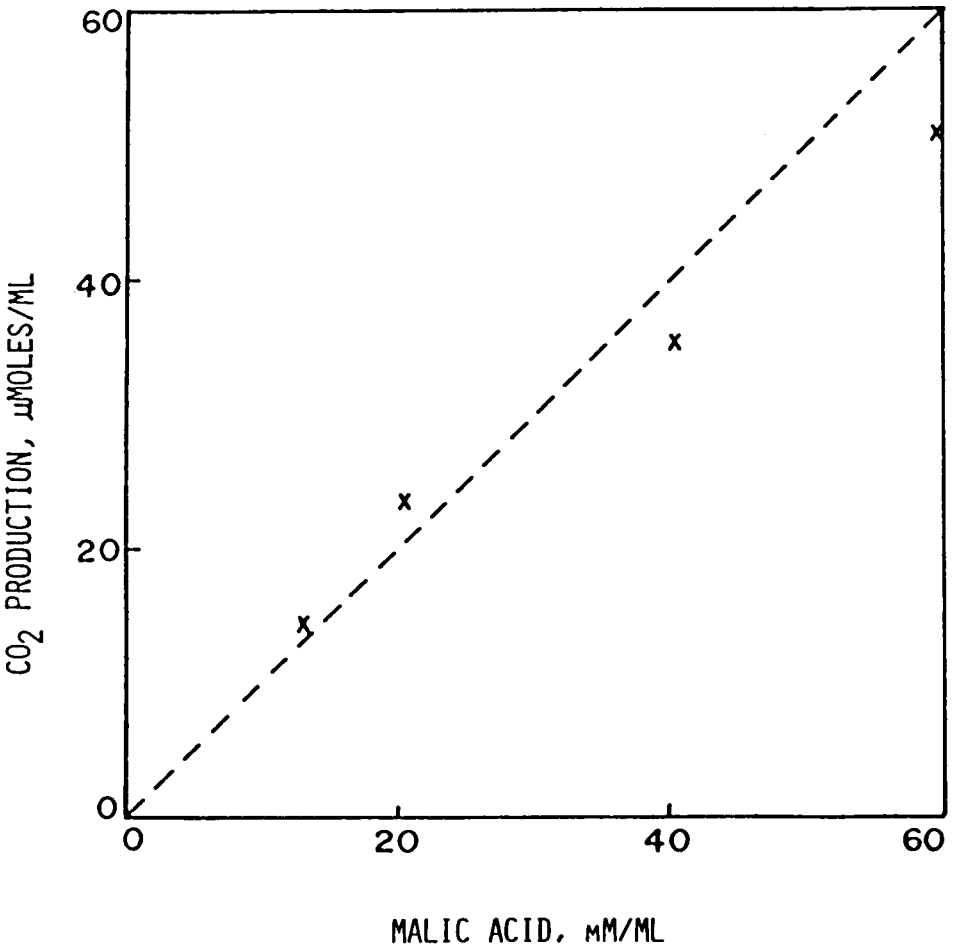

FIG. 1. CO_2 production in cucumber juice supplemented with 6.0% NaCl and malic acid. Determinations were made after incubation at 30 C for 7 d. The dashed line shows the expected CO_2 production if 1 mol of CO_2 were produced per mol of malic acid. From McFeeters et al. (1982b).

may have significant economic advantages since mechanical purging of dissolved CO_2 in commercial brine tanks currently is necessary to ensure bloater-free fermentations. Elimination of purging requirements would result in savings to the industry.

Nonmalolactic cultures. The inability of lactic acid bacteria to decarboxylate malate is rare, as indicated by the surveys of McFeeters et al. (1984) and Caspritz and Radler (1983). With this in mind, Daeschel et al. (1984a) developed nonmalolactic cultures by mutating existing strains of *L. plantarum* containing desirable fermentation characteristics so they no longer possessed malolactic activity. A differential medium designated "MD medium" (Table 1) containing malic acid, glucose, and pH indicator (bromocresol green) as key components was developed to distinguish between malolactic and nonmalolactic strains of lac-

