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Lactate Metabolism by Pediococci Isolated from Cheese†

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***Pediococcus pentosaceus* is commonly found among the adventitious microflora of Cheddar cheese. When this organism was incubated with L-(+)-lactate under anaerobic conditions, L-(+)-lactate was rapidly converted to D-(−)-lactate until racemic (DL) lactate was present. Under aerobic conditions this initial reaction was followed by a slower reaction resulting in the use of both lactate isomers and in the production of acetate and CO_2. With intact cells the lactate oxidation system had an optimum pH of 5 to 6, depending on the initial lactate concentration. Cells grown anaerobically possessed lactate-oxidizing activity which increased two- to fourfold as sugar was exhausted from the medium. Aerobic growth further increased specific activities. Cheddar cheese was made with the deliberate addition of *P. pentosaceus*. When the resulting cheese was grated to expose a large surface area to O_2, lactate was converted to acetate at a rate which depended on the density of pediococci in the cheese. The lactate oxidation system remained active in cheese which had been ripened for 6 months.**

Cheddar cheese provides a highly selective environment for the growth of adventitious bacteria which may either survive pasteurization of the cheese milk or gain access as postpasteurization contaminants. Selectivity is mainly due to (i) the low pH and redox potential (both produced by the starter bacteria), (ii) low moisture, (iii) high salt concentration, and (iv) storage at low temperature. However, certain adventitious organisms thrive in this environment, often attaining densities of $>10^7$ CFU/g of cheese, several weeks after manufacture. The predominant organisms are usually lactobacilli (*Lactobacillus casei*, *Lactobacillus plantarum*), pediococci (*Pediococcus pentosaceus*), or both (2, 6, 7). In other food fermentations, such as the manufacture of certain meat and vegetable products, pediococci and lactobacilli are added as starters (12, 16).

There have been many investigations into the role of the nonstarter microflora on flavor development in Cheddar cheese, although few studies have involved aseptic vats where strict bacteriological control is achieved (7, 15, 18). Some of these reports suggested that homolactic nonstarter bacteria had little or no effect, although others concluded that certain organisms enhanced flavor development. A surprisingly limited amount of information exists on the metabolic activities of nonstarter organisms which might affect cheese ripening. Recent research has shown that during Cheddar cheese ripening, adventitious bacteria can have a significant role in lactose fermentation, especially if starter metabolism has been inhibited by relatively high salt levels (22, 23). Nonstarter bacteria, especially pediococci, may also transform up to 0.7% of the cheese mass by converting L-(+)-lactate (produced by the starter) to D-(−)-lactate by use of two stereospecific lactate dehydrogenases (21). This substantial transformation indicates the potential which adventitious bacteria have to influence the composition of cheese during the long period of time required for ripening. More information is needed concerning other metabolic activities of these organisms. As we examined lactate racemization by *P. pentosaceus* (formerly known as *Pediococcus cerevisiae*), suspensions of clump-forming strains were shaken, whereupon lactate use occurred. Since there appear to be no reports of lactate catabolism by pediococci, the present study was undertaken.

MATERIALS AND METHODS

Organisms. *P. pentosaceus* NCDO 559, 813, 990 (type strain), 1220, and 1850 were obtained from the National Institute for Research in Dairying, Reading, England. Strains 559 and 1220 were originally isolated from Cheddar cheese. Culture stocks were stored in broth at −45°C.

Isolation and characterization of pediococci from cheese. Cheddar cheeses aged 2 to 6 months were obtained from different commercial plants in New Zealand and the United States. The nonstarter microflora in cheeses was examined by blending aseptically drawn plugs (10% [wt/vol] of cheese in 2% sodium citrate) and plating on LBS agar (BBL Microbiology Systems, Cockeysville, Md.) which is selective for lactobacilli and pediococci. Plates were incubated anaerobically for 5 days at 30°C. For each cheese sample, 100 adjacent colonies were picked into MRS broth (Difco Laboratories, Detroit, Mich.), and the cultures were incubated for up to 4 days at 30°C. Gas formation was checked (Durham tubes), and cell morphology was examined by using a phase-contrast microscope. The predominant nonstarter bacteria in cheeses produced no gas and were either rods (lactobacilli) or organisms with morphological features typical of pediococci, i.e., spherical cocci often arranged in tetrads.

