Antibacterial Activity of Cabbage Juice Against Lactic Acid Bacteria

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ABSTRACT

Fresh juice of Cecile cultivar cabbage (*Brassica oleracea*) was inhibitory to the growth of four species (including seven strains) of lactic acid bacteria. Inhibition was eliminated when the cabbage was heated (steamed 10 min) before juice extraction. When *Leuconostoc mesenteroides* C33 was tested in juices of three other cultivars of cabbage, growth inhibition was invariably noted in fresh juices, but was variable in heated juices. Fresh cabbage juice (CJ) adjusted to higher initial pH (6.5–7.5) showed greater antibacterial activities than at pH 5.0 to 6.0. When pH 4.0 precipitate from fresh CJ was added to noninhibitory CJ (cabbage heated before extraction), antibacterial activity was restored. A heat-labile and pH-dependent factor seemed responsible for activating a precursor into an inhibitory compound.

Key Words: cabbage juice, antibacterial activity, lactic acid bacteria, *Leuconostoc, Lactobacillus*

INTRODUCTION

THE PRESENCE of antibacterial activity in cabbage was demonstrated initially by Sherman and Hodge (1936), and since has been the subject of many studies (Pederson and Fisher, 1944a, b; Lucas and Lewis, 1944; Little and Grubaugh, 1946; Dickerman and Liberman, 1952; Yildiz and Westhoff, 1981; Liu et al., 1986). Reports concerning inhibitory activity of cabbage have been conflicting. Sherman and Hodge (1936) and Pederson and Fisher (1944a, b) reported that the antibacterial substance was destroyed by heating. Yildiz and Westhoff (1981) reported that fresh filter-sterilized cabbage juice (CJ) was a better growth medium for lactic acid bacteria (LAB) than heated CJ. The antibacterial activity was reported to be more pronounced against Gram-negative bacteria than against Gram-positive types (Pederson and Fisher, 1944a, b). The growth inhibitory substance of cabbage was suggested to be carbohydrate in nature (Dickerman and Liberman, 1952) and of low molecular weight (Dickerman and Liberman, 1952; Liu et al., 1986). However, the identity of the inhibitory compound has not been elucidated.

Among cabbage components, glucosinolate hydrolysis products have been reported to be antimicrobial (Virtanen, 1962; Zsolnai, 1966). Fresh cabbage was reported to contain 300–1070 μg/g of total glucosinolate compounds (Van Etten et al., 1976, 1980) which, upon hydrolysis, produce isothiocyanates, nitriles, and thiocyanates. Glucosinolates are goitrogens which inhibit iodine uptake of the thyroid gland. Zsolnai (1966) reported that glucosinolate hydrolysis products inhibited Gram-positive bacteria and fungi more than Gram-negative bacteria. However, Pederson and Fisher (1944a, b) reported that CJ was more inhibitory to Gram-negative bacteria than Gram-positive bacteria.

Our objectives were to determine the inhibitory activity of juice from four cultivars (cvs.) of cabbage to growth by *Leuconostoc mesenteroides* and other bacteria involved in the fermentation of cabbage to sauerkraut, and to characterize the system involved in inhibitor formation.

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MATERIALS & METHODS

Materials

Brutus, Galaxy, and Bentley cabbage cvs. were obtained from Castle Harvester Company, Inc. (Seneca Castle, NY), and Cecile cv. was obtained from Shiocton Kraut Company, Inc. (Shiocton, WI). Juices from fresh or steamed cabbage were extracted by an electrical centrifuge-type juice extractor (Braun Type 4290, Germany) which expressed free juice, leaving coarse pulp. Juice for bacterial growth studies was made from inner leaves. Fresh CJ was filter-sterilized (0.2 μm, Costar Bottle Filter, Costar Corp., Cambridge, MA) following centrifugation at 47,800 × g for 25 min. The time lapse between extraction of fresh CJ and bacterial inoculation was ≈1 hr. Fresh, uninoculated CJ, became milky in appearance soon after incubation at 30°C, and a precipitate began to appear after 6 to 12 hr. A similar result was reported with onion distillate (Kohman, 1947).