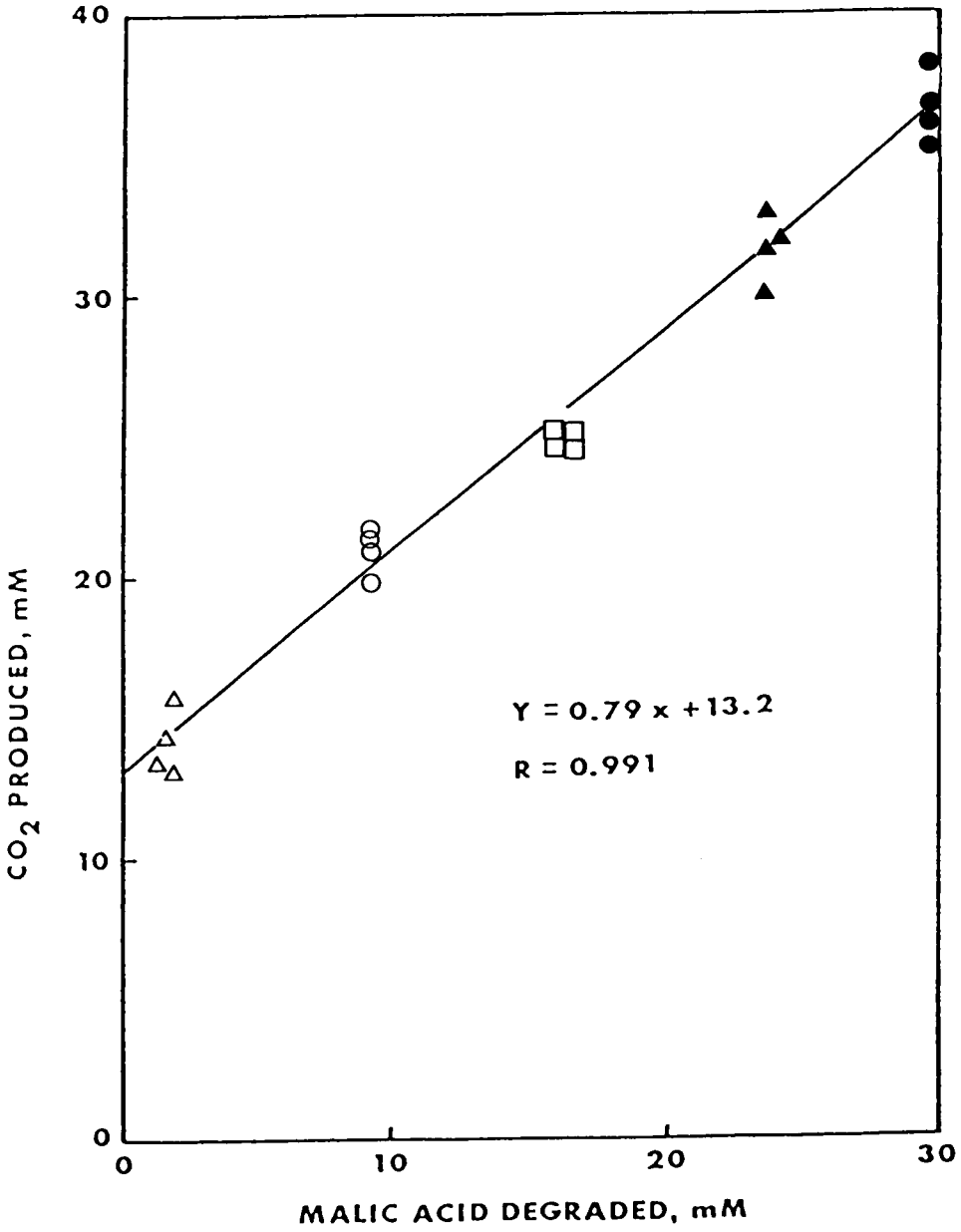

FIG. 2. Relationship between malic acid degradation and CO_2 formation in cucumber fermentations. Cucumbers without added malic acid were fermented with *L. plantarum* 965 (△) and WSO (○). Cucumbers supplemented with 7 (□), 14 (▲), and 21 m*M* (●) malic acid were fermented with *L. plantarum* WSO only. From McFeeters et al. (1984).

tic acid bacteria (Table 2). The differential ability of the medium is based upon the fact that when malate is decarboxylated, there is uptake of a proton. Thus, a lactic acid bacterium that decarboxylates malate will neutralize the lactic acid

