APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1992, p. 3845–3849
0099-2240/92/123845-05$02.00/0

Vol. 58, No. 12

Competitive Growth of Genetically Marked Malolactic-Deficient *Lactobacillus plantarum* in Cucumber Fermentations

FRED BREIDT AND HENRY P. FLEMING*

Food Fermentation Laboratory, Agricultural Research Service, U. S. Department of Agriculture, and North Carolina Agricultural Research Service, Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695-7624

Received 22 May 1992/Accepted 4 September 1992

Procedures were developed for the differential enumeration of an added strain of *Lactobacillus plantarum* and indigenous lactic acid bacteria (LAB) during the fermentation of brined cucumbers. The added strain was an *N,N*-nitrosoguanidine-generated mutant that lacked the ability to produce CO_2 from malic acid (MDC^-). The MDC^- phenotype is desirable because CO_2 production from malic acid decarboxylation has been shown to contribute to bloater formation in fermented cucumbers. A basal medium containing malic acid and adjusted to pH 4.0 permitted growth of indigenous LAB (predominantly MDC^+), but not growth of the added MDC^- culture. Transformation of the MDC^- culture by electroporation with cloning vector pGK12 conferred chloramphenicol resistance, which permitted selective enumeration of this culture. The reversion frequency of the MDC^- mutation was determined by a fluctuation test to be less than 10^{-10}. The level of retention of plasmid pGK12 was greater than 90% after 10 generations in cucumber juice medium at 32°C. With the procedures developed, we were able to establish the ratio of MDC^- to MDC^+ LAB that results in malic acid retention in fermentations of filter-sterilized cucumber juice and unsterilized whole cucumbers under specified conditions.

Most commercial cucumber fermentations result from growth of indigenous lactic acid bacteria (LAB). Bloater damage, softening, and other spoilage problems can result from the presence of indigenous microorganisms, including various species of LAB (4). *Lactobacillus plantarum* has been suggested as the preferred species of LAB for possible use as a brine starter culture because of its relatively high acid tolerance, rapid growth, and inability to produce CO_2 from hexoses.

McFeeters et al. (14) showed that CO_2 production by *Lactobacillus plantarum* in cucumber juice was due to decarboxylation of malic acid, a natural component of cucumbers, by the malolactic reaction. It was demonstrated that the CO_2 produced by *Lactobacillus plantarum*, in addition to that produced by the cucumber tissue, was sufficient to cause bloater formation in fermented cucumbers (13).

Daeschel et al. (2) developed a procedure for isolating mutants of selected *Lactobacillus plantarum* strains that do not decarboxylate malic acid (MDC^-). When some of these strains were evaluated for use as starter cultures, they exhibited longer lag and generation times than their parent strains (11). Such cultures may prove to be useful in cucumber fermentations, depending upon the numbers of indigenous LAB with which they must compete. No simple, rapid method has been described previously for the differential enumeration of indigenous MDC^+ and added MDC^- strains of LAB.

Previously, streptomycin resistance was used as a marker to follow the growth of *Lactobacillus plantarum* WSO (MDC^+) inoculated into cucumber fermentations (5, 10). Naturally occurring streptomycin-resistant mutants were selected by plating the organisms onto media containing increasing concentrations of streptomycin. However, the relatively small numbers of indigenous MDC^+ LAB could not be selectively enumerated in the presence of the added culture.

The recent advent of electroporation provides a simple and rapid method for transforming LAB (9). Foegeding and Stanley (6) used electroporation to introduce plasmid pGK12 into *Listeria innocua*. This plasmid confers resistance to chloramphenicol and erythromycin, and the transformed *Listeria innocua* culture was proposed as a useful indicator organism to study lethality of thermal processes, having the advantage of easy selection among a large, complex background microflora (6). Foegeding et al. (7) used a similar method to enumerate a pGK12-transformed strain of *Listeria monocytogenes* in dry fermented sausage.

The objectives of this study were to develop practical procedures for enumerating an MDC^- starter culture of *Lactobacillus plantarum* and the indigenous LAB (MDC^+) during cucumber fermentation. We found that an acidified medium supplemented with malic acid permitted growth of indigenous MDC^+ LAB but not growth of an added MDC^- strain. The use of a pGK12-marked MDC^- mutant strain permitted selective enumeration of this strain on chloramphenicol-containing medium. The effectiveness of the enumeration system was demonstrated by the growth of mixtures of MDC^+ and MDC^- strains in sterile cucumber juice and by competition of the MDC^- strain with indigenous MDC^+ LAB in the fermentation of whole cucumbers.

