

# Diversity of Sulfur Compound Production in Lactic Acid Bacteria<sup>1</sup>

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## ABSTRACT

Volatile sulfur compounds such as methanethiol, dimethyl disulfide, dimethyl trisulfide, and hydrogen sulfide constitute an important fraction of Cheddar cheese flavor. These compounds are products of the catabolism of methionine and cysteine by bacteria in the cheese matrix. The objectives of this study were to examine the levels and types of volatile sulfur compounds produced from methionine by lactic acid bacteria frequently used in cheese making and to investigate cystathionine degrading activity, which may be responsible for the liberation of these compounds. Gas chromatography with headspace sampling was used to determine volatile sulfur compounds (VSC) produced by whole cells of 24 strains of lactobacilli and 13 strains of lactococci incubated with methionine. Total VSC production varied widely in the species and subspecies tested. Nearly all strains produced VSC from methionine, but the enzyme responsible for this activity remains unclear. Cystathionine-degrading ability and the effect of methionine concentration on this ability of some of the strains was investigated. Increasing the concentrations of methionine inhibited the cystathionine-degrading ability of lactococci, but not of lactobacilli. Lactococci were found to require methionine for growth, while lactobacilli required both methionine and cysteine. Because of the low level of cystathionine-degrading activity in lactobacilli and the inhibition of this activity by methionine in lactococci, VSC production is likely due to enzymes other than cystathionine  $\beta$ - and  $\gamma$ -lyase in whole cells.

**(Key words:** cystathionine lyase, lactococci, lactobacilli, volatile sulfur compounds)

**Abbreviation key:** CDM = chemically defined medium, CFE = cell-free extract, CL = cystathionine lyase, DMDS = dimethyl disulfide, DMTS = dimethyl trisul-

fide, DTNB = 5, 5'-dithio-bis-2-nitrobenzoate, MGL = methionine- $\gamma$ -lyase, OD = optical density, VSC = volatile sulfur compounds.

## INTRODUCTION

Methanethiol and other volatile sulfur compounds constitute an important fraction of Cheddar cheese flavor. The catabolic pathway(s) of methionine in lactococci and lactobacilli is not well characterized. Methionine is a precursor to methanethiol and other volatile sulfur compounds (VSC) as determined by studies with <sup>35</sup>S-methionine and <sup>13</sup>C-methionine (Gao et al., 1998; Grill et al., 1967). Possible pathways involve transaminases, methionine  $\gamma$ -lyase (EC 4.4.1.11), and cystathionine  $\gamma$ -lyase (EC 4.4.1.1) or cystathionine  $\beta$ -lyase (EC 4.4.1.8). Several bacteria commonly found in cheese use methionine to make VSC. *Brevibacterium linens* was the first cheese-ripening bacterium identified with the ability to produce methanethiol from methionine in cheese (Sharpe et al., 1977). Lactobacilli, *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, and micrococci produce lesser amounts of methanethiol (Dias and Weimer, 1998a; Law and Sharpe, 1978).

The transamination of methionine by aminotransferases leads to the formation of 4-methylthio 2-oxobutyric acid, which can degrade both enzymatically and nonenzymatically to form methanethiol (Gao et al., 1998). Aromatic aminotransferases in lactococci exhibit substantial activity with methionine (Gao and Steele, 1998; Yvon et al., 1997), as do branch chain aminotransferases (Rijnen et al., 1999). Inactivation of the aromatic aminotransferase leads to a 50% reduction in methionine degradation (Rijnen et al., 1999). Aminotransferase activity is inhibited by high levels of methionine in the growth media in at least one strain of *L. lactis* ssp. *cremoris* (Dias and Weimer, 1998a).

Methionine  $\gamma$ -lyase (MGL) directly deaminates and decarboxylates methionine, producing methanethiol,  $\alpha$ -ketylbutyrate, and ammonia. Collin and Law (1989) partially purified the enzyme from *B. linens* NCDO 739, and recently Dias and Weimer (1998b) purified the enzyme from *B. linens* BL2 to homogeneity. They demonstrated methionine  $\gamma$ -lyase from *B. linens* BL2 is active in the pH, temperature, and salt concentration condi-

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tions that exist in aging Cheddar cheese. Further, the addition of MGL or *B. linens* BL2 to the slurries enhanced VSC production in Cheddar cheese slurries (Dias and Weimer, 1999). MGL activity of *B. linens* is enhanced with addition of methionine to the growth medium (Weimer et al., 1997).