Precipitation was slowed when fresh CJ was stored at low temperature or adjusted to higher pH. Heated CJ was made by quartering cabbage and steaming (≈100°C) it in an autoclave at atmospheric pressure for 10 min. Juice from heated cabbage was extracted as for fresh CJ. Heated CJ was used either immediately or after being frozen (−20°C).

Bacterial strain and culture conditions

Lactic acid bacteria (Table 1) were obtained from the culture collection maintained by the Food Fermentation Laboratory (Raleigh, NC). They were stored at −84°C in MRS broth (Difco Laboratories, Detroit, MI) containing 16% glycerol. Stock cultures of bacteria were streaked onto MRS agar. An isolated colony was transferred to filter-sterilized, heated CJ and subcultured for 16 hr before each experiment.

Aliquots (10 mL) of CJ were dispensed into 16 mm × 150 mm glass culture tubes with caps and were statically incubated (30°C) after inoculation. Undiluted or appropriately diluted culture (100 μL) served as inoculum.

Adjustment of initial pH of CJ

Freshly extracted CJ (unadjusted pH was 6.01) was adjusted to pH 5.0, 5.5, 6.5, 7.0, 7.5 with 0.5N NaOH. The pH of heated CJ (unadjusted pH of heated CJ was 5.65–6.10) for growth inhibition studies was always adjusted to 6.5.

Table 1—Strains of lactic acid bacteria used and their sources

Bacterial strains used[a]	Source
Leuconostoc mesenteroides (LA10)	C33[b]
Leuconostoc mesenteroides (LA81)	ATCC 2893
Leuconostoc mesenteroides (LA145)	NCK 293[c]
Lactobacillus plantarum (LA23)	WSO[d]
Lactobacillus plantarum (LA70)	ATCC 14917
Lactococcus lactis (LA138)	NCK 400[e]
Pediococcus pentosaceus (LA76)	ATCC 33316

[a] Lab code number in parentheses.
[b] Stamer et al. (1971).
[c] Originally isolated from sauerkraut, Harris et al. (1992b).
[d] Fleming and Etchells (1967).
[e] Nisin-producing strain. Originally isolated from sauerkraut, Harris et al. (1992a).

