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Catabolism of aromatic amino acids in cheese-related bacteria: aminotransferase and decarboxylase activities

Áine C Curtin and Paul LH McSweeney*

Department of Food Science, Food Technology and Nutrition, University College, Cork, Ireland

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Bacterial decarboxylases and aminotransferases may be involved in the production of flavour or off-flavour compounds from aromatic amino acids during cheese ripening. Transamination is one of the first steps in amino acid catabolism for both lactococci and lactobacilli (Gao et al. 1997; Klein et al. 2001). Biologically active amines, produced by decarboxylation, such as tyramine, phenylethylamine, tryptamine, histamine, cadaverine and putrescine, known as biogenic amines, have been found in cheese and can cause migraine and hypertension in susceptible consumers (McSweeney & Sousa, 2000).

In this study, we investigated whether several cheese-related bacteria, including lactic acid bacteria (LAB) and strains isolated from the surface of smear cheese, were capable of transamination and decarboxylation of aromatic amino acids. The activity of amino acid catabolic enzymes from smear strains has not been studied previously and compared with the activities of LAB. The production of biogenic amines by selected strains was also studied in a chemically defined medium, which was designed to simulate cheese-ripening conditions. It has been shown that under simulated cheese ripening conditions, certain lactobacilli and lactococci produce enzymes active in catabolism of sulphur-containing and aromatic amino acids (Gao et al. 1997; Gummala & Broadbent, 1999, 2001), however, the enzymes of smear strains have not been studied in this manner.

Materials and Methods

LAB were grown at 30 °C (37 °C for *Lactobacillus helveticus* NCDO1243 and *Lb. helveticus* NCDO87) overnight in MRS broth (Oxoid Ltd., Basingstoke, Hampshire, England). Strains from the surface of smear cheese were incubated at 30 °C, pH 7·2, under shaking conditions (150 rpm) in a medium containing 5 g peptone/l, 2·5 g yeast extract/l, 30 g NaCl/l, 1 g skim milk powder/l, 1 g glucose/l and 10 g casein hydrolysate/l. Cells were harvested and lysed (using lysozyme) as described by Rattray (1996). Protein concentration was determined using the Bradford method with bovine serum albumin as standard (Bradford, 1967).

The methods of Nakazawa et al. (1977) and Gao et al. (1997) were used to determine decarboxylase and aminotransferase activities, respectively. Assays were performed in triplicate at pH 9 (decarboxylase) or pH 8·5 (aminotransferase). Controls without substrate and without cell extracts were included. One-way ANOVA was performed on the log data of the activity results. Tukey's Pairwise Comparisions were performed in cases where $P<0{\cdot}05$.

A chemically defined medium (CDM) was prepared with pH (5·2) and NaCl concentration (4%) similar to those of many cheeses (e.g. Cheddar or young smear-ripened cheese) during ripening (Gao et al. 1997; Gummalla & Broadbent, 2001). The CDM was spiked with one of the aromatic amino acids (5 mM) and inoculated with a strain shown to have good decarboxylase activity on that amino acid. The CDM was incubated at 15 °C and samples were taken on days 0, 7, 14, 21 and 28. The cells were harvested and activity of medium supernatant determined; pH was also recorded. Numbers of viable cells were determined by plating (smear strains on agar prepared from the broth described above, and LAB on Rogosa agar (Merck KgaA, 64271 Darmstadt, Germany).

HPLC analyses were performed according to Gao et al. (1997). Samples (50 µl) were injected onto column directly, and after dilution with concentrated HCl (1:50). Separation on HPLC was accomplished using a C_{18} Symmetry 300 column (5 µm) using a Shimadzu HPLC system (Kyoto, Japan). The separation programme followed the method of Antolini et al. (1999). Concentrations were calculated by reference to standard curves prepared using known concentrations of tryptamine, tyramine and phenylethylamine.

Results and Discussion

Certain products of the catabolism of aromatic amino acids can be significant in flavour or off-flavour development during cheese ripening.

*For correspondence; e-mail: p.mcsweeney@ucc.ie

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Table 1. Aminotransferase activity at pH 8·5 and 30 °C of several cheese-related bacteria on aromatic amino acids