Cystathionine  $\beta$ -lyase (Alting et al., 1995) and cystathionine  $\gamma$ -lyase (Bruinenberg et al., 1997) produce methanethiol,  $\alpha$ -ketobutyrate, and ammonia from methionine with slight activity under cheese-like conditions, and together are responsible for cystathionine lyase (CL) activity. Cystathionine  $\beta$ -lyase was purified (Alting et al., 1995) and cloned (*metC*) (Fernández et al., 2000) from *Lc. lactis* ssp. *cremoris*. MetC is responsible for CL activity, but a knockout mutant lacking the *metC* gene still produced VSC, indicating that these strains contain other lyases capable of producing VSC (Fernández et al., 2000). Cystathionine  $\gamma$ -lyase was purified from *Lc. lactis* ssp. *cremoris* SK11 (Bruinenberg et al., 1997) and *Lactobacillus fermentum* DT41 (Smacchi and Gobbetti, 1998). Cystathionine lyase activity is inhibited in the presence of methionine in at least one strain of *Lc. lactis* ssp. *cremoris* (Dias and Weimer, 1998a).

The purpose of this study was to examine the VSC-producing ability of lactococci and lactobacilli, and to measure the CL ability of lactococci and lactobacilli. These studies help define the ability lactococci and lactobacilli have to generate free thiols during growth.

## MATERIALS AND METHODS

### Bacterial Strains and Media

Bacterial strains and their growth conditions are listed in Table 1. Strains were stored at  $-70^{\circ}\text{C}$  in 10% nonfat milk containing 30% glycerol and were subcultured twice in either Elliker (lactococci) or MRS (lactobacilli) broth (Difco Laboratories, Detroit, MI). Chemically defined medium (CDM) was prepared according to Gao et al. (1997) with slight modifications. Modifications included using glucose at 2% instead of lactose, eliminating the  $\beta$ -glycerophosphate and adjusting the pH to 7.0. Chemically defined medium was used to grow lactococci and lactobacilli for cystathionine degrading-ability assays and methionine auxotrophy experiments.

### Methionine and Cysteine Auxotrophy

Strains used for auxotrophy determination (chosen for either their high VSC-producing ability or their frequent study in our laboratory) were grown twice under optimal conditions (Table 1) and transferred to CDM for 24 h at 30 or  $37^{\circ}\text{C}$ , depending on the strain (Table 1). Cells were harvested by centrifugation ( $3,500 \times g$