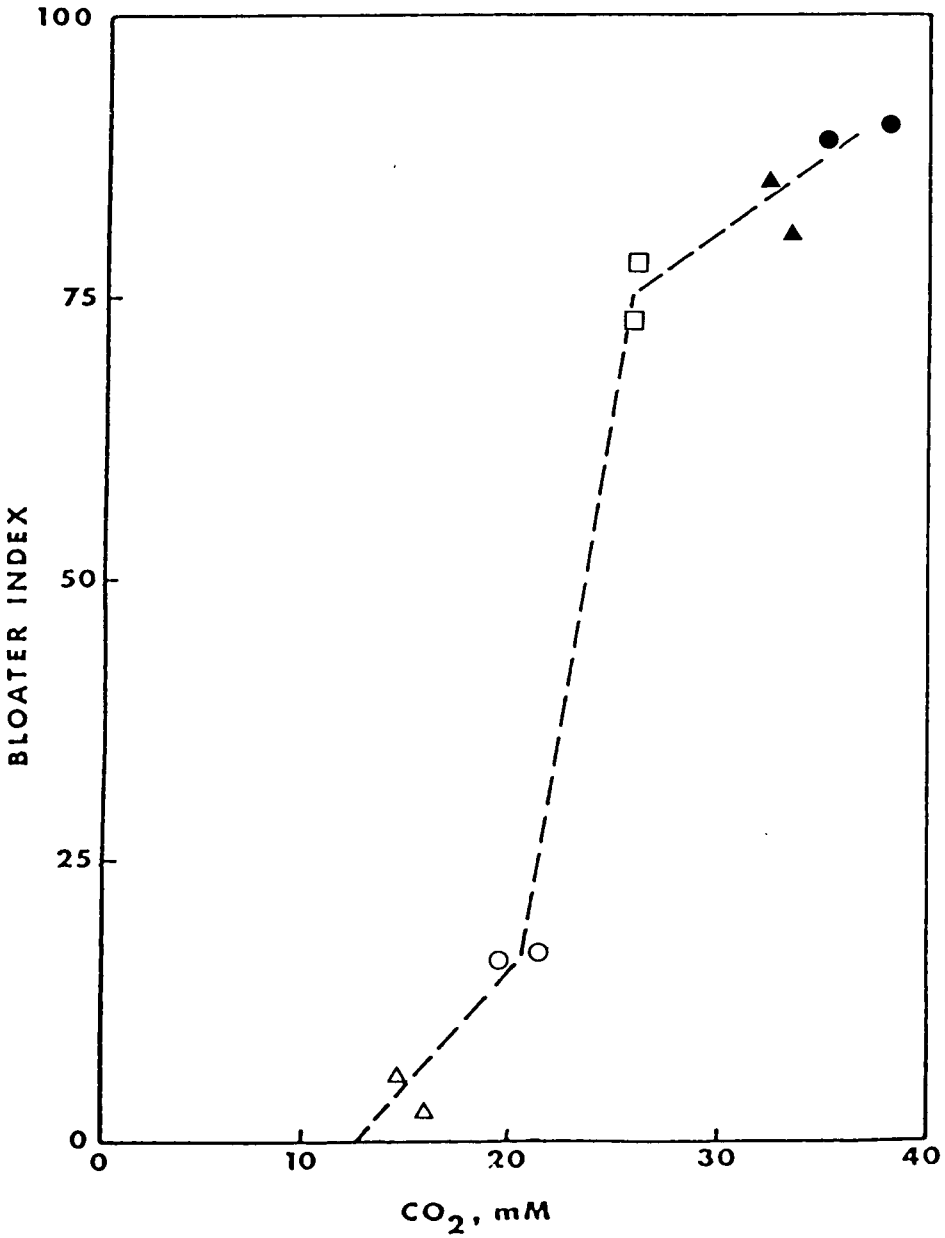

FIG. 3. Relationship between CO_2 production and bloating. Cucumbers without added malic acid were fermented with *L. plantarum* 965 (△) and WSO (○). Cucumbers supplemented with 7 (□), 14 (▲), and 21 m*M* (●) malic acid were fermented with *L. plantarum* WSO only. From McFeeters et al. (1984).

produced from glucose so the pH will not decrease and the medium will remain dark blue. Strains that do not decarboxylate malate will decrease the pH of the medium because the lactic acid from glucose will not be neutralized. The effect

will be that the medium will become yellow-green in color. This differential system was used to detect nonmalolactic mutants of *L. plantarum* that had been mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine. The strains giving acid reactions (Table 3) did not produce significant amounts of CO_2, indicating a loss in ability to decarboxylate malate.