MATERIALS AND METHODS

Strains. *Lactobacillus plantarum* MOP3 (MDC^+) and its *N,N*-nitrosoguanidine-generated mutant, strain MOP3-M6 (MDC^-), have been described elsewhere (2, 5, 11). Strains MOP3CE and M6CE were constructed in this study by electroporation of strains MOP3 and MOP3-M6 with antibiotic plasmid pGK12 (8). Strain WSO-MU8, and MDC^- mutant of *Lactobacillus plantarum* WSO, was obtained from the U. S. Food Fermentation Laboratory Culture Collection,

* Corresponding author.

Raleigh, N.C. Strain WSO-MU8 was generated previously by *N,N*-nitrosoguanidine mutagenesis (1).

Media. MRS agar (MRS broth [Difco Laboratories, Detroit, Mich.] containing 1.5% agar) was supplemented with 0.005% sodium azide (Fisher Scientific Co., Fair Lawn, N.J.) or 0.01% cycloheximide (Sigma Chemical Co., St. Louis, Mo.). Cucumber juice broth was prepared by extracting juice from fresh cucumbers, diluting it to 60% (vol/vol) (15), and then supplementing it with 53 mM acetic acid, 18 mM $Ca(OH)_2$, and 2% NaCl; the pH was adjusted to 4.75. Malate selective (MS) agar contained (per liter) 5 g of glucose (Sigma), 20 g of L-malate (Sigma), 6 g of Casamino Acids (Difco), 6 g of yeast extract (Difco), 1 ml of Tween 80 (Sigma), 100 ml of a mineral salts supplement (containing [per 100 ml] 0.6 g of KH_2PO_4, 0.45 g of KCl, 0.13 g of $CaCl_2 \cdot 2\,H_2O$, 0.13 g of $MgSO_4$, 0.01 g of $MnSO_4$) (Sigma), and 16 g of agar. All of the medium components except agar and mineral salts were prepared in volumes of 500 ml (one-half the final volume), and the pH was adjusted to 4.0. The resulting basal medium broth was then filtered through a 0.22-μm-pore-size sterile filter; 16 g of agar in 400 ml of water and 100 ml of mineral salts supplement were autoclaved separately. All medium components were then equilibrated at 50°C and mixed, and the mixtures were poured into petri plates.

Brining procedures. Washed size 2B (diameter, 3.5 to 3.8 cm) cucumbers from commercial sources were brined in 19-liter plastic pails; the brine was prepared as described by Fleming et al. (5). All brine preparations contained 18 mM $Ca(OH)_2$, 53 mM acetic acid, and 2% NaCl (upon equilibration of the brine with the fruit). The initial pH of the brine was 4.75. All fruit were of good quality and free of obvious mechanical damage. A pack-out ratio (ratio of weight of cucumbers to volume of brine) of 55:45 was used. For inoculated brine preparations the starter culture was added directly to the brine immediately prior to brining. The brine preparations were purged with N_2 at a rate of 25 ml/min. After the pails were opened and sampled, the flow rate for purging was increased to 75 ml/min for 1 h to remove O_2 and to prevent film yeast growth.

Assays. Samples were removed aseptically from cucumber juice or fermentation brine and diluted in sterile saline for plating. Cucumber samples were removed from fermentation pails with sterile forceps and washed to remove the brine; two cucumbers were removed per sample and were washed in a sterile breaker with 5 volumes of sterile saline. The beaker was covered and placed on a shaker table at 100 rpm for 2 min. After three washes, the cucumbers were aseptically homogenized in a Waring blender with 2 volumes of saline. The resulting homogenate was filtered through sterile, Whatman no. 1 filter paper in a syringe filter, and the filtrate was plated onto selective media or prepared for high-performance liquid chromatography analysis.