**Table 1.** Bacterial strains and culture conditions.

Strain	Source	Growth condition
<i>Lactococcus lactis</i> ssp. <i>cremoris</i>		
HP	USU Collection	$30^{\circ}\text{C}$ , Elliker
Wg2	USU Collection	$30^{\circ}\text{C}$ , Elliker
Fg2	USU Collection	$30^{\circ}\text{C}$ , Elliker
UC317	USU Collection	$30^{\circ}\text{C}$ , Elliker
S1	USU Collection	$30^{\circ}\text{C}$ , Elliker
S2	USU Collection	$30^{\circ}\text{C}$ , Elliker
<i>L. lactis</i> ssp. <i>lactis</i> bv. <i>diacetylactis</i>		
11007	ATCC <sup>1</sup>	$30^{\circ}\text{C}$ , Elliker
DRC-1	USU Collection	$30^{\circ}\text{C}$ , Elliker
18-16	USU Collection	$30^{\circ}\text{C}$ , Elliker
<i>L. lactis</i> ssp. <i>lactis</i>		
S3	USU Collection	$30^{\circ}\text{C}$ , Elliker
11454	ATCC	$30^{\circ}\text{C}$ , Elliker
C2	U. of Minnesota	$30^{\circ}\text{C}$ , Elliker
ML3	USU Collection	$30^{\circ}\text{C}$ , Elliker
<i>Lactobacillus casei</i> ssp. <i>casei</i>		
LC301	Rhodia <sup>2</sup>	$30^{\circ}\text{C}$ , MRS
LC202	Rhodia	$30^{\circ}\text{C}$ , MRS
334	ATCC	$30^{\circ}\text{C}$ , MRS
393	ATCC	$30^{\circ}\text{C}$ , MRS
4913	ATCC	$30^{\circ}\text{C}$ , MRS
4940	ATCC	$30^{\circ}\text{C}$ , MRS
39392	ATCC	$30^{\circ}\text{C}$ , MRS
39539	ATCC	$30^{\circ}\text{C}$ , MRS
<i>Lactobacillus helveticus</i>		
LH212	Rhodia	$30^{\circ}\text{C}$ , MRS
CNRZ32	USU Collection	$37^{\circ}\text{C}$ , MRS
L89	USU Collection	$37^{\circ}\text{C}$ , MRS
8018	ATCC	$37^{\circ}\text{C}$ , MRS
10386	ATCC	$37^{\circ}\text{C}$ , MRS
10797	ATCC	$37^{\circ}\text{C}$ , MRS
10705	ATCC	$37^{\circ}\text{C}$ , MRS
12046	ATCC	$37^{\circ}\text{C}$ , MRS
15009	ATCC	$37^{\circ}\text{C}$ , MRS
15807	ATCC	$37^{\circ}\text{C}$ , MRS
<i>Lactobacillus paracasei</i>		
25302	ATCC	$37^{\circ}\text{C}$ , MRS
25598	ATCC	$37^{\circ}\text{C}$ , MRS
<i>Lactobacillus rhanmnosus</i>		
10863	ATCC	$37^{\circ}\text{C}$ , MRS
11981	ATCC	$37^{\circ}\text{C}$ , MRS
11982	ATCC	$37^{\circ}\text{C}$ , MRS
12116	ATCC	$37^{\circ}\text{C}$ , MRS

<sup>1</sup>American Type Culture Collection.

<sup>2</sup>Madison, WI.

for 10 min at  $4^{\circ}\text{C}$ ), washed twice with sterile 0.85% NaCl, and resuspended to an optical density (OD) of  $600 \text{ nm} = 1.0$ . This suspension was inoculated (1%) into CDM containing (per liter) 20 mg of L-methionine and L-cysteine, L-methionine with no L-cysteine, L-cysteine with no L-methionine, or no L-methionine and L-cysteine, and the CDM was incubated at 30 or  $37^{\circ}\text{C}$ , depending on the organism being tested (Table 1). Growth was followed by monitoring OD at 600 nm.

### Cystathionine Lyase Assays

Cell-free extracts (CFE) of thiol-producing strains were assayed for CL activity. Cells for CFE were grown

to mid-log phase in 50 ml of CDM with 0.002%, 0.02%, or 0.2% L-methionine at 30 or 37°C, depending on the strain (Table 1), harvested by centrifugation (5,000 × *g* for 10 min at 4°C), washed twice with 30 ml of 0.05 *M* potassium phosphate buffer (pH 7.2), and resuspended in 1.5 ml of buffer. This cell suspension was disrupted by sonication on ice for 6 to 15 min on pulsed mode, 65% duty cycle, with an output of 6.5 with a Branson Cell Disrupter 200 (Danbury, CT), centrifuged at (4°C for 30 min. at 8,200 × *g*), and the supernatant was tested for protein content with a BCA protein assay kit (Pierce Chemical Co., Rockford, IL) according to the manufacturer's directions with BSA as the standard.

Cystathionine lyase activity was determined by measuring the amount of free thiol [measured with 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB)] (Ferchichi et al., 1985)) formed from cystathionine in 2 h with incubation at 30 or 37°C, depending on the organism being tested (Table 1). The reaction mixture consisted of 0.05 *M* potassium phosphate (pH 7.2), 2 mM cystathionine, 20 μM pyridoxal phosphate, 100 μl of CFE, and 0.28 mM DTNB in 1 ml total volume.