Specific activity was expressed as units of activity per mg protein

Strain	Activity on phenylalanine	Activity on tyrosine	Activity on tryptophan	*P*
Brevibacterium linens ATCC 9174	$16{\cdot}93\pm1^{Aa}$	$11{\cdot}28\pm0{\cdot}6^{Ba}$	$65{\cdot}36\pm5^{Cad}$	***
Corynebacterium sp. subgroup *flavescens* CA8	$16{\cdot}24\pm0{\cdot}2^{Ab}$	$7{\cdot}62\pm2^{Bb}$	$111{\cdot}43\pm6^{Ca}$	***
Microbacterium gubbeenense DPC5288	$25{\cdot}62\pm0{\cdot}2^{Aa}$	$14{\cdot}29\pm1^{Ba}$	$88{\cdot}86\pm6^{Ca}$	***
Lactobacillus casei DPC2766	ND	ND	$3{\cdot}84\pm0{\cdot}3^{b}$	*
Lactobacillus casei subsp. *casei* DPC2786	ND	ND	$342{\cdot}14\pm3^{c}$	NS
Lactobacillus curvatus DPC2770	ND	ND	ND	NS
Lactobacillus curvatus DPC2767	ND	ND	ND	NS
Lactococcus lactis C2	$110{\cdot}41\pm4^{A}$	$59{\cdot}75\pm1^{B}$	$46{\cdot}21\pm5^{a}$	***
Lactococcus lactis HP952	$17{\cdot}82\pm0{\cdot}8^{Abc}$	$6{\cdot}61\pm0{\cdot}3^{B}$	$46{\cdot}21\pm0{\cdot}2^{Cd}$	***
Lactococcus lactis 18-16 (Cit^{+})	$7{\cdot}43\pm0{\cdot}3^{A}$	$1{\cdot}31\pm0{\cdot}3^{B}$	$29{\cdot}50\pm2^{Cde}$	***
Lactobacillus helveticus NCD087	$22{\cdot}96\pm2^{Aac}$	$21{\cdot}18\pm1^{A}$	$32{\cdot}70\pm0{\cdot}4^{Abe}$	**
Lactobacillus helveticus NCDO1243	$117{\cdot}53\pm0{\cdot}1^{A}$	$105{\cdot}22\pm4^{B}$	$94{\cdot}52\pm0{\cdot}7^{c}$	***
P	***	***	***	

Values in a row followed by the same capital letter are not significantly different ($P>0{\cdot}05$). Values in a column followed by the same lowercase letter were not significantly different. *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$. ND – not detected; NS – not significant

Of the 12 bacteria studied, 10 had aminotransferase activity on at least one of the aromatic amino acids. After comparative evaluation of the strains, the majority (10) had greater aminotransferase activity on tryptophan and phenylalanine than on tyrosine (Table 1). Each of the three smear strains, *Brevibacterium linens* ATCC9174, *Microbacterium gubbeenense* DPC5288 and *Corynebacterium* sp. subgroup *flavescens* CA8 had highest activity on tryptophan, as did 3 of the LAB – *Lactococcus lactis* HP952, *Lc. lactis* 18-16 (Cit^{+}) and *Lb. helveticus* NCDO87. *Lb. helveticus* NCDO1243 and *Lc. lactis* C2 had greatest activity on phenylalanine, while none of the strains appeared to prefer tyrosine as a substrate. These results are similar to the results of Gummalla & Broadbent (2001), who reported that specific activity of phenylalanine aminotransferase was higher than tyrosine aminotransferase for lactobacilli.

Decarboxylase activity of the strains on the 3 aromatic amino acids is shown in Table 2. Activity was not as widespread as aminotransferase activity amongst the strains. Joosten & Northolt (1987) reported that the majority of lactobacilli studied did not have decarboxylase activity. More strains (8) were active on tryptophan than on phenylalanine (6) and tyrosine (6). Only extracts from *Brevi. linens* ATCC9174 and *Lb. curvatus* DPC2767 were active on all 3 substrates, while no activity was detected for *Lc. lactis* 18-16 (Cit^{+}) on any of the 3 amino acids. *Lb. helveticus* NCDO1243 had by far the greatest activity on tryptophan. *Brevi. linens* ATCC9174 had the highest activity on phenylalanine and tyrosine. Differences between the activities of strains on the same substrates generally had low statistical significance.

Selected strains were inoculated into CDM containing one aromatic amino acid. *Brevi. linens* ATCC9174 and *Micro. gubbeenense* DPC5288 were inoculated into CDM with phenylalanine, while *Corynebacterium* sp. subgroup *flavescens* CA8 and *Lb. casei* DPC2766 were incubated in the medium with tyrosine. The medium containing tryptophan was inoculated with *Lb. casei* DPC2786 and *Lb. helveticus* NCDO1243. For all inoculated samples, pH increased over the incubation period. For example, the pH of the medium spiked with 5 mM tyrosine and inoculated with *Lb. casei* DPC2766 increased from pH 5·1 at day 0 to pH 5·72 at the end of incubation. Most strains did not grow in the CDM. The plate counts of the smear strains maintained their original numbers, while cell numbers of lactobacilli decreased over the incubation period. Failure to grow or even to maintain cell numbers may not be vital for biogenic amine production as cell death and lysis may be necessary for the enzyme to have an effect on the flavour development in the cheese. Alting et al. (1995) reported that cell lysis was necessary optimal activity of a lactococcal cystathionine β-lyase in cheese.

Samples of the CDM containing strains were assayed for enzyme activity over time. *Lb. casei* DPC2766 and *Lb. casei* subsp. *casei* DPC2786 were the only strains in which decarboxylase activity was detected during incubation. None of the strains showed the same level of decarboxylase activity in the CDM as they did in the initial screening. This may be due to the incubation conditions used (pH 5·2; salt-in-moisture concentration of 4%; temperature of 15 °C).