Aqueous samples were prepared for high-performance liquid chromatography analysis by filtration through a 0.22-μm-pore-size syringe filter to remove suspended particulate matter. Organic acid concentrations were determined by the method of McFeeters (12), using a Phenomenex Resex H^+ anion-exchange column (30 cm by 7.8 mm; Phenomenex, Rancho Palos Verdes, Calif.). The solvent for the column, 1.6 mM heptaflourobutyric acid (Aldrich Chemical Co., Milwaukee, Wis.), was run through the column at a flow rate of 0.7 ml/min, and the column temperature was 65°C. Samples were injected with a model 7125 injector (Rheodyne, Cotati, Calif.) equipped with a 10-μl injection loop. The organic acids eluted from the column were detected with a model II conductivity detector (Dionex Corp., Sunnyvale, Calif.) supported by a Dionex model AMMS-II suppressor cell. The suppressor cell used 5 mM tetrabutylammonium hydroxide (Aldrich) that was pumped at a rate of 5 ml/min with positive N_2 pressure. The conductivity detector was set up for a full-scale range of 30 μS. Data were collected with a model Chrom-1AT chromatography acquisition board (Galactic Industries Corp., Salem, N.H.). The peak areas were determined with LabCalc software (Galactic) that was run on a model 486/25C computer (Gateway 2000, Sioux City, S.D.).

Electroporation. Strains MOP3 and MOP3-M6 were transformed with plasmid pGK12 by using an electroporation apparatus (GIBCO BRL Life Technologies, Inc., Grand Island, N.Y.) equipped with an accompanying voltage booster. The method of Luchansky et al. (9) was modified as described below. MRS broth (20 ml) was inoculated to an A_{600} of 0.05 (approximately 5×10^6 CFU/ml) by using a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). The cells were grown to an A_{600} of 1.3 (approximately 2×10^9 CFU/ml), washed three times in 0.5 volume of 0.3 M sucrose–1 mM $CaCl_2$, and then resuspended in 0.1 volume of 1 M sucrose–3 mM $CaCl_2$. A DNA solution (1 μl) containing approximately 0.5 μg of pGK12 in water was added to 25 μl of cell suspension for electroporation. The cell-DNA mixture was electroporated by using a 330-μF capacitance setting (setting on the voltage booster, 4,000 Ω). The capacitor was discharged at 300 V (with the fast charge and low Ohm switch settings) to give 12,000 V/cm across the 0.15-cm cuvette gap with the voltage booster. Following electroporation, the cells were plated directly onto MRS agar plates containing 6 μg of chloramphenicol per ml.

Plasmid stability. Strain M6CE was grown in sterile cucumber juice medium at 32°C and was transferred daily for 14 days by using 1% inocula. Cells were plated at each transfer in duplicate onto chloramphenicol-containing and nonselective MRS agar plates to determine the percentage of cells that retained the plasmid. The regression line and the 95% confidence limits for the data were determined by using SAS software (SAS Institute, Cary, N.C.).

Fluctuation analysis. Strain M6CE was inoculated into 20 1-ml portions of cucumber juice at a concentration of 10^3 CFU/ml. Cultures were grown for approximately 36 h at 32°C until maximum turbidity was reached, giving 2×10^9 CFU/ml; this required 21 generations. The 20 1-ml cultures were then harvested; each culture was resuspended in 0.1 ml of sterile saline, and the entire 0.1 ml was plated onto an MS agar plate. The MS plates were incubated at 32°C for 5 days. To estimate the reversion frequency (μ), we calculated the probability (P) that no revertant would be found on 20 plates (16) as follows: $P = (1 - \mu)^{N_0} \times (1 - \mu)^{2N_0} \times \ldots \times (1 - \mu)^{2^{T-1}N_0}$, and therefore, $P = (1 - \mu)^{(2^T - 1)N_0}$, where N_0 is the number of cells in the initial population (10^3 CFU/ml) and T is the number of generations (21 generations) required for that population to grow to a maximum cell density of 2×10^9 CFU/ml.

Plating efficiency. Strain MOP3 was grown overnight in MRS medium to a density of approximately 2×10^9 CFU/ml. Comparable dilutions were plated onto 10 plates containing MS medium and 10 plates containing MRS medium. After 36 h of incubation, the numbers of colony-forming units were determined, and the mean was calculated for each set of 10 plates. The plating efficiency was defined as the number of cells growing on MS medium compared with the number of cells growing on MRS medium.

