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Metabolism of amino acids by resting cells of non-starter lactobacilli in relation to flavour development in cheese

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Abstract

Amino acid metabolism by one strain each of *Lactobacillus casei* and *Lactobacillus paracasei* subsp. *paracasei* in resting cell suspensions was studied. The experiment was performed under cheese like conditions in terms of pH, salt concentration, temperature and carbohydrate starvation using a mixture of L-amino acids as substrate. The effect of supplementing the amino acids mixture with α-ketoglutarate was estimated. Asparagine, serine and glutamine were utilised in the suspension with only amino acids. In the suspension with both amino acids and α-ketoglutarate, the degradation of leucine and lysine was observed. Production of metabolites that could be important for cheese flavour such as carbon dioxide, ammonia, organic acids and volatiles was measured. The suspensions with amino acids were characterised by high production of acetoin and ammonia and both increased even more when α-ketoglutarate was added. Carbon dioxide was produced in high amounts in the suspension with both amino acids and α-ketoglutaric acid.

Keywords: Amino acid metabolism; Non-starter Lactobacilli; Cheese flavour

1. Introduction

Catabolism of amino acids by the starter and non-starter microflora is involved in cheese flavour formation. Catabolic reactions such as deamination, decarboxylation, transamination and side chain modification may yield keto acids, ammonia, amines, aldehydes, acids and alcohols, which are essential compounds in cheese taste and aroma (Hemme, Bouillane, Metro, & Desmazeaud, 1982).

Amino acid catabolism has been studied in starter lactococci (Roudot-Algaron & Yvon, 1998; Yvon, Thirouin, Rijnen, Fromentier, & Gripon, 1997; Gao et al., 1997). However, relatively little is known about the ability of non-starter lactobacilli to metabolise amino acids. These are the predominant microorganisms in mature Cheddar (Fox, McSweeney, & Lynch, 1998). The addition of selected lactobacilli to cheese milk with the objective to accelerate or improve cheese quality resulted in increased levels of free amino acids in cheese, accompanied by increased flavour intensity (Muir, Banks, & Hunter, 1996; Trepanier, El Abboudi, Lee, & Simard, 1992; Fernandez-Espla & Fox, 1998).

It is uncertain which source of energy is used by the non-starter lactobacilli during their growth in cheese after lactose depletion. A number of lactobacilli were shown to utilise some amino acids and peptides as an energy source in addition to galactose and ribose residues, *N*-acetyl-galactosamine and sialic acid, derived from nucleic acid and casein degradation (Williams, Withers, & Banks, 2000).

Transamination was described to be one of the possible mechanisms for amino acid breakdown by lactococci in cheese (Gao et al., 1997; Yvon et al., 1997). α-Ketoglutarate was found to be an effective amino group acceptor for lactoccocal aminotransferases and enhanced the conversion of amino acids to flavour compounds in experimental cheeses (Yvon, Berthelot, & Gripon, 1998).

Most publications about amino acid metabolism by lactic acid bacteria focus on catabolism of single amino acids by cell-free extracts and demonstrate that some cheese microorganisms have the enzymatic potential to carry out reactions leading to the formation of cheese flavour compounds (Christensen, Dudley, Pederson, & Steele, 1999). However, the conditions in cheese are

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more complex since cheese contains proteins, peptides, amino acids and bacterial cells that are able to grow under stress conditions in an environment lacking fermentable carbohydrates.

In this study, the amino acid metabolism by whole cells of two non-starter lactobacilli in an amino acid mixture was investigated. Our study focused on the potential products of amino acid metabolism including organic acids and volatile compounds, since these might significantly contribute to cheese flavour. The other objective was to evaluate the effect of α-ketoglutarate addition on amino acid catabolism and formation of metabolites.

2. Materials and methods

2.1. Bacterial strains and media

Two strains of non-starter mesophilic and facultatively heterofermentative lactobacilli were used. *Lactobacillus paracasei* subsp. *paracasei* INF-15D (APILAB PLUS ver. 3.2.2., BioMèrieux, France) isolated from Norvegia cheese was obtained from the culture collection of the Department of Food Science, Agricultural University of Norway, Aas, Norway (Narvhus, Hulbaekdal, & Abrahamsen, 1993). *Lactobacillus casei* 2756 isolated from Cheddar cheese was a gift from the Department of Food Chemistry, University College, Cork, Ireland (Gobbetti, Fox, & Stepaniak, 1996). The cultures were maintained in MRS broth (Difco Lab., Detroit, USA), kept in refrigerator at 4°C and subcultured weekly.