Tyramine, tryptamine and phenylethylamine were quantified by HPLC of extracts to determine if decarboxylase activity could be linked to a fall in concentrations of amino acids and an increase in levels of biogenic amines. For five of the six strains studied, there was a decrease in concentrations of the spiked amino acid over the incubation period. However, there was no corresponding increase in biogenic amine content (results not shown). Other reactions or enzyme activities may have been acting to alter the amino acid levels, for example, by transamination or incorporation for nutritional requirements during cell

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Table 2. Decarboxylase activity at pH 9 and 30 °C of several cheese-related bacteria on aromatic amino acids

Specific activity was expressed as units per mg protein

Strain	Activity on phenylalanine	Activity on tyrosine	Activity on tryptophan	*P*
Brevibacterium linens ATCC 9174	$8{\cdot}53\pm1^{Aa}$	$15{\cdot}07\pm1^{Aa}$	$0{\cdot}59\pm0{\cdot}06^{Bab}$	**
Corynebacterium sp. subgroup *flavescens* CA8	$4{\cdot}4\pm2^{Aac}$	$4{\cdot}4\pm0{\cdot}6^{Aa}$	ND	NS
Microbacterium gubbeenense DPC5288	$6{\cdot}83\pm2^{Aa}$	ND	$1{\cdot}29\pm0{\cdot}4^{Ba}$	*
Lactobacillus casei DPC2766	$1{\cdot}28\pm0{\cdot}8^{Abc}$	$2{\cdot}94\pm0{\cdot}2^{Bab}$	ND	*
Lactobacillus casei subsp. *casei* DPC2786	ND	ND	$5{\cdot}36\pm0{\cdot}5^{a}$	NS
Lactobacillus curvatus DPC2770	ND	ND	$4{\cdot}02\pm0{\cdot}2^{a}$	NS
Lactobacillus curvatus DPC2767	$0{\cdot}02\pm0{\cdot}005^{b}$	$0{\cdot}42\pm0{\cdot}08^{bc}$	$0{\cdot}02\pm0{\cdot}001^{a}$	NS
Lactococcus lactis C2	ND	ND	$2{\cdot}98\pm0{\cdot}4^{a}$	NS
Lactococcus lactis HP952	ND	$0{\cdot}2\pm0{\cdot}1^{c}$	ND	NS
Lactococcus lactis 18-16 (Cit^{+})	ND	ND	ND	NS
Lactobacillus helveticus NCD087	ND	$0{\cdot}13\pm0{\cdot}4^{c}$	$0{\cdot}19\pm0{\cdot}006^{b}$	NS
Lactobacillus helveticus NCDO1243	$1{\cdot}83\pm0{\cdot}08^{Aac}$	ND	$163{\cdot}85\pm40^{B}$	*
P	***	***	***	

Values in a row followed by the same capital letter are not significantly different ($P>0{\cdot}05$). Values in a column followed by the same lowercase letter were not significantly different. *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$. ND – not detected; NS – not significant

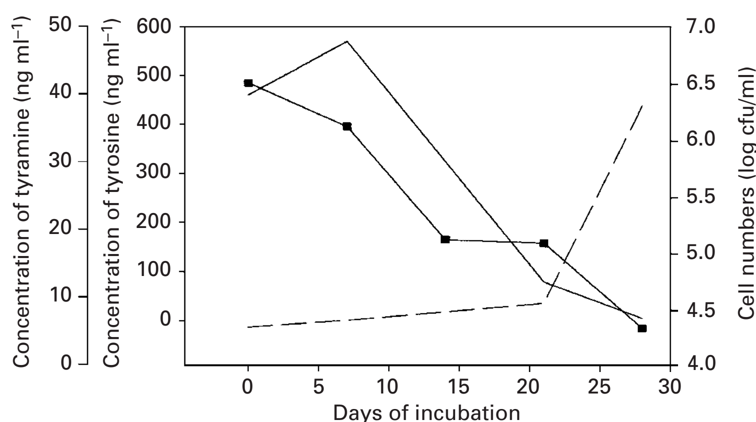

Fig. 1. Changes in medium containing 5 mM tyrosine inoculated with *Corynebacterium* sp. subgroup *flavescens* CA8. ■ cell numbers (log cfu/ml); — concentration of tyrosine (ng/ml); – – concentration of tyramine (ng/ml).

growth. *Corynebacterium* sp. subgroup *flavescens* CA8 was the only sample to show a decrease in tyrosine and a corresponding increase in tyramine concentrations (Fig. 1). The concentration of tyrosine increased initially (from 460 to 570 ng/ml) then decreased to a final value of ~9 ng/ml. The concentration of tyramine increased almost 8-fold over the same period.

In this study, the majority of strains screened had decarboxylase and transaminase activities on the three aromatic amino acids. Following incubation of strains under cheese-like conditions, *Corynebacterium* sp. subgroup *flavescens* CA8 which had been incubated with tyrosine, was the only strain which caused an increase in concentrations of biogenic amines (tyramine). It appears that the strains studied here may not have the potential to produce biogenic amines from aromatic amino acids used in the model system in this study. It would be useful to perform further experiments in a real cheese with a controlled microflora.

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