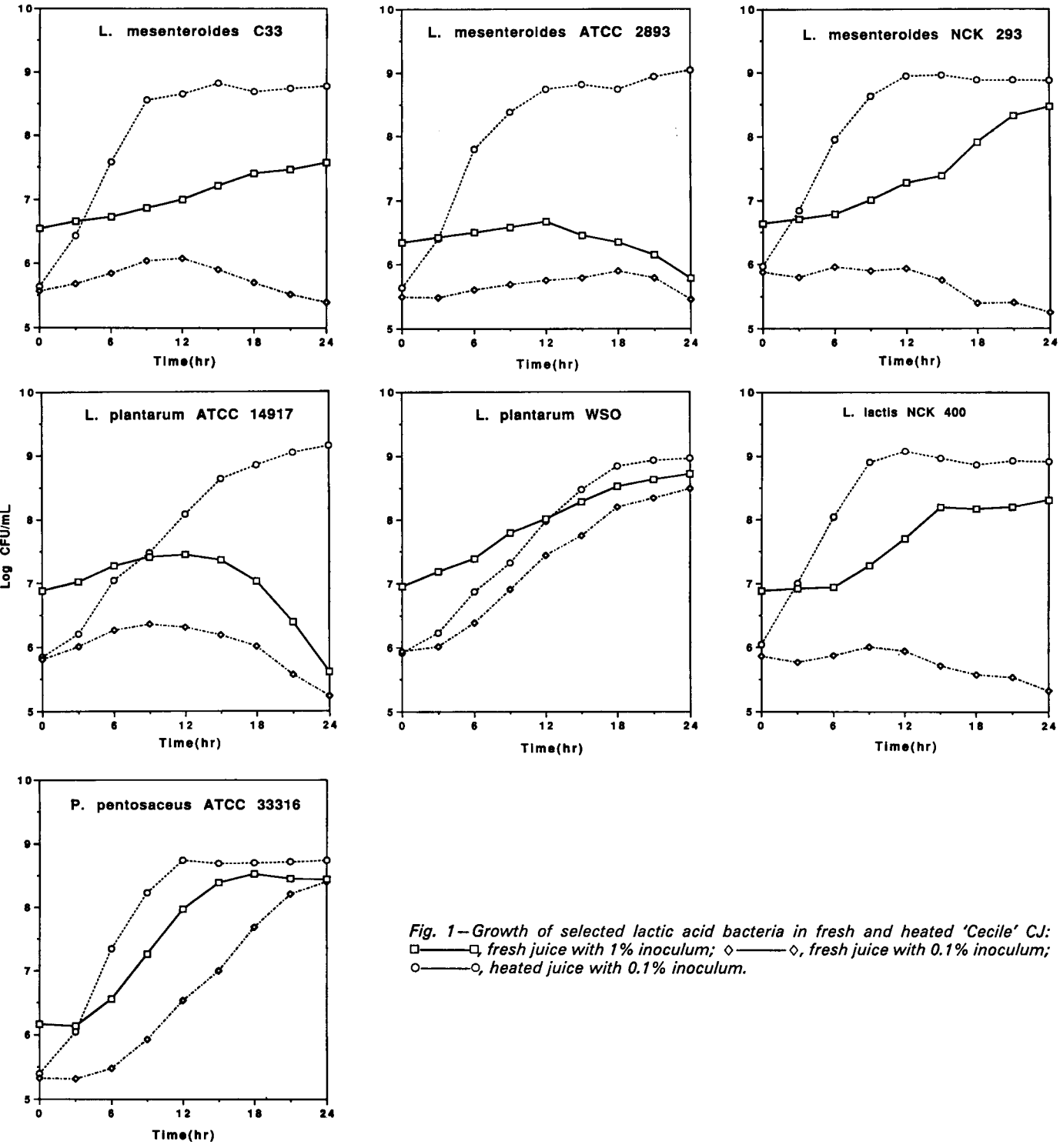

Fig. 1—Growth of selected lactic acid bacteria in fresh and heated 'Cecile' CJ: □——□, fresh juice with 1% inoculum; ◇——◇, fresh juice with 0.1% inoculum; ○——○, heated juice with 0.1% inoculum.

Preparation of pH 4.0 precipitate and pre-treatment

Fresh CJ was adjusted to pH 4.0 (Fujiwara et al., 1954), which resulted in a precipitate. The juice was centrifuged at 12,000 × *g* for 10 min, and the supernatant was decanted. The precipitate was washed twice with distilled water. Precipitate obtained from 20 mL of fresh CJ was added to 10 mL of heated CJ. For heat inactivation studies, the precipitate was suspended in heated CJ and then heated at 60°C or 100°C for 10 min in a water bath.

Bacterial count

Viable cell numbers were counted as colony forming units (cfu)/mL with a Spiral Plater (Spiral System, Inc., Cincinnati, OH) on MRS agar plates.