2.2. Preparation of resting cell suspension

Lactobacillus strains were subcultured twice in MRS broth, at 30°C before inoculation. Bacteria were grown overnight in static culture without mixing to minimise aeration in 2 L of MRS broth, in screw-capped bottles (1% inoculum) at 30°C. The growth of bacteria was monitored by optical density at 650 nm (OD_{650}) using a Shimadzu UV-210 spectrophotometer (Shimadzu Corp., Kyoto, Japan). Cells in late logarithmic phase were harvested by centrifugation ($6000 \times g$, 10 min, 4°C), washed twice in 0.2 M sodium phosphate buffer (deareated by heating for 30 min at 70°C, pH 5.6) and resuspended in 100 mL of the appropriate experimental solutions A, B and C as described below. The final cell density was 10^9 cfu mL^{-1} in all experiments performed.

2.3. Experimental design

Three different experimental solutions A, B and C were used. Solution A consisted of phosphate buffer 0.2 M (pH 7.2) supplemented with L-amino acids (mixture of aspartic acid, glutamic acid, asparagine, serine, histidine, glycine, threonine, alanine, arginine, tyrosine, valine, methionine, tryptophan, phenylalanine, leucine, isoleucine, lysine and proline). The concentration of each amino acid was 2 mmol L^{-1} and this was equal to the average concentration of free amino acids found in a six-month old Gouda cheese (Engels & Visser, 1996). NaCl (1.5% w/v) was added to the buffer and the pH was adjusted with lactate to 5.6, similar to conditions found in ripened cheese. The solution was filter-sterilised (0.2 μm pore-size syringe filter, Advantec MFS, Inc.). Solution B contained the compounds of solution A supplemented with 0.9 mg mL^{-1} of α-ketoglutarate (Yvon et al., 1998). Solution C was blank and did not contain any added amino acids or α-ketoglutarate.

All solutions had the same pH and salt content, however the amount of added lactate to adjust pH differed slightly. The experiments with all three solutions were repeated twice for both strains and all analyses were performed in duplicate. 10 mL of resting cells were dispensed into vials, sealed and incubated for 144 h in a water bath at 14°C. Amino acids and α-ketoglutarate were purchased from Sigma-Aldrich, USA.

2.4. Analytical methods

Ammonium production was measured by Nessler's reagent (Merck, Darmstadt, Germany) as described by Brendehaug and Langsrud (1985) with the following modifications: 0.5 mL of the cell suspension was deproteinized by 1 mL of trichloroacetic acid (10%) and centrifuged in Micromax centrifuge (IEC, Needahm Heights, MA, USA) at $4000 \times g$ for 10 min. To 0.5 mL supernatant was added 2.5 mL distilled water and 2.0 mL diluted Nessler's reagent (1 : 5 with 0.1 M KOH). Light absorption was measured by a Shimadzu UV-210 spectrophotometer at 490 nm after 10 min. A solution of NH_4Cl (3 μmol mL^{-1}) was used for preparation of the standard curve. The ammonium content was determined at time 0 and after 144 h.

Quantitative analysis of carbon dioxide was performed by measuring the production of CO_2 in 10 mL of resting cells with an infrared gas analyser (ADC 225 Mk3, Analytical development Co. Ltd., Hoddesdon, Hertfordshire, UK) as described by Narvhus, Hulbaekdal, Baugeroed, and Abrahamsen (1991). The carbon dioxide production was measured every 24 h.

Organic acids were analysed every 24 h by high performance liquid chromatography (Perkin Elmer Series 410, Norwalk, USA) according to Marsili, Ostapenko, Simmons, and Green (1981) as modified by Narvhus, Osteraas, Mutukumira, and Abrahamsen (1998).

Volatile compounds were analysed using headspace gas chromatography (Gas Chromatograph 5300, Carlo

Erba, CE Instruments; Headspace Autoanalyser, Dani 3950, Lab-Data; Hydrogengenerator, Whatman 75–32, KeboLab; Interface Series 900, Perkin Elmer; Data Programme version 4.1, Perkin Elmer) according to the method described by Narvhus et al. (1998) with minor modifications. 10 mL of resting cells were transferred into a headspace vial and sealed with a Teflon-coated septum and aluminium ring. After 144 h of incubation, vials were equilibrated for 45 min at 50°C before the headspace sampling. The gas chromatography was performed with a gradient of 53°C for 1 min, an increase by 15°C min^{-1} to 70°C that then was kept for 2 min, a second increase by 22°C min^{-1} to 130°C that was held for 3 min. The identification of compounds was indicated by analysing reference standards (Sigma-Aldrich, USA).