### Volatile Sulfur Production

**Cell preparation.** Cells were grown in optimal conditions in media (Table 1) to early stationary phase (~18 h), and 10 ml was harvested by centrifugation (3500 × *g* at 4°C for 10 min). Cell pellets were washed with two 10-ml volumes of sterile 0.05 *M* potassium phosphate buffer (pH 7.2), and resuspended to an OD<sub>650</sub> = 0.8 in sterile 0.05 *M* potassium phosphate buffer (pH 7.2). The assay mixture contained 0.2 ml of 100 mM L-methionine or 0.2 ml of distilled water added to 1.8 ml of cell suspension in a 22-ml headspace vial with Teflon-lined silicone septa (Tekmar, Cincinnati, OH).

The VSC produced from CFE were also examined. The CFE was made by growing strains as for whole-cell assays. Washed cell pellets from 10 ml overnight culture were resuspended in 1.5 ml of sterile 0.05 *M* potassium phosphate buffer (pH 7.2). This cell suspension was sonicated as described for CL assays to make CFE. The assay mixture contained 0.2 ml of 100 mM L-methionine or 0.2 ml of sterile distilled water added to 0.2 ml of CFE and 1.4 ml of sterile distilled water in a 22-ml headspace vial with Teflon-lined silicone septa.

**Gas chromatography.** Headspace vials (22 ml) containing cells or CFE plus methionine were crimped, flushed with argon filtered through a 0.22-μm filter for 2 min with shaking, and incubated 1 h at 30°C or 37°C, depending on the strain (Table 1). Vials were loaded into the headspace autosampler for runs, which began every 45 min, for a total analysis time of about 12 h, with samples held at room temperature while awaiting

analysis. Samples were analyzed as described by Dias and Weimer (1999) except the vials were equilibrated at 70°C for 15 min before injection. Total volatile sulfur was calculated by adding the -SH contributed by methanethiol, dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS).

**Statistical analysis.** Data sets for VSC production by lactobacilli, lactococci, and CFE (with one replicate) were submitted to analysis of variance, using a completely randomized design, as implemented in the SAS (SAS Institute, Inc., Cary, NC) computer software. An assessment of significant differences in the VSC production by each strain was performed according to the least significant differences method ( $P \leq 0.05$ ).

## RESULTS

### Methionine Auxotrophy

All lactococci tested were able to grow in the absence of cysteine, but not methionine, and are therefore auxotrophic for methionine (Table 2). All lactobacilli tested were unable to grow in the absence of methionine and cysteine, and are therefore auxotrophic for both (Table 2).

### Cystathionine Degradation

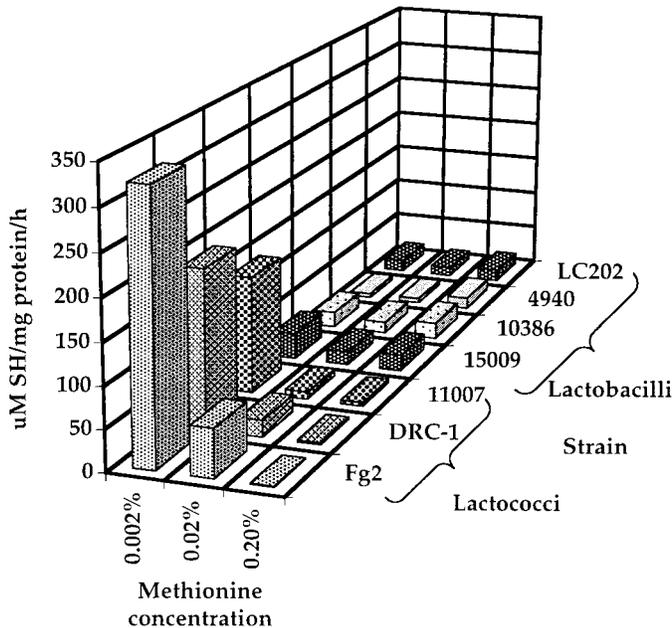
Lactococci possessed more CL activity than lactobacilli (Figure 1). Increasing methionine levels in the growth medium reduced this activity in lactococci. In *Lc. lactis* ssp. *cremoris* Fg2, 18% of the original activity was found when the methionine concentration was in-

**Table 2.** Growth of lactococci and lactobacilli in chemically defined medium with and without methionine and cysteine.