RESULTS & DISCUSSION

Growth of LAB in fresh and heated CJ

The growth of seven strains of LAB including four species (*Lactobacillus plantarum*, *L. mesenteroides*, *Lactococcus lactis*, and *Pediococcus pentosaceus*; Table 1) was tested first in fresh and heated juices of Cecile cv. cabbage (Fig. 1). All bacteria grew well in heated CJ without inhibition but were inhibited in fresh CJ to varying extents. The degree of bacterial growth inhibition in fresh CJ depended upon strain of bacteria and on initial cell concentration in the growth medium. With small initial populations (about 5.0×10^5 cells/mL) the viable cells of *L. mesenteroides* C33 and *L. lactis* NCK400 and *L.*

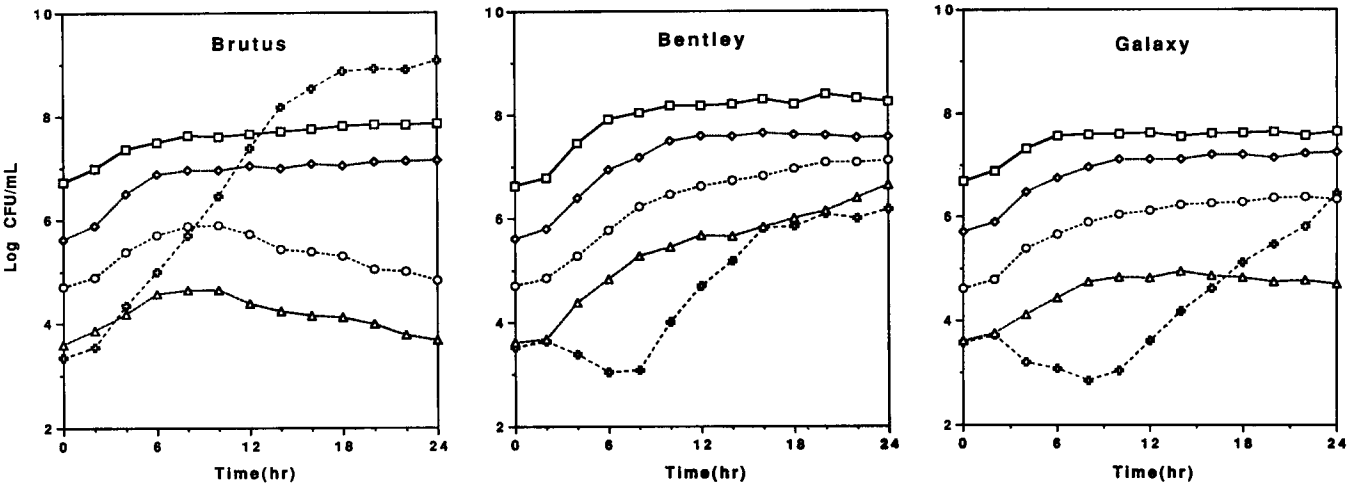

Fig. 2—Growth of L. mesenteroides *C33 in juice of different cultivars of cabbage.* □——□, *fresh juice with 1% inoculum;* ◇——◇, *fresh juice with 0.1% inoculum;* ○——○, *fresh juice with 0.01% inoculum;* △——△, *fresh juice with 0.001% inoculum;* #——#; *heated juice with 0.001% inoculum.*

mesenteroides NCK293 declined after some initial growth. With large initial populations (about 5.0×10^6 cells/mL), they grew to some extent. In the case of *L. mesenteroides* ATCC2893 and *L. plantarum* ATCC14917, growth was inhibited and viable cell numbers declined after limited growth, regardless of initial populations. The growth of *L. plantarum* WSO and *P. pentosaceus* ATCC 33316 was inhibited, but to a lesser extent than the other cultures tested (Fig. 1).