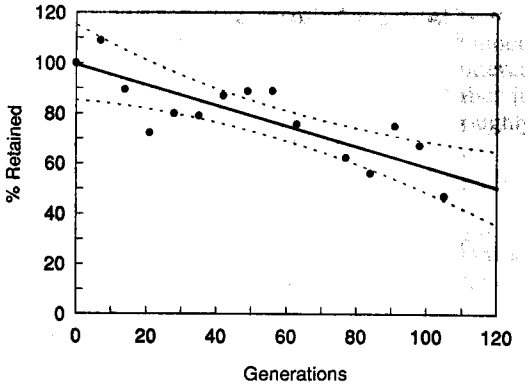

FIG. 1. Stability of pGK12 in strain M6CE grown in cucumber juice. The percentage of cells that retained the plasmid was determined from plate counts on MRS agar with and without chloramphenicol, as described in Materials and Methods. The dashed lines indicate the 95% confidence limits of the regression (solid line).

RESULTS

Plating efficiency on MS medium. To determine the plating efficiency of strain MOP3 on MS medium, cells were plated onto MS and MRS media. The average colony counts of strain MOP3 (corrected for dilution) were 4.8×10^9 CFU/ml on MRS medium and 5.1×10^9 CFU/ml on MS medium, giving a plating efficiency of approximately 100%. Colonies on MS medium were somewhat smaller than colonies on MRS medium at 36 h after plating. Malolactic mutant strains of *Lactobacillus plantarum*, including strains MU8, MOP3-M6, and M6CE, were completely inhibited on MS medium.

Stability of pGK12 in strain M6CE. Plasmid pGK12 is a broad-host-range vector developed for lactic streptococci by Kok et al. (8). This plasmid confers chloramphenicol and erythromycin resistance in gram-positive hosts and had been transformed previously into strain MOP3 (8). More than 90% of the cells retained the plasmid for 10 generations (Fig. 1), the expected number of generations for a culture inoculated at a concentration of 10^6 CFU/ml into a cucumber fermentation before the maximum cell density of 10^9 CFU/ml is reached. No apparent differences were observed in the growth rates of MDC^- mutant MOP3-M6 and its pGK12 transformant, strain M6CE, as determined spectrophotometrically in cucumber juice medium (data not shown).

Stability of the malolactic mutation. To determine the stability of the malolactic mutation, a fluctuation test was employed. Colonies which had reverted to the parental malolactic phenotype (MDC^+) grew on MS medium, while colonies that retained the MDC^- phenotype did not grow. The plates were incubated at 32°C for 5 days to ensure that revertants would be detected. No revertants were observed on the MS plates; however, on control plates on which strain MOP3 (MDC^+) cells were added to a turbid 1-ml culture of strain MOP3-M6 at a concentration of 10 CFU/ml, the parent cells were detected (data not shown). If the reversion frequency is 10^{-10}, the probability of seeing no revertants in one 1-ml culture (P) is 0.8108, and the probability of seeing no revertants in 20 independent cultures (P^{20}) is 0.0151. Therefore, there is $1 - P^{20}$ (0.985 or 98.5%) chance that the reversion frequency for the MDC^- phenotype is less than 10^{-10}.

Competitive growth of strains M6CE and MOP3 in cucumber juice. Having established the stability of the plasmid marker and the stability of the malolactic mutation, we wished to determine how strain MOP3 and its MDC^- mutant, strain M6CE, would grow when they were in competition for the same nutrients. In addition, we were interested in defining the predominance of the malolactic mutant strain in terms of malate usage in the mixed culture.

Cucumber juice prepared as described in Materials and Methods contained 10 to 20 mM malic acid. At an initial strain MOP3 (MDC^+) concentration of 3.2×10^3 CFU/ml (Table 1, culture 4) or higher, no residual malate remained in cucumber juice 10 days after inoculation. At an initial MDC^+ strain concentration of 3.2×10^2 CFU/ml (culture 3) or lower, 58 to 68% of the initial concentration of malic acid remained after 5 months. Only about 71% residual malic acid occurred at this time in the culture containing only the MDC^- mutant (culture 1). Apparently, the mutant metabolized a small fraction of the malic acid. However, only 86% of the malic acid was retained in uninoculated juice (culture 8), so malate stability may have been influenced by nonmicrobial factors.