Strain	NMNC <sup>1</sup>	M	C	MC
<i>Lactococcus lactis</i> ssp. <i>lactis</i>				
by <i>diacetylactis</i>				
11007	— <sup>2</sup>	+++	—	+++
DRC-1	—	+++	—	+++
<i>Lactococcus lactis</i> ssp. <i>cremoris</i>				
Fg2	—	+++	—	+++
S1	—	+	—	++
S2	—	+++	—	+++
<i>Lactococcus lactis</i> ssp. <i>lactis</i> S3				
<i>Lactobacillus casei</i> ssp. <i>casei</i>				
LC202	—	—	—	+
4940	—	—	—	+++
<i>Lactobacillus helveticus</i>				
15009	—	—	—	+++
10386	—	—	—	++

<sup>1</sup>NMNC = No L-methionine and L-cysteine, M = 20 mg/L of L-methionine but no L-cysteine, C = 20 mg/L of L-cysteine but no L-methionine, MC = 20 mg/L of L-methionine and L-cysteine.

<sup>2</sup>The number of (+) indicates the amount of growth relative to other strains and (—) indicates no growth.



**Figure 1.** Cystathionine degradation by lactococci and lactobacilli.

creased from 0.002 to 0.02%. Similarly, DRC-1 retained 10% and 11007 retained 6%. When the methionine concentration was increased to 0.20%, the strains retained 0.2, 1.2, and 2.9% of their original activity, respectively. Among the lactobacilli, *Lb. helveticus* 15009 had the highest activity and *Lb. casei* ssp. *casei* LC202 had the lowest activity. Increasing methionine concentrations in the medium had little effect on the CL activity of lactobacilli.

### Volatile Sulfur Production

The VSC-producing ability was characterized for lactobacilli and lactococci with methionine as the substrate. The VSC-producing ability varied widely among strains and species, with *Lactobacillus helveticus* 15009 having the highest VSC production and with very low producers present among all species (Figure 2A). Significant differences were found in all species except *Lb. paracasei* ( $P \leq 0.05$ ). The greatest diversity was found among strains of *Lb. helveticus* (group 4). Within the VSC for all lactobacilli, DMDS contributed an average of 11.5% of the -SH with a SD of 11.0%, while DMTS contributed an average of 28% of the -SH with a SD of 14% (data not shown).

Lactococci as a group had greater VSC production than lactobacilli (Figure 2B), but because they were precultured in different growth medium, the results are not directly comparable. Significant differences were found in each subspecies of lactococci ( $P \leq 0.05$ ), indicat-

ing diverse VSC production in this species. Within the VSC for all lactococci, DMDS contributed an average of 2.8% of the -SH with a SD of 1.0%, while DMTS contributed an average of 7.9% of the -SH with a SD of 2.1% (data not shown).