Two pediococcus isolates, designated P_5 and P_{22} and obtained from New Zealand and U.S. cheeses, respectively, were further characterized by using the tests described by Garvie (9) and the Rapid CH system (DMS Laboratories, Inc., Flemington, N.J.) to test for use of different carbon sources. Both isolates showed physiological and biochemical properties characteristic of *P. pentosaceus* (9). All pediococcus isolates possessed catalase activity when grown on MRS agar with limiting (0.2%) glucose and tested by the method of Whittenbury (24).

Culture conditions. MRS broth was modified by increasing the buffering capacity and reducing the sugar concentration

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so that growth was sugar limited. Broth containing acetate (PP broth) consisted of (per liter): glucose, 3.6 g, unless otherwise specified; polypeptone (BBL), 10 g; beef extract (Scott Laboratories, Inc., Richmond, Calif.), 10 g; yeast extract (BBL), 5 g; Tween 80, 1 g; Na_2HPO_4, 5.5 g; KH_2PO_4, 5.0 g; sodium acetate · $3H_2O$, 5 g; diammonium citrate, 2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; and $MnSO_4 \cdot 4H_2O$, 0.05 g. Broth without acetate (PM broth) contained (per liter): glucose, 3.6 g; polypeptone (BBL), 5 g; beef extract (Scott), 2 g; yeast extract (BBL), 5 g; Tween 80, 1 g; Na_2HPO_4, 8.5 g; KH_2PO_4, 2 g; diammonium citrate, 2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; and $MnSO_4 \cdot 4H_2O$, 0.05 g. The media were autoclaved in two parts, and the sugar-$MgSO_4$-$MnSO_4$ part was added aseptically to the bulk media. The initial pH values of PP and PM broths were 6.7 and 7.1, respectively. The beef extract used was chosen for its relatively low L-(+)-lactate content (1%). L-(+)-Lactate was a major component (10 to 13% [wt/wt]) of some of the beef extract preparations examined (unpublished data).

Experimental cultures were grown aerobically in PP broth (100 ml) unless otherwise indicated. The inoculum cells were from a culture incubated for 16 h at 30°C under static conditions. Aerobic cultures in 500-ml flasks were shaken (300 rpm) at 30°C in a water bath shaker (model G76; New Brunswick Scientific Co., Inc., Edison, N.J.). Anaerobic cultures were grown at 30°C in freshly autoclaved medium which was overlaid with sterile Vaspar (paraffin/mineral oil ratio, 50:50). Resazurin was reduced in these cultures.

Cell suspensions. Aerobically grown cells were harvested by centrifugation 1 to 2 h after glucose exhaustion. To examine lactate use, cells were washed twice in 50 mM phosphate–25 mM citrate buffer (pH 5.0) and resuspended in the same buffer at 4°C. At zero time, 1.0 M L-(+)-lactate solution (pH 5.0) was added, and the suspension (10 ml in a 125-ml screw-capped flask) was aerated by using a water bath shaker (300 rpm) at 30°C. Samples were removed at intervals. To determine O_2 use and CO_2 production, cell suspensions (6.0 mg [dry weight] of bacteria per ml) were placed in flasks containing 0 to 250 μmol of L-(+)-lactate. Flasks were sealed with silicone rubber-teflon septa, which were impermeable to O_2 and CO_2, and shaken for 6 h at 30°C. Headspace gas was removed with a syringe and analyzed for O_2 and CO_2. Peroxidase was added to suspensions, and the headspace gas was assayed for O_2 to check for the presence of hydrogen peroxide. The suspension was analyzed to check that lactate was completely used.

To determine the effect of pH on the activity of the lactate oxidation system, cells were washed twice in salt solution (20 mM NaCl, 10 mM $MgCl_2$) and resuspended in the same solution at a density of 45 mg (dry weight) of bacteria per ml. At zero time, samples (0.2 ml) of the suspension were added to the experimental buffer (1.8 ml; 50 mM phosphate and 25 mM citrate adjusted to the appropriate pH) containing L-(+)-lactate (final concentrations, 0, 20, 100, and 500 mM). The suspensions were shaken at 30°C for 20 min and then assayed for acetate.