Competitive growth of strains M6CE and MOP3 in cucumber fermentations. We wished to extend our results with cucumber juice and pure cultures to an actual cucumber

TABLE 1. Malate retention in mixed-culture fermentations of cucumber juice by *Lactobacillus plantarum* MOP3 and its malate-negative mutant, strain M6CE[a]

Culture	Initial fermentation			1 Day			10 Days			% Retention after 5 months
	Strain MOP3 concn (log CFU/ml)	Strain M6CE concn (log CFU/ml)	% Retention[b]	Strain MOP3 concn (log CFU/ml)	Strain M6CE concn (log CFU/ml)	% Retention	Strain MOP3 concn (log CFU/ml)	Strain M6CE concn (log CFU/ml)	% Retention	
1	0.00	5.53	100.0	0.00	8.16	98.15	0.00	8.21	89.95	70.61
2	1.51	5.53	100.0	4.45	8.37	89.80	3.86	8.20	82.90	67.64
3	2.51	5.53	100.0	5.53	8.13	64.92	5.17	8.23	58.98	57.94
4	3.51	5.53	100.0	6.75	8.28	65.77	7.31	8.18	0.00	0.00
5	4.51	5.53	100.0	7.88	8.19	0.00	7.22	8.10	0.00	0.00
6	5.51	5.53	100.0	8.75	8.17	0.00	7.84	8.47	0.00	0.00
7	5.51	0.00	100.0	8.68	0.00	0.00	7.94	0.00	0.00	0.00
8	0.00	0.00	100.0	0.00	0.00	100.00	0.00	0.00	92.29	86.49

[a] The strain MOP3 (MDC^+) populations were enumerated on MS agar, and the strain M6CE (MDC^-) populations were enumerated on MRS agar containing chloramphenicol.

[b] The initial malic acid concentration in the cucumber juice was 11.86 mM; the percent retention values were calculated by defining this initial concentration as 100%.

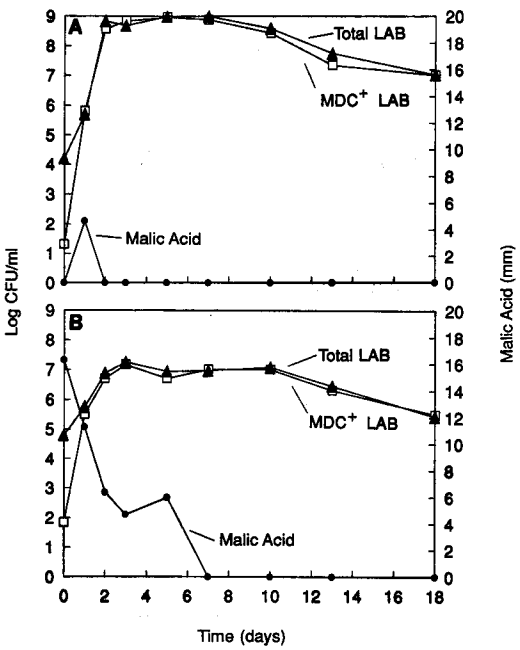

FIG. 2. Growth of indigenous LAB and depletion of malic acid in uninoculated, brined cucumbers. (A) Brine. (B) Cucumbers.

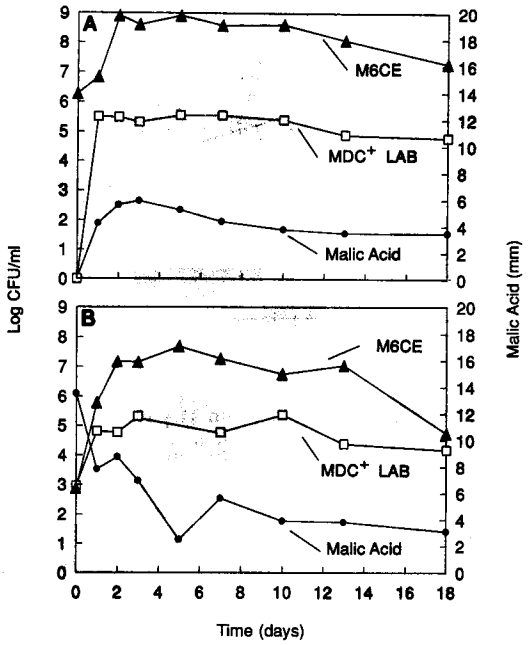

FIG. 3. Growth of added MDC$^-$ culture (strain M6CE) and indigenous MDC$^+$ LAB and depletion of malic acid in brined cucumbers. (A) Brine. (B) Cucumbers.

fermentation. To enumerate the bacteria accurately in the fermentation, we sampled both the brine and the fruit.