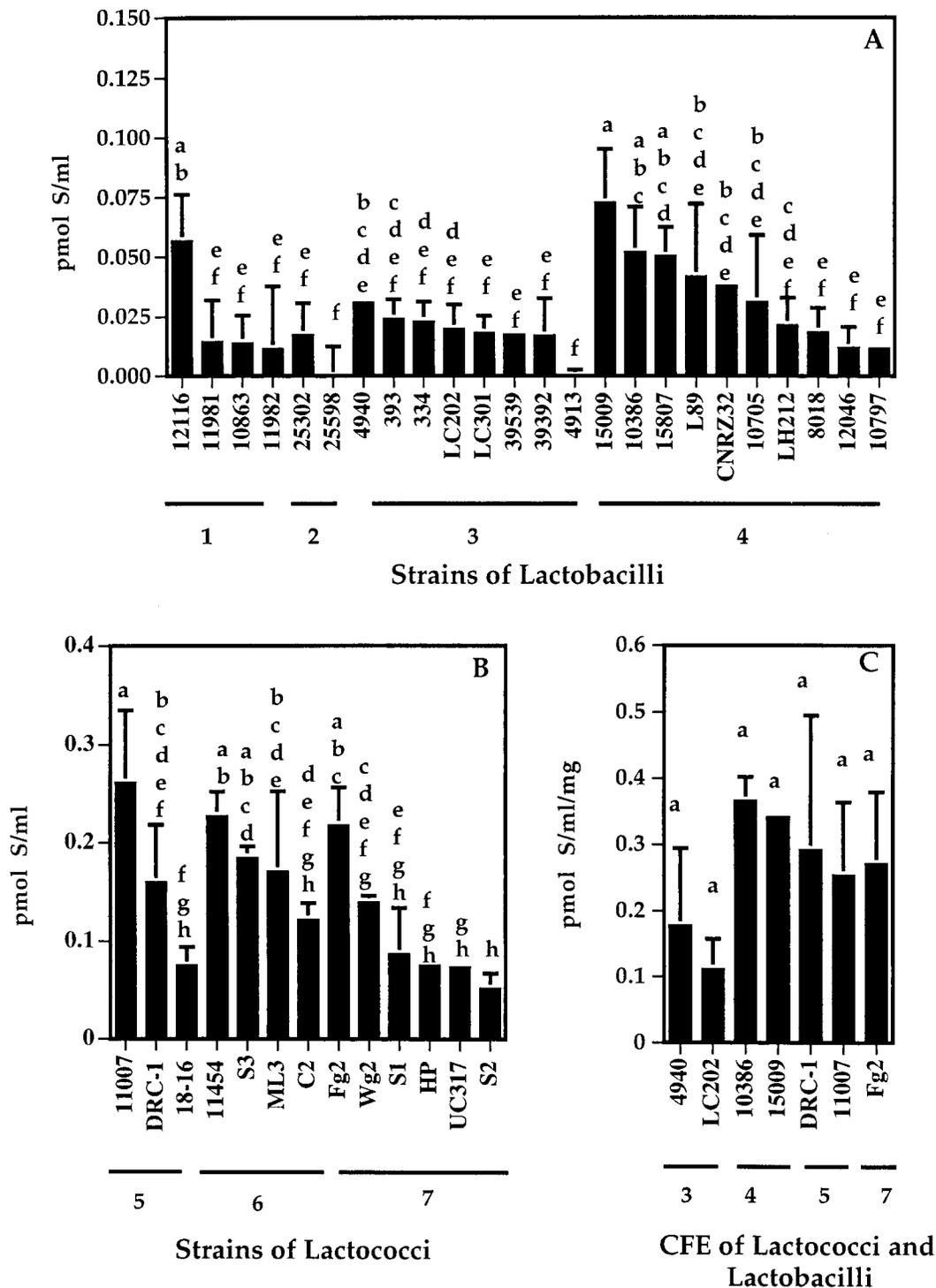
Cell-free extracts also show a diverse pattern of VSC production (Figure 2C), but due to variation between trials, strains were not significantly different ( $P \geq 0.05$ ). In lactobacilli, DMDS contributed 5.4% of the -SH in the total VSC with a SD of 3.5%, while DMTS contributed 12.6% with a SD of 5.0%. In lactococci, DMDS contributed 2.5% of the -SH in the total VSC with a SD of 0.7% while DMTS contributed 7.3% with a SD of 1.1%.

### DISCUSSION

All *Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris* strains tested were found to be auxotrophic for methionine, in accordance with several investigators (Alting et al., 1995; Anderson and Elliker, 1953; Deguchi and Morishita, 1992; Reiter and Oram, 1962). However, Dias and Weimer (1998a) were able to culture *Lc. lactis* ssp. *cremoris* S2 in the absence of methionine. This may be due to a difference in the initial pH of the medium (6.6 vs. 7.0 in this study). Chopin (1993) found that while arginine and methionine stimulated the growth of 36 strains of *Lc. lactis* ssp. *lactis*, omission of these nutrients allowed growth at very low rates, indicating *Lc. lactis* ssp. *lactis* possess the genes needed for biosynthesis of these amino acids. Chopin (1993) found growth requirements in general to be strain specific. Auxotrophy for histidine and branched-chain AA is the result of accumulated mutations and deletions within the region of the necessary genes (Delorme et al., 1993; Godon et al., 1993). The strains in this study may have grown at very low rates in the absence of methionine.