Similar phenomena were reported with garlic and onion extracts. A1-Delaimy and Ali (1970) reported that onion extract completely destroyed *Shigella dysenteriae* and *Staphylococcus aureus* when the inoculum size was small, but not when it was large. The ability of *S. aureus* to initiate growth in BHI broth with supplemented garlic extract was dependent on the size of initial populations (Mantis et al., 1978). When the initial population was $>1.0 \times 10^6$ cells/mL, *S. aureus* in BHI broth with 2% garlic extract multiplied after a lag period. However, when the initial population was $< 9.9 \times 10^4$ cells/mL, multiplication was not possible and viable cell number declined. In BHI broth with 1% garlic extract, *S. aureus* grew when initial population was $>3.8 \times 10^3$ cells/mL, but not when it was $< 1.0 \times 10^3$ cells/mL. Karaioannoglou et al. (1977) reported essentially the same result when they tested *L. plantarum* in BHI broth with garlic extract. The results of those three cases with onion and garlic extracts were similar to the results of our work with CJ. The inhibitory principles in the three different vegetables apparently have common biological characteristics. The delay in antibacterial effect of fresh CJ could be due to the capability of bacterial cells to reproduce prior to permanent damage and death of cells, to delayed generation of inhibitory compound from an inactive precursor, or both. Pederson and Fisher (1944a), who extensively studied the antibacterial activity of cabbage reported that such activity of cabbage was destroyed by heating. They reported that some varieties of cabbage were pronounced in antibacterial action, while others showed less effect. The antibacterial effect varied depending on variety, growing season, age, and individual head of cabbage. Sherman and Hodge (1936), who initially reported the antibacterial activity of cabbage, mentioned that the activity was destroyed by heating.

Growth of *L. mesenteroides* in fresh and heated CJ

We established that heated CJ supported the growth of LAB well without inhibition while fresh CJ inhibited them to varying extent. A strain (C33; Stamer et al., 1971) of *L. mesenteroides* which has been known to be important in the primary fermentation of sauerkraut (Pederson and Fisher, 1944a; Stamer et al., 1971; Fleming et al., 1988) was chosen for the test organism for further experiments. Figure 2 shows the growth characteristics of *L. mesenteroides* C33 in fresh and heated juices of three different cabbage cvs. The bacterium grew well in heated juice of some cabbage (Brutus cv.), but was inhibited in those of other cabbage cvs. (Bentley and Galaxy). In cases of both Bentley and Galaxy cvs., an initial decrease and later recovery in cell number was noted, as it was in the data of Pederson and Fisher (1944a). The growth of *L. mesenteroides* was more inhibited in heated CJ than in fresh CJ in 'Galaxy', as was reported by Yildiz and Westhoff (1981). They found that autoclaved CJ of Glory variety was more inhibitory toward LAB including *L. mesenteroides* than filter-sterilized fresh CJ. We could not explain these varying effects.

The heated CJ of 'Brutus', like that of 'Cecile', was not inhibitory to growth of *L. mesenteroides* even with very small initial cell numbers (3.0×10^3 cells/mL; Fig. 2). Heating apparently either inactivated the natural inhibitory compound of cabbage or destroyed some factor (e.g., an enzyme) which activates a precursor of an inhibitory compound. Stamer et al. (1969) reported that some cabbage hybrids, when made into sauerkraut, failed to undergo proper fermentation and resulted in inferior quality products. They suggested that their observations may be due to growth inhibitory substances in the cabbage or lack of essential nutrients. However, good growth of the bacterium in heated CJ in our study seemed to exclude the possibility that *L. mesenteroides* was unable to grow in fresh CJ due to lack of nutrients. Conceivably, however, heat could release nutrients essential or stimulatory to growth.

Effect of initial pH of fresh CJ on the growth of *L. mesenteroides*

In order to evaluate the effect of pH on antibacterial activity of cabbage, the initial pH of fresh 'Brutus' CJ was adjusted to a range of pH values, as indicated, before addition of *L. mesenteroides*. The effect of initial pH of fresh CJ on antibacterial activity of fresh CJ was very evident (Fig. 3). At pH values lower than the natural pH of cabbage, *L. mesenteroides* grew rapidly to 10^8 cells/mL, which was $\approx 10 \times$ higher than that in juice that was not pH-adjusted. At higher pH values (6.5, 7.0, 7.5) *L. mesenteroides* did not show appreciable growth but began to decline in viable cell number starting 2 hr after inoculation and continuing throughout the experimental period (24 hr). At these high pH values, there was not much difference in degree of antibacterial activity among the pH values