For anaerobic incubation, phosphate-citrate buffer (pH 5.0) was boiled for 15 min and cooled under CO_2. Aerobically grown cells were washed and suspended (by sparging with N_2) in this buffer at a density of 5.2 mg (dry weight) of bacteria per ml. After the addition of L-(+)-lactate (20 mM final concentration), a stream of N_2, previously passed over heated copper turnings, was bubbled into the suspension, which was incubated for 6 h at 30°C. The bacterial dry weight in cell suspensions was determined by using membrane filters.

Activity of the lactate oxidation system. Except when examining the effect of pH, the activity of the lactate oxidation system was determined spectrophotometrically by recording the rate of 2,6-dichlorophenolindophenol (DCPIP) reduction (17). The reaction mixture contained the following (in a 3-ml volume): 50 mM phosphate buffer (pH 6.5), 0.033 mM DCPIP, saturating (250 mM) L-(+)-lactate, and washed cells. Activity was proportional to cell density up to a turbidity (600 nm; 10-mm light path) of 1.0 (corresponding to 0.25 mg [dry weight] of bacteria per ml). The assay temperature was 25°C, and the reaction was recorded by using a Beckman Acta III spectrophotometer at a wavelength of 600 nm. The rate of DCPIP reduction was corrected for endogenous activity. The specific activity of the lactate oxidation system was expressed as nanomoles of DCPIP reduced per milligram (dry weight) of bacteria per minute.

Analytical procedures. Enzymatic analyses were used to assay for L-(+)- and D-(−)-lactate (23), acetate (5), and sugars (23). CO_2 and O_2 in headspace samples were determined by gas chromatography (Hewlett-Packard 5840 gas chromatograph with a stainless steel column [2.4 m by 3 mm] packed with Carbosieve B [Supelco, Inc., Bellefonte, Pa.], helium carrier gas, and a thermal conductivity detector; oven temperature was at 35°C for 4 min and then raised linearly over a 6-min period to 185°C where it was held for 1 min).

Cheese manufacture and analysis. Cheddar cheese was made in 350-liter vats as described previously (21, 23). The starter strains used were *Streptococcus cremoris* 134 and 584 (2% inoculum). Diluted PP broth cultures of *P. pentosaceus* 1220 and P_5 were added in separate vats so that the initial density of pediococci was 100 CFU/ml. The milk was mixed thoroughly before the addition of rennet. Care was taken not to cross contaminate vats during manufacture. Curd was hooped and pressed overnight, at ambient temperature, into 10-kg blocks. Cheeses were then wrapped and sealed in plastic film (cellophane coated with polyvinylidene chloride), boxed, and stored at 10 ± 1°C. Cheeses contained typically 32% fat, 38% moisture, and 1.9% salt. After 14 days the cheese pH was 5.0 to 5.1, and lactose was undetectable.

At intervals during ripening, the nonstarter microflora in cheeses was examined as described above. To examine acetate formation, cheese was removed from inside the block and grated aseptically, and samples (1 g) were placed in sterile, screw-capped tubes (50 ml). A separate tube was used for each time point. The cheese was spread out, and the tube was incubated at 30°C. Acetate was determined in cheese extracts made by adding water (9 ml) to the grated cheese and holding at 60°C for 20 min with occasional mixing. No yeasts or molds were detected (<100/g of cheese) when grated cheese samples, incubated at 30°C for 50 h, were plated on potato dextrose agar (pH 3.5; Difco).

RESULTS

Lactate metabolism in cell suspensions. *P. pentosaceus* 1220 rapidly converted L-(+)-lactate to the D-(−) isomer until racemic (DL) lactate was present (Fig. 1). Under these aerobic conditions, a slower reaction resulted in the use of both lactate isomers and in the production of acetate. The initial linear rate of acetate production was 1.75 μmol of acetate per mg (dry weight) of bacteria per h. The pH of the suspension (Fig. 1) after 0 and 6 h was 5.02 and 5.26, respectively. In separate experiments cell suspensions were incubated in gas-tight flasks with 0 to 250 μmol of L-(+)-lactate, and after lactate exhaustion, the headspace gas was

analyzed for O_2 and CO_2. The data (not shown) indicated that the oxidation of 1 mol of L-(+)-lactate consumed 1 mol of O_2 and produced 1 mol of CO_2 plus 1 mol of acetate. Hydrogen peroxide was not detected as a product. Without added lactate, no detectable acetate or CO_2 production occurred in the phosphate-citrate buffer (pH 5.0). The oxidation of L-(+)-lactate to acetate and CO_2 was observed with all seven *P. pentosaceus* strains used in the present study. Under anaerobic conditions, lactate racemization occurred as in Fig. 1, but no strains produced detectable acetate when cells were incubated in phosphate-citrate buffer (pH 5.0).