Amino acids were analysed at time 0 and after 144 h by reverse phase HPLC (Perkin Elmer Auto Injector ISS 200, Pump LC Series 410, Fluoroscence Detector LC 240, Column Oven Series 200, Series Interface 900) with precolumn derivatisation, with OPA and FMOC using the method described by Bütikofer and Ardö (1999). The reagents were purchased from Hewlett Packard Agilent Technologies and KeboLab (Oslo).

Bacteria were enumerated on LBS™ agar (Becton Dickinson & Co., Cockeysville, USA) after anaerobic incubation at 30°C for 48 h. The extent of autolysis was estimated by measuring the decrease in OD_{650} after 144 h. Results were expressed as a percentage calculated by the formula described by Kang, Vezinz, Laberge and Simard (1998).

Statistical analysis of organic acids was performed by principal component analysis (PCA) using Unscrambler 7.5 (Camo, Oslo, Norway). The data were validated using cross validation and variables were standardised in order to give all the variables equal variance and thereby equalise the influence of each variable on the estimation of the components. One-way analysis of variance (ANOVA) was performed on the results from amino acids, volatiles, organic acids and carbon dioxide analysis using Minitab for Windows 95, release 12.22 (Minitab Inc., State College, PA, USA).

3. Results

3.1. Amino acids

The differences in the amino acid contents between time 0 and after 144 h, in the resting cell suspension of *L. paracasei* subsp. *paracasei* INF-15D and *L. casei* 2756 is shown in Fig. 1. Co-elution of tryptophan and isoleucine interfered with precise determination of these two amino acids.

Both strains reduced the amount of asparagine, serine and glutamine in suspension A. In solution B, (α-ketoglutarate added) a decrease of lysine, leucine and tryptophan or isoleucine or both was found for both strains. In addition, valine was utilised by *L. paracasei* and methionine by *L. casei*. The blank suspension of resting cells (*C*) had no added amino acids and thus no degradation was found. However, small amounts of amino acids were detected already at time 0, probably due to lysis or leakage from the bacterial cell, resulting in release of intracellular amino acids into the sample.

In addition to amino acid degradation, an increase in certain amino acids was observed (Fig. 1). In all three solutions tested, the amounts of proline and alanine showed a significant increase ($p < 0.05$). A higher content of aspartic acid was found in solution A and of glutamic acid in both suspensions with added amino acids (A and B). Additionally, in suspension A and C of *L. casei* the level of glycine and lysine, respectively, increased and in the suspensions of *L. paracasei*, methionine increased in A, and both histidine and phenylalanine in B.

No significant changes in the content of arginine and threonine were observed in any of the three suspensions of the strain tested.

3.2. Organic acids

The development of pyroglutamate, pyruvate, lactate and acetate was determined (periodically) every 24 h. Fig. 2 shows the score plot from the PCA for organic acids, produced by *L. casei* 2756 (X) and *L. paracasei* subsp. *paracasei* INF-15D (Y) at time 24, 48, 72, 96,120 and 144 h in solutions A, B and C. The score plot explains 75% of the total variance. The clustered samples with the *L. casei* strain in solution B were characterised by a high amount of pyroglutamic acid and pyruvic acid during incubation. The *L. casei* strain differed markedly from the *L. paracasei* strain under similar conditions. The amount of lactate increased with time in all samples of solution A and B and slightly in C, for both strains tested. Higher amounts of acetate were associated with suspension C (blank). The samples of solution C were clearly separated from the solutions, which contained amino acids (A and B).

Fig. 3 shows the production of pyruvate by *L. casei* and *L. paracasei* in solutions A, B and C during the incubation (average values from duplicate samples). The initial content of pyruvic acid drastically decreased during 24 h and then slightly increased after 96 h. This increase was observed only for suspensions A and B and was not found in solution C.

3.3. Volatile compounds

Volatile compounds considered to be important for cheese flavour such as acetoin, acetone, acetaldehyde and ethanol were detected. In addition, compounds such as 3-methylbutanal and 2-methylbutanal, originating