TABLE 1. *Formulation and preparation of "MD medium" for the detection of strains and mutants of lactic acid bacteria that do and do not decarboxylate malic acid*[a]

Component	Manufacturer	Amount/Liter
L-Malic acid	Sigma	20 g
Trypticase	BBL	10 g
D(+)Glucose	Sigma	5 g
Casamino acids	Difco	3 g
Phytone	BBL	1.5 g
Yeast extract	Difco	1 g
Tween 80	Atlas	1 g
Bromocresol green	Fisher	20 ml[b]
Agar (when desired)	Difco	20 g

[a] Adjust pH to 7.0 with 10 N KOH. Autoclave at 15 psi for 15 min. Can be stored at room temperature. From Daeschel et al. (1984a).
[b] Stock solution (solubilize 0.1 g in 30 ml of 0.01 N NaOH).

TABLE 2. *Reaction of lactic acid bacteria in MD medium after incubation at 30 C for 1 wk*

Bacterial Strain	pH Reaction	Color	Ability to Decarboxylate Malic Acid
Pediococcus cerevisiae 61	8.58	Blue	+
Pediococcus cerevisiae 39	8.48	Blue	+
Leuconostoc paramesenteroides NCDO 803	8.34	Blue	+
Leuconostoc mesenteroides 43	5.53	Green	–
Leuconostoc mesenteroides LC-33	5.54	Green	–
Leuconostoc dextranicum ATCC 19255	5.52	Green	–
Leuconostoc oenos PSU-1	6.82	Blue	+

From Daeschel et al. (1984b).

TABLE 3. *pH Reaction, CO_2 produced and reducing sugar concentration in MD broth fermented by strains and mutants of* L. plantarum *at 30 C for 7 d*

Strains	pH	CO_2 (mg/100 ml)	% w/v Reducing Sugar	Broth Color
965	5.19	16.9	0.02	Lt. green
WSO	6.82	596.6	0.02	Dk. blue
WSO-M-34[a]	5.28	25.3	0.02	Lt. green
WSO-M-35[a]	5.23	18.9	0.02	Lt. green
Uninoculated control	6.96	15.5	0.48	Dk. blue

From Daeschel et al. (1984a).
[a] Mutants of WSO.

Although mutagenesis is nonselective in its action, we were able to obtain mutants that retained the vigor and desirable fermentation characteristics of the parent and that have not reverted back to the malolactic phenotype. Mutant strains were tested (Daeschel et al. 1984b) for their ability to produce CO_2 from cucumber juice containing the natural malate concentration or additional malate (Table 4). The mutant *L. plantarum* WSO M35 did not produce significant amounts of CO_2 from the cucumber juice with or without additional malate.

TABLE 4. *CO_2 produced in filter-sterilized cucumber juices fermented by strains and mutants of* L. plantarum *at 30 C for 7 d*

Strains	CO_2 mg/100 ml	
	Cucumber Juice[a]	Cucumber Juice with Added Malic Acid[b]
965	22.96	22.85
WSO	98.91	294.5
WSO-M-35	35.23	33.58
Uninoculated control	19.85	6.31

From Daeschel et al. (1984b).
[a] Contains 20 m*M* malic acid.
[b] Additional malic acid added to give a 70 m*M* concentration.

Conclusions

An understanding of the mechanisms of cucumber bloater damage has provided additional parameters on which to base the development of starter cultures for brined cucumbers. Prevention of malate decarboxylation during fermentation by using selected starter strains should reduce the need for mechanical purging systems now employed commercially to prevent bloater damage. Current research (USDA, North Carolina) is directed toward the pilot-scale level using whole cucumbers to determine if the nonmalolactic mutants can successfully mediate bloater-free fermentations.

Acknowledgments

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