The pH of cell suspensions had a marked effect on the rate of lactate oxidation to acetate (Fig. 2). The optimum pH moved from 5 to 6 as the initial L-(+)-lactate concentration was increased from 20 to 500 mM. No endogenous acetate was produced. Similar data were obtained with *P. pentosaceus* 1850 and P_5. The low optimum pH for lactate oxidation by intact cells with low substrate concentrations may be primarily an effect on transport, since the substrate is taken up by bacterial cells as the protonated species, i.e., as lactic acid (11).

Induction of the lactate oxidation system. When the experiment shown in Fig. 1 was carried out with cells harvested from the exponential growth phase in aerated cultures or with stationary-phase cells from static cultures, the rate of lactate oxidation was lower, suggesting that the system was inducible. During aerobic growth of *P. pentosaceus* 1220 on glucose, the specific activity of the lactate oxidation system was 6 nmol of DCPIP reduced per mg (dry weight) of bacteria per min (Fig. 3). As glucose became exhausted, there was a threefold increase in the specific activity of this system. Acetate then began to accumulate, and there was a decrease in the concentration of total lactate (Fig. 3). At the point of glucose exhaustion, the ratio of D-(−) and L-(+) isomers of lactate was 2:1 and lactate accounted for 92% of the glucose fermented. In addition to lactate oxidation to acetate, it appears that the D-(−) isomer was converted to the L-(+) isomer (Fig. 3). In this experiment, the doubling time during exponential growth was 89 min, and the pH at the point of glucose exhaustion was 6.24.

The specific activity of the lactate oxidation system was determined during and after aerobic and anaerobic growth on different sugars (Table 1). Cells from the exponential growth phase had similar specific activities whether grown

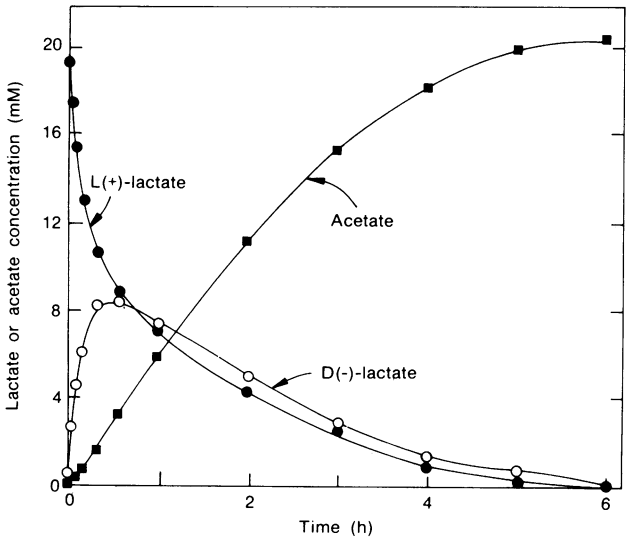

FIG. 1. Aerobic metabolism of L-(+)-lactate by cell suspensions of *P. pentosaceus* 1220. Cells grown aerobically were washed and suspended in phosphate-citrate buffer (pH 5.0) at a density of 3.4 mg (dry weight) of bacteria per ml. L-(+)-Lactate was added, and the suspension was shaken at 30°C.

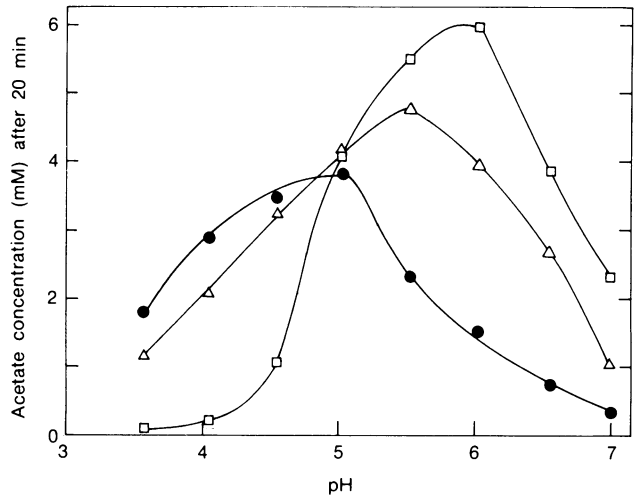

FIG. 2. Effect of pH on activity of the lactate oxidation system in *P. pentosaceus* 1220. Acetate concentrations were determined after cell suspensions (4.5 mg [dry weight] of bacteria per ml) had been shaken at 30°C for 20 min with an initial L-(+)-lactate concentration of 20 (●), 100 (△), or 500 mM (□).