In uninoculated brined cucumbers (Fig. 2), malic acid appeared in the brine (diffused from the cucumber) after 1 day, but was absent thereafter. The malic acid concentration in the cucumbers gradually decreased over a 6-day period, and malic acid was absent after 7 days. Malic acid depletion apparently was due to the indigenous MDC$^+$ LAB, which approximated the total LAB population (Fig. 2), as enumerated on MS and MRS media. We have not isolated any MDC$^-$ LAB from cucumber fermentations and presume that natural occurrence of such strains in this environment is not common, although a thorough systematic search has not been conducted.

In brined cucumbers inoculated with the strain M6CE (MDC$^-$) culture (Fig. 3), the malic acid concentration began to stabilize after about 6 days. After 18 days, the brine and cucumber malic acid concentrations were 3.4 and 3.1 mM, respectively. Since the raw cucumbers contained about 14.9 mM malic acid, which was diluted 45% by brining, the residual malic acid after 18 days represents about 40% retention of the initial concentration.

Interestingly, the concentration of indigenous MDC$^+$ LAB (LAB able to grow on MS medium) did not exceed 5×10^5 CFU/ml in the brine or in the cucumbers when the strain M6CE (MDC$^-$) inoculum was added (Fig. 3). In contrast, the concentration of indigenous MDC$^+$ LAB approached 1×10^9 CFU/ml in the brine of uninoculated cucumbers.

To ensure that chloramphenicol-resistant bacteria in the indigenous microflora were not affecting the colony counts on MRS medium containing 6 μg of chloramphenicol per ml (used for strain M6CE enumeration), we routinely plated similar dilutions of brine and cucumber homogenate samples from the uninoculated 19-liter fermentation onto plates containing MRS medium supplemented with chloramphenicol. At the dilutions used to enumerate the strain M6CE culture, no chloramphenicol-resistant bacteria were isolated from the uninoculated pails.

DISCUSSION

To ensure that we were accurately counting the MDC$^+$ strains, the plating efficiency on MS medium was determined. The MDC$^-$ mutant was not able to initiate growth on MS medium, as the other MDC$^-$ strains which we tested were (data not shown), presumably because of the low pH of the medium (pH 4.0). MDC$^+$ LAB are able to ferment the malate in MS medium via the malolactic enzyme fermentation, which raises the medium pH when decarboxylation occurs, as observed with MD medium (2), thereby allowing the cells to initiate growth. The segregational stability of pGK12 was also determined, as a loss of the plasmid by the MDC$^-$ mutant during growth in the fermentation would result in an underestimation of the mutant population on plates containing MRS agar supplemented with chloramphenicol.

The genetic stability of the MDC$^-$ phenotype was determined by a fluctuation analysis. This method allowed an estimation of the frequency at which the mutant reverted to the parental MDC$^+$ phenotype and supplemented the previous characterization of this strain (11). The reversion frequency was determined because MDC$^+$ revertants should contribute to the decarboxylation of malate. We found that reversion of the mutant to the parental phenotype probably did not occur in the cucumber fermentations with initial inocula of 10^6 CFU/ml and ultimate, maximum cell densities around 10^9 CFU/ml.

A study of competitive growth of strains MOP3 and M6CE in cucumber juice allowed us to determine the conditions that prevented malic acid from being fermented for a given ratio of parent cells to mutant cells in the inoculum. We inoculated the mutant strain at a concentration of approxi-

mately 10^6 CFU/ml in every case because this is the cell concentration that has been deemed practical as an inoculum for commercial cucumber fermentation (3). It is interesting that for every ratio of mutant to parent in cucumber juice, growth of the parent strain was restricted to roughly 10 generations, the same number of generations required for the mutant to grow from a concentration of 10^6 CFU/ml to a concentration of 10^9 CFU/ml. Presumably, the depletion of nutrients and the accumulation of lactic acid prevented further growth of the parent strain once the mutant strain achieved a concentration of 10^9 CFU/ml in the mixed culture.

The ability of the MDC^- mutant to compete against indigenous organisms in cucumber fermentation was determined in a 19-liter fermentation of freshly brined cucumbers. Malate was completely fermented in the uninoculated fermentation, while in the inoculated fermentation malate was present after 18 days. At this time, both the inoculated and uninoculated fermentations were essentially complete, as determined by the absence of fermentable carbohydrate and lactic acid concentrations in excess of 100 mM (data not shown).