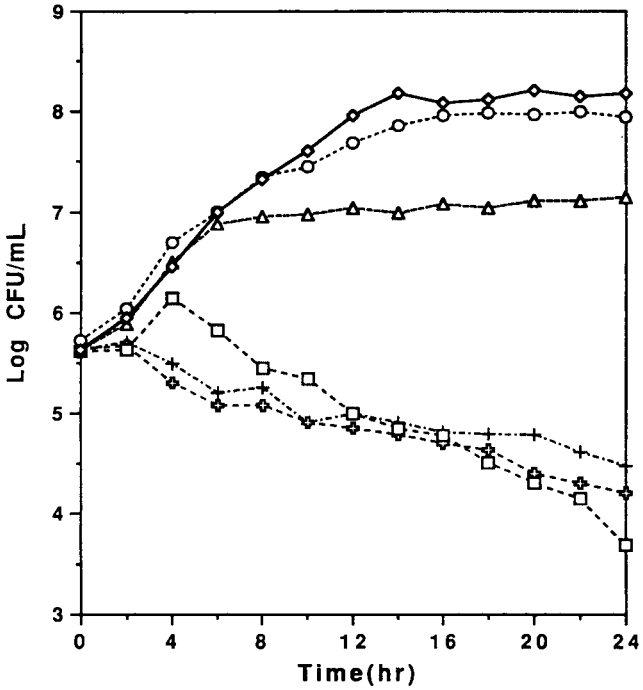

Fig. 3—Growth of L. mesenteroides *C33 in fresh juice of 'Brutus' cabbage with varying initial pH. ◇———◇, 5.0; ○———○, 5.5; △———△, 6.0 (unadjusted); □———□, 6.5; #———#, 7.0; +———+, 7.5.*

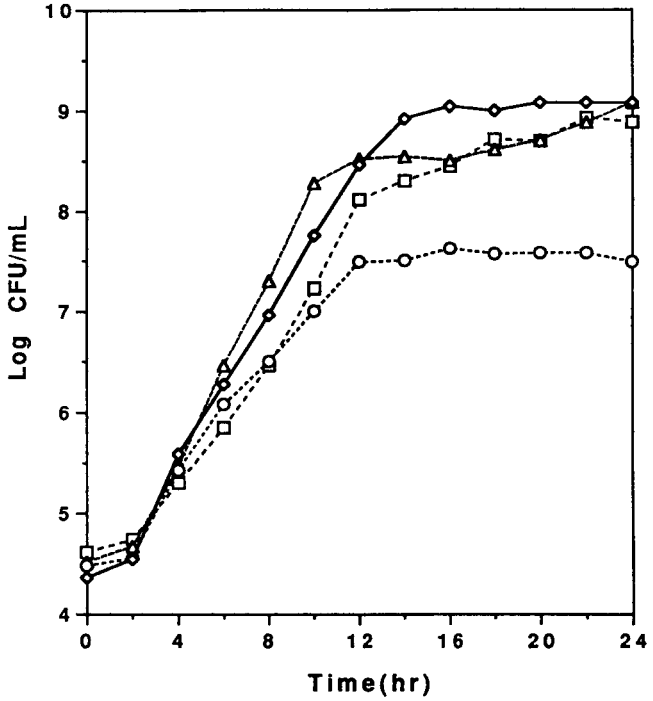

Fig. 4—Growth of L. mesenteroides *C33 in heated 'Brutus' juice with pH 4.0 precipitate treated differently. ◇———◇, CJ control; □———□, heated pH 4.0 precipitate (60°C); △———△, heated pH 4.0 precipitate (100°C); ○———○, unheated pH 4.0 precipitate.*

tested. Fresh 'Brutus' CJ seemed somewhat growth inhibitory at pH values even below 5.5, showing a final viable cell number of 1.5×10^8 cells/mL, which was 10 × lower than the final viable cell number of 1.2×10^9 cells/mL in heated CJ. This may be due to growth inhibitory factors released by enzymatic activity before pH adjustment but after juice extraction.