Lactobacilli were auxotrophic for both methionine and cysteine, meaning they either lack the enzymes needed for biosynthesis of these amino acids or the pathways are interrupted, as reported histidine and branched chain amino acids biosynthesis in lactococci (Delorme et al., 1993; Godon et al., 1993). In studies involving a single strain, *Lb. casei* has been reported to require cysteine but not methionine (Morishita et al., 1981) and to require neither (Imamoto et al., 1989). In a study involving a single strain of *Lb. helveticus*, it was found to require methionine but not cysteine (Morishita et al., 1981). It is likely that these are strain dependent requirements for *Lb. casei* and *Lb. helveticus*.

Cystathionine  $\gamma$ -lyase is a cysteine biosynthetic enzyme, catalyzing the  $\alpha, \gamma$ -elimination reaction of L-cystathionine to produce L-cysteine,  $\alpha$ -ketobutyrate, and am-



**Figure 2.** A, Volatile sulfur compound-producing ability of lactobacilli strains from 20  $\mu$ moles methionine/20-ml vial. Sulfur compounds are the sum of methanethiol, dimethyl disulfide, and dimethyl trisulfide. Group 1, *Lactobacillus rhamnosus*; group 2, *Lb. paracasei* ssp. *paracasei*; group 3, *Lb. casei* ssp. *casei*; group 4, *Lb. helveticus*. Strains with the same letter are not significantly different ( $P \leq 0.05$ ). B, Volatile sulfur compound-producing ability of lactococci strains from 20  $\mu$ moles methionine/20-ml vial. Sulfur compounds are the sum of methanethiol, dimethyl disulfide, and dimethyl trisulfide. Group 5, *Lactococcus lactis* ssp. *lactis* bv. *diacetylactis*; group 6, *Lc. lactis* ssp. *lactis*; group 7, *Lc. lactis* ssp. *cremoris*. Strains with the same letter are not significantly different ( $P \leq 0.05$ ). C, Volatile sulfur compound-producing ability of cell-free extracts of lactobacilli and lactococci strains from 20  $\mu$ moles methionine/20-ml vial. Sulfur compounds are the sum of methanethiol, dimethyl disulfide, and dimethyl trisulfide. Group 3, *Lactobacillus casei* ssp. *casei*; group 4, *Lb. helveticus*; group 5, *Lc. lactis* ssp. *lactis* bv. *diacetylactis*; group 7, *Lc. lactis* ssp. *cremoris*. Strains with the same letter are not significantly different ( $P \leq 0.05$ ).

monia. Cystathionine  $\beta$ -lyase is involved in methionine biosynthesis, catalyzing the  $\alpha,\beta$ -elimination reaction of L-cystathionine to produce homocysteine, pyruvate, and ammonia. Both cystathionine  $\gamma$ -lyase and cystathionine  $\beta$ -lyase have the ability to demethylate methionine to form methanethiol (Alting et al., 1995; Bruinenberg et al., 1997; Smacchi and Gobetti, 1998), but this activity in lactococci is greatly reduced in comparison to its primary function (Alting et al., 1995; Bruinenberg et al., 1997). All lactococci tested had CL activity, in contrast to Gao et al. (1998), who found no activity in the strains S1, S3, HP, and 11007, probably due to a less sensitive detection method. Dias and Weimer (1998a) reported CL activity in lactococci and lactobacilli, with lower levels of activity in lactobacilli. A knockout mutant for *metC* showed that cystathionine  $\beta$ -lyase is important for cystathionine degradation, but does not account for all of the VSC formed from methionine (Fernández et al., 2000).

Free methionine is present in the cheese matrix at 0.02 to 3% (Christensen et al., 1995; Wood et al., 1985), meaning production of these enzymes in lactococci would be suppressed in the cheese matrix. The contribution these enzymes makes to VSC in the cheese matrix is most likely minimal.

A wide variety of lactococci and lactobacilli were found to have the potential for VSC production from methionine. *Lc. lactis* ssp. *cremoris* S1 and S2 and *Lc. lactis* ssp. *lactis* S3 were found to produce VSC by Dias and Weimer (1998a) using colorimetric assays, and S3 was also reported to produce methanethiol from methionine using GC assays (Gao et al., 1998). Law and Sharpe (1978) found that three out of six lactococcal strains tested produced trace amounts of methanethiol from methionine using GC.