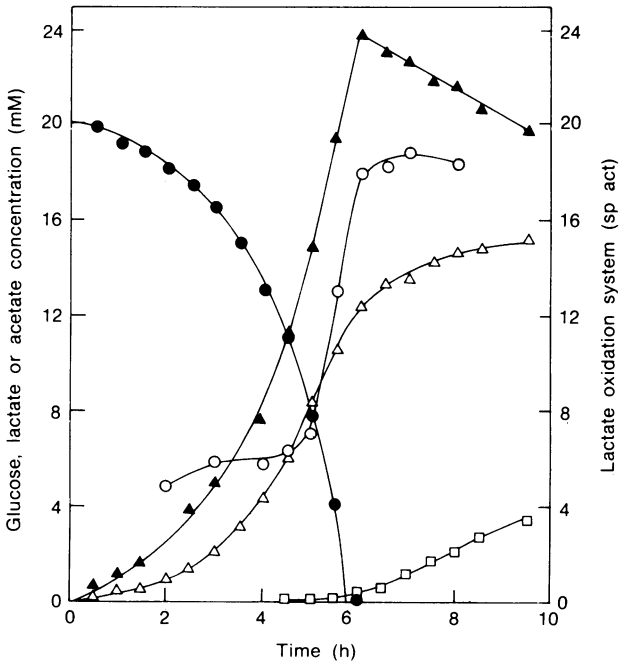

FIG. 3. Induction of the lactate oxidation system during aerobic growth of *P. pentosaceus* 1220 on glucose. Cells from a 16-h static culture (10 ml) in PP broth were pelleted and suspended in 200 ml of experimental medium (PM broth containing 20 mM glucose). The culture was aerated by shaking the 1-liter flask at 30°C, and samples were removed to measure the specific activity of the lactate oxidation system (○), glucose (●), L-(+)-lactate (△), D-(−)-lactate (▲), and acetate (□).

TABLE 1. Specific activity of the lactate oxidation system in *P. pentosaceus* strains grown in PP broth, under aerobic or anaerobic conditions, on different sugars

Strain	Sugar ingrowth medium[a]	Lactate oxidation system sp act[b] different growth in conditions[c]			
		Aerobic		Anaerobic	
		E	S	E	S
1220	Glucose	7 ± 1	24 ± 7	7 ± 1	20 ± 3
	Galactose	23 ± 4	49 ± 7	19 ± 2	32 ± 3
	Lactose	16 ± 1	49 ± 2	12 ± 2	34 ± 3
P_5	Lactose	11 ± 1	50 ± 6	8 ± 1	28 ± 2
P_{22}	Lactose	15 ± 4	55 ± 5	12 ± 2	31 ± 2
990	Glucose	29 ± 2	131 ± 18	20 ± 3	36 ± 6

[a] Glucose, 20 mM; galactose, 20 mM; lactose, 10 mM.
[b] Specific activities are expressed as nanomoles of DCPIP reduced per milligram (dry weight) of bacteria per minute and are the means ± standard deviations from three independent experiments.
[c] Exponential cells (E) were harvested from cultures at one-third of the maximum turbidity, whereas stationary cells (S) were harvested 1 to 2 h after growth stopped (culture pH, 5.9).

aerobically or anaerobically. Compared with growing cells, stationary-phase cells contained higher specific activities, especially when grown aerobically. With *P. pentosaceus* 1220, cells grown on either galactose or lactose had higher specific activities than glucose-grown cells. Among the different strains examined, *P. pentosaceus* 990 (the type strain) had the most active lactate oxidation system. The maximum cell density reached in aerobic cultures was higher (10 to 20%) than in anaerobic cultures with the same initial sugar concentration, suggesting that cells derive energy from the oxidation of lactate to acetate, which can be coupled to growth. Attempts to grow *P. pentosaceus* 1220 and 990 aerobically on L-(+)-lactate, i.e., in PP broth without added sugar, were unsuccessful. Up to 200 mM L-(+)-lactate was used in media having an initial pH value of 6.0.