The inoculated fermentation showed a 60% decrease in total malate concentration at 18 days, and the concentration of MDC^+ cells never exceeded 5×10^5 CFU/ml. The residual level of malate seen in the inoculated fermentation is analogous to the residual in malate concentration seen in Fig. 2B with 10^5 CFU/ml of strain MOP3 (MDC^+). If significantly greater numbers of MDC^+ LAB were permitted in the brine, it is likely that malate would be completely degraded in the brine, but further experiments will be needed to clarify this relationship, as the added complexity of cucumbers offering a solid support for colonization by LAB prevented a direct comparison of the cucumber juice experiments and the brine fermentation experiments.

A method was established for the differential enumeration of indigenous, MDC^+ LAB and an added starter culture of MDC^- *Lactobacillus plantarum* during the fermentation of brined cucumbers. This method should be useful in determining the conditions necessary for predominant growth by the MDC^- culture and the tolerance levels for indigenous LAB to preclude the production of unacceptably high concentrations of CO_2 from degradation of malic acid.

ACKNOWLEDGMENTS

We thank Bruce Weir for supplying advice and the equations used in the statistical analysis of the MDC^- reversion frequency data. We acknowledge Dean Toler for excellent secretarial assistance.

This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, Ill.

REFERENCES

1. **Daeschel, M. A., and H. P. Fleming.** 1992. Personal communication.
2. **Daeschel, M. A., R. F. McFeeters, H. P. Fleming, T. R. Klaenhammer, and R. B. Sanozky.** 1984. Mutation and selection of *Lactobacillus plantarum* strains that do not produce carbon dioxide from malate. Appl. Environ. Microbiol. **47:**419–420.
3. **Etchells, J. L., T. A. Bell, H. P. Fleming, R. E. Kelling, and R. L. Thompson.** 1973. Suggested procedure for the controlled fermentation of commercially brined pickling cucumbers—the use of starter cultures and reduction of carbon dioxide accumulation. Pickle Pak Sci. **3:**4–14.
4. **Fleming, H. P.** 1991. Mixed cultures in vegetable fermentations, p. 69–103. *In* J. G. Zeikus and E. A. Johnson (ed.), Mixed cultures in biotechnology. McGraw-Hill, Inc., New York.
5. **Fleming, H. P., R. F. McFeeters, M. A. Daeschel, E. G. Humphries, and R. L. Thompson.** 1988. Fermentation of cucumbers in anaerobic tanks. J. Food Sci. **53:**127–133.
6. **Foegeding, P. M., and N. W. Stanley.** 1991. *Listeria innocua* transformed with an antibiotic resistance plasmid as a thermal-resistance indicator for *Listeria monocytogenes*. J. Food Prot. **54:**519–523.
7. **Foegeding, P. M., A. B. Thomas, D. H. Pilkington, and T. R. Klaenhammer.** 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. **58:**884–890.
8. **Kok, J., J. M. B. M. van der Vossen, and G. Venema.** 1984. Construction of plasmid cloning vectors for lactic streptococci which also replicate in *Bacillus subtilis* and *Escherichia coli*. Appl. Environ. Microbiol. **48:**726–731.
9. **Luchansky, J. B., P. M. Muriana, and T. R. Klaenhammer.** 1988. Application of electroporation for transfer of plasmid DNA to *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Listeria*, *Pediococcus*, *Bacillus*, *Staphylococcus*, *Enterococcus*, and *Propionibacterium*. Mol. Microbiol. **2:**637–646.
10. **McDonald, L. C., H. P. Fleming, and M. A. Daeschel.** 1991. Acidification effects on microbial populations during initiation of cucumber fermentation. J. Food Sci. **56:**1353–1356, 1359.
11. **McDonald, L. C., D.-H. Shieh, H. P. Fleming, and R. F. McFeeters.** 1992. Personal communication.
12. **McFeeters, R. F.** Unpublished data.
13. **McFeeters, R. F., H. P. Fleming, and M. A. Daeschel.** 1984. Malic acid degradation and brined cucumber bloating. J. Food Sci. **49:**999–1002.
14. **McFeeters, R. F., H. P. Fleming, and R. L. Thompson.** 1982. Malic acid as a source of carbon dioxide in cucumber juice fermentations. J. Food Sci. **47:**1862–1865.
15. **McFeeters, R. F., R. L. Thompson, and H. P. Fleming.** 1984. Liquid chromatographic analysis of sugars, acids, and ethanol in lactic acid vegetable fermentations. J. Assoc. Off. Agric. Chem. **67:**710–714.
16. **Weir, B. S.** 1992. Personal communication.