Marks et al. (1992) reported that both the decrease of S-methyl-L-cysteine sulfoxide (SMCSO) and the increase in methyl methanethiolsulfinate in brussels sprout extract were much more pronounced at high pH (8.0) than at lower pH. This was explained as being due to the pH optimum (8.0–8.5) of cystine lyase (Hamamoto and Mazelis, 1986) in *Brassica.* Methyl methanethiolsulfinate is a known antibacterial compound with minimum inhibitory concentrations <5 ppm for many microorganisms (Small et al., 1947). Those findings support our observation that the growth inhibitory activity in CJ was more pronounced at high pH values than at lower pH.

Various hypotheses were considered to explain the greater antimicrobial activity at higher pH. Perhaps the enzyme responsible for the precursor activation has a high optimum pH. A more potent antibacterial compound(s) may be formed at higher pH. The antibacterial compound(s) could penetrate the bacterial cells more easily at higher pH than at lower pH. From previous information (Hamamoto and Mazelis, 1986; Marks et al., 1992), the effect of pH on enzyme activity seems to be most probable.

Effects of pH 4.0 precipitate from fresh CJ on heated CJ

Cysteine sulfoxide lyase (also known as alliinase and cystine lyase) has an isoelectric point of pH 4.0 where it precipitates. The precipitate gave a clear solution when dissolved in buffer at pH 6.4 (Stoll and Seebeck, 1951). When garlic or scallion protein obtained by acidification to pH 4.0 was added to extracts of heated plants, allicin was immediately produced (Fujiwara et al., 1954). Allicin and methyl methanethiolsulfinate are examples of antibacterials which react with −SH groups of proteins. Cysteine was shown to react with these compounds. Allicin and methyl methanethiolsulfinate were reported to lose antibacterial activity when they were treated with cysteine (Cavallito et al., 1944; Ostermayer and Tarbell, 1960; Small et al., 1949), giving mixed disulfides (Cavallito et al., 1944; Chin and Lindsay, 1994).

The possible participation of enzymatic activity, e.g., cysteine sulfoxide lyase, was considered. The pH 4.0 precipitate from fresh CJ was added to the heated 'Brutus' CJ after adjustment to pH ≥ 6.5. When pH 4.0 precipitate was heated (60 and 100°C) before it was added into heated CJ, it did not restore antibacterial activity (Fig. 4). Neither was antibacterial activity restored when the pH of heated CJ was not adjusted. The antibacterial activity of heated 'Brutus' CJ was restored only when unheated pH 4.0 precipitate was added to heated CJ and the pH was adjusted ≥ 6.5.

These findings suggest that an inactive precursor, possibly SMCSO, exists in CJ and that this precursor is activated by some factor, possibly cysteine sulfoxide lyase, in the pH 4.0 precipitate from fresh CJ. Since the activating principle was precipitated by pH 4.0 and inactivated by heating, it was thought to be protein. Earlier reports (Stoll and Seebeck, 1951; Fujiwara et al., 1954) and our results suggest that the pH 4.0 precipitate from fresh CJ may contain cysteine sulfoxide lyase enzyme which has been reported in cabbage (Mazelis, 1963; Pederson and Albury, 1969; Hall and Smith, 1983; Marks et al., 1992).

CONCLUSIONS

JUICES of fresh cabbage and some heated cabbage inhibited the growth of LAB to varying degrees. The antibacterial activity of fresh CJ was dependent upon a heat-labile and pH-dependent factor which was contained in a precipitate resulting when pH of fresh CJ was adjusted to 4.0. This factor may be an enzyme which converts a precursor to the inhibitory com-

pound. The inhibitory factor may influence the types of LAB involved in sauerkraut fermentation.

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