DCPIP reducing activity was observed with cell suspensions of *P. pentosaceus* 1220 when either D-(−)-lactate or pyruvate was added as the substrate.

Lactate oxidation by pediococci in cheese. Strains of *P. pentosaceus*, which were originally isolated from Cheddar cheese, were added to cheese milk to examine their potential for lactate oxidation in the resulting cheese. At weekly intervals after manufacture, samples were removed to determine the density of nonstarter bacteria in the different cheeses and the proportion of the population which was pediococci (Table 2). At 3 weeks after manufacture, the nonstarter bacteria in cheeses to which *P. pentosaceus* 1220 and P_5 were added had attained maximum cell densities of 1.2×10^8 and 5.6×10^7 CFU/g, respectively. All of the recovered nonstarter bacteria in these 3-week-old cheeses were identified as pediococci. The organisms presumably resulted from growth of the pediococci added to the cheese milk. Cheese made in the control vat (no added nonstarter) had a much lower density of nonstarter bacteria after 3 weeks (7×10^6 CFU/g), only half of which were pediococci.

When the 3-week-old cheese was grated to expose a large surface area to O_2 and was held at 30°C, the acetate concentration in the experimental cheeses increased linearly with time and at rates which were faster than for the control cheese (Fig. 4). When grated samples of the experimental cheeses were incubated anaerobically (H_2-CO_2), there was no detectable increase in acetate concentration within 50 h at 30°C. There was a decrease in the concentration of DL-lactate in cheese samples exposed to O_2, which corresponded to the increase in acetate concentration (data not shown). It seems likely that the faster rates of lactate oxidation to acetate in the experimental cheeses were due to higher densities of pediococci. When the incubation temperature of

TABLE 2. Nonstarter microflora in Cheddar cheese made with added *P. pentosaceus* 1220 and P_5

Strain added	Age of cheese (wk)	Density of NSLAB[a] (CFU per g of cheese [$\times 10^{-7}$])	NSLAB as pediococci (%)
None (control)	3	0.7	54
	13	5.5	36
1220	3	12.0	100
	13	4.4	83
P_5	3	5.6	100
	13	5.0	92

[a] NSLAB, Nonstarter lactic acid bacteria.

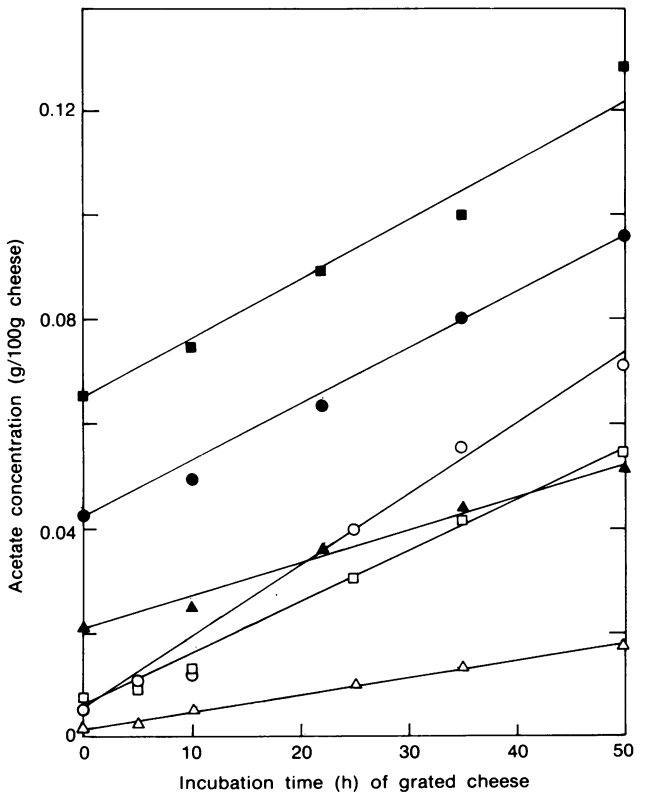

FIG. 4. Acetate formation in cheese made with added pediococci. Cheddar cheese was made by traditional procedures but with added *P. pentosaceus* 1220 (○, ●) or P_5 (□, ■) or without added pediococci (△, ▲). After 21 (open symbols) and 90 days (closed symbols), cheese samples were removed and grated aseptically and then held in sealed tubes at 30°C, and the acetate content was determined at intervals.