VSC production by whole cells of lactobacilli has not been widely reported in the literature. Most strains in this study produced VSC. Out of 36 strains tested by Law and Sharpe (1978), only three were found to produce trace amounts of methanethiol by GC. However, the GC method in that study was less sensitive due to the lack of concentration of the headspace. Additionally, Dias and Weimer (1998a) found VSC production by *Lb. casei* LC301 and *Lb. helveticus* CNRZ32 by a colorimetric assay.

CFE of lactococci and lactobacilli were also found to produce VSC. This is in contrast to other researchers (Dias and Weimer, 1998a; Gao et al., 1998) who found that some strains produced VSC with whole cells but not with CFE. Cell-free extract of *Lc. lactis* ssp. *lactis* bv. *diacetylactis* 11007 produced VSC in this study, but did not in a study by Gao et al. (1998).

Total VSC were calculated as the sum of -SH contributed by methanethiol, DMDS and DMTS because the

methanethiol can oxidize to form DMDS and DMTS (Chin and Lindsay, 1994; Parliment et al., 1982). In addition, all three compounds contribute to the flavor profiles of many cheeses (Urbach, 1993). In a study using cheese slurries inoculated with *Lc. lactis* ssp. *cremoris* S2, Dias and Weimer (1999) found dimethyl sulfide levels to remain constant while methanethiol, DMDS, and DMTS levels increased with time. Dimethyl trisulfide levels mirrored those of methanethiol and were correlated ( $R^2 = 0.80$ ). These trends indicate that VSC measurements should include methanethiol, DMDS, and DMTS in order to obtain a more accurate picture of the total VSC-producing ability of a strain.

Dimethyl disulfide and DMTS contributed less to the total VSC produced by lactococci in comparison to lactobacilli. Additionally, there was less variation in the contribution of DMDS and DMTS in lactococci than with lactobacilli. The contribution of DMDS and DMTS to total VSC in was similar for whole cells and CFE in lactococci, but not in lactobacilli, which showed higher percentages of DMDS and DMTS in assays using whole cells. This may be due to the small number of lactobacilli strains used for the assays with CFE, as these values were quite variable in the assays with whole cells.

Cystathionine-lyase activity was found to be inhibited by the presence of methionine and cysteine in one strain of *Lc. lactis* ssp. *cremoris* (Dias and Weimer, 1998a). Therefore, the effect of methionine concentration in the growth medium on CL activity was investigated. The CL activity of all lactococci was inhibited as methionine concentrations in the growth medium increased. Because the enzymes responsible for CL activity are biosynthetic enzymes, methionine in the growth media may inhibit their expression. In *Escherichia coli*, expression of *metC* is controlled by methionine concentration in the media (Saint-Girons et al., 1988). Fernández et al. (2000) show that *metC* and *cysK* (encoding cysteine synthase) form an operon, which may well be controlled by methionine and cysteine concentrations in the media.

Cystathionine  $\gamma$ -lyase has been purified from *Lactobacillus fermentum* (Smacchi and Gobetti, 1998). Lactobacilli possess CL activity, despite being unable to synthesize cysteine or methionine, suggesting these enzymes may not be a part of the biosynthetic pathway for these organisms. Cystathionine-degrading activity, although minimal compared to lactococci, was not greatly affected by methionine concentration in the growth medium in lactobacilli. Unlike the lactococcal enzymes, this enzyme had a greater activity on L-methionine and L-cysteine compared with the activity on L-cystathionine (Smacchi and Gobetti, 1998), and therefore may not be involved in cysteine biosynthesis. Cys-

tathionine  $\beta$ -lyase has not been purified from lactobacilli.

In conclusion, we observed that lactococci and lactobacilli produce VSC from methionine and possess CL activity. The activity of these enzymes is lower when strains are grown under methionine concentrations present in cheese. Based on the auxotrophy, inhibition of VSC production by increasing methionine concentrations, and the total amount of CL activity, it is likely that these enzymes have an insignificant contribution to the VSC production in cheese. Other enzymes, such as aminotransferases in lactococci, methionine  $\gamma$ -lyase in *Brevibacterium*, or a yet undiscovered enzyme, make a greater contribution to VSC in cheese.

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