the grated cheese was reduced from 30 to 20 and 10°C, the rate of acetate formation was reduced two- and fourfold, respectively. Cheese aged 13 weeks contained higher basal acetate concentrations, which increased on exposure to O_2 (Fig. 4). By this time the density of nonstarter bacteria in the control cheese was similar to that in the experimental cheeses (Table 2), probably accounting for the increased acetate concentrations in the control cheese. The acetate concentration in cheese aged 24 weeks also increased on exposure to O_2 but at a slower rate (data not shown). By 24 weeks, the density of nonstarter lactic acid bacteria had decreased 10-fold, and pediococci accounted for half of the recoverable nonstarter bacteria. Cheese manufacture in an aseptic vat would be required to attain full control over the nonstarter lactic acid bacteria growing in the cheese. However, this was not possible in the present study.

DISCUSSION

Intact cells of *P. pentosaceus* oxidized 1 mol of lactate, consuming 1 mol of O_2 and producing 1 mol each of acetate and CO_2. The term "lactate oxidation system" is used only to describe this capability of intact cells, as several enzymes are likely to be involved. Both L-(+) and D-(−) isomers of lactate and pyruvate were oxidized with either O_2 or an appropriate dye serving as the electron acceptor. Glycerol is the only substrate previously reported to be oxidized by pediococci (4). Several other lactic acid bacteria oxidize lactate. These include *L. casei* (20) and *L. plantarum* (10) which, along with *P. pentosaceus*, are commonly found among the nonstarter microflora of Cheddar cheese. The different enzyme systems involved in lactate oxidation by lactic acid bacteria have been reviewed by Condon (3) and Kandler (13). The system studied in most detail (*L. plantarum*) includes pyruvate oxidase, which is a flavoprotein catalyzing the phosphate-dependent, oxidative decarboxylation of pyruvate producing acetyl phosphate, CO_2, and H_2O_2.

Synthesis of the lactate oxidation system was regulated in all four *P. pentosaceus* strains examined. Strain 1220 induced the system as soon as glucose became exhausted in the growth medium. With this strain, cells had their highest specific activity after aerobic growth on either galactose or lactose. Thus, formation of the lactate oxidation system appears to be controlled by catabolite repression and by oxygen, as in *Streptococcus faecium* (17). Unlike *S. faecium* (17) and *Streptococcus faecalis* (1), *P. pentosaceus* cannot grow aerobically at the expense of lactate, although the effect of haematin was not examined. However, *P. pentosaceus* may gain energy from aerobic lactate use, if cells possess pyruvate oxidase and acetate kinase, which could help these organisms survive in dairy manufacturing plants.

The concentration of acetate in Cheddar cheese increases during ripening (15, 18). The CO_2 concentration in cheese, made by using starters which do not produce CO_2, also increases with time (19). Cheese is a highly complex biological system, and these compounds can arise in different ways. However, experiments in which cheese was made in aseptic vats and in which nonstarter lactic acid bacteria were deliberately added showed that these organisms are responsible for the bulk of the acetate normally produced (15, 18). Of the different nonstarter organisms studied, pediococci gave the highest acetate concentrations in the cheese (15). In the present study, where *P. pentosaceus* formed the predominant microflora in cheese, an active lactate oxidation system persisted for at least 6 months. One of the substrates for this system is present at saturating concentrations since the lactate concentration in the moisture phase of Cheddar cheese is 0.5 M (21). However, since cheese is essentially anaerobic (8, 14), the other substrate (O_2) must be limiting. The slow diffusion of O_2 during the months involved in cheese ripening or the presence of other electron acceptors in cheese could permit some oxidation of lactate, thus accounting for the slow increase in acetate and CO_2 concentrations. Maintenance of a low redox potential is considered important for satisfactory ripening of Cheddar cheese (14). Starter bacteria are responsible for the initial lowering of redox potential (8), but their effect in Cheddar cheese appears to be short-lived (14). The present results suggest that nonstarter bacteria such as pediococci, which maintain an active lactate oxidation system in cheese for months, may hold the redox potential at a low value, thereby contributing to ripening.

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LITERATURE CITED

1. **Bryan-Jones, D. G., and R. Whittenbury.** 1969. Haematin-dependent oxidative phosphorylation in *Streptococcus faecalis*. J. Gen. Microbiol. **58**:247–260.
2. **Chapman, H. R., and M. E. Sharpe.** 1981. Microbiology of cheese, p. 157–243. *In* R. K. Robinson (ed.), Dairy microbiology, vol. 2: the microbiology of milk products. Applied Science Publishers, London.
3. **Condon, S.** 1983. Aerobic metabolism of lactic acid bacteria. Irish J. Food Sci. Technol. **7**:15–25.
4. **Dobrogosz, W. J., and R. W. Stone.** 1962. Oxidative metabolism in *Pediococcus pentosaceus*. I. Role of oxygen and catalase. J. Bacteriol. **84**:716–723.
5. **Fordyce, A. M., V. L. Crow, and T. D. Thomas.** 1984. Regulation of product formation during glucose or lactose limitation in nongrowing cells of *Streptococcus lactis*. Appl. Environ. Microbiol. **48**:332–337.
6. **Franklin, J. G., and M. E. Sharpe.** 1963. The incidence of bacteria in cheese milk and Cheddar cheese and their association with flavour. J. Dairy Res. **30**:87–99.
7. **Fryer, T. F.** 1969. Microflora of Cheddar cheese and its influence on cheese flavour. Dairy Sci. Abstr. **31**:471–490.
8. **Galesloot, T. E.** 1960. The oxidation-reduction potential of cheese. Neth. Milk Dairy J. **14**:111–140.
9. **Garvie, E. I.** 1974. Nomenclatural problems of the pediococci. Request for an opinion. Int. J. Syst. Bacteriol. **24**:301–306.
10. **Gotz, F., B. Sedewitz, and E. F. Elstner.** 1980. Oxygen utilization by *Lactobacillus plantarum*. I. Oxygen consuming reactions. Arch. Microbiol. **125**:209–214.
11. **Harold, F. M., and E. Levin.** 1974. Lactic acid translocation: terminal step in glycolysis by *Streptococcus faecalis*. J. Bacteriol. **117**:1141–1148.
12. **Jay, J. M.** 1978. Modern food microbiology, 2nd ed., p. 253–290. Van Nostrand Co., New York.
13. **Kandler, O.** 1983. Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek **49**:209–224.
14. **Kristoffersen, T.** 1967. Interrelationships of flavor and chemical changes in cheese. J. Dairy Sci. **50**:279–284.
15. **Law, B. A., M. Castanon, and M. E. Sharpe.** 1976. The effect of non-starter bacteria on the chemical composition and flavour of Cheddar cheese. J. Dairy Res. **43**:117–125.
16. **Liepe, H. U.** 1983. Starter cultures in meat production, p. 399–424. *In* H. J. Rehm and G. Reed (ed.), Biotechnology, vol. 5. Food and feed production with microorganisms. Verlag Chemie, Deerfield Beach, Fla.
17. **London, J.** 1968. Regulation and function of lactate oxidation in *Streptococcus faecium*. J. Bacteriol. **95**:1380–1387.

18. **Reiter, B., T. F. Fryer, A. Pickering, H. R. Chapman, R. C. Lawrence, and M. E. Sharpe.** 1967. The effect of the microbial flora on the flavour and free fatty acid composition of Cheddar cheese. J. Dairy Res. **34**:257–272.
19. **Robertson, P. S.** 1957. The carbon dioxide content of New Zealand Cheddar cheese. J. Dairy Res. **24**:235–241.
20. **Strittmatter, C. F.** 1959. Flavin-linked oxidative enzymes of *Lactobacillus casei*. J. Biol. Chem. **234**:2794–2800.
21. **Thomas, T. D., and V. L. Crow.** 1983. Mechanism of D(−)-lactic acid formation in Cheddar cheese. N. Z. J. Dairy Sci. Technol. **18**:131–141.
22. **Thomas, T. D., and K. N. Pearce.** 1981. Influence of salt on lactose fermentation and proteolysis in Cheddar cheese. N. Z. J. Dairy Sci. Technol. **16**:253–259.
23. **Turner, K. W., and T. D. Thomas.** 1980. Lactose fermentation in Cheddar cheese and the effect of salt. N. Z. J. Dairy Sci. Technol. **15**:265–276.
24. **Whittenbury, R.** 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J. Gen. Microbiol. **35**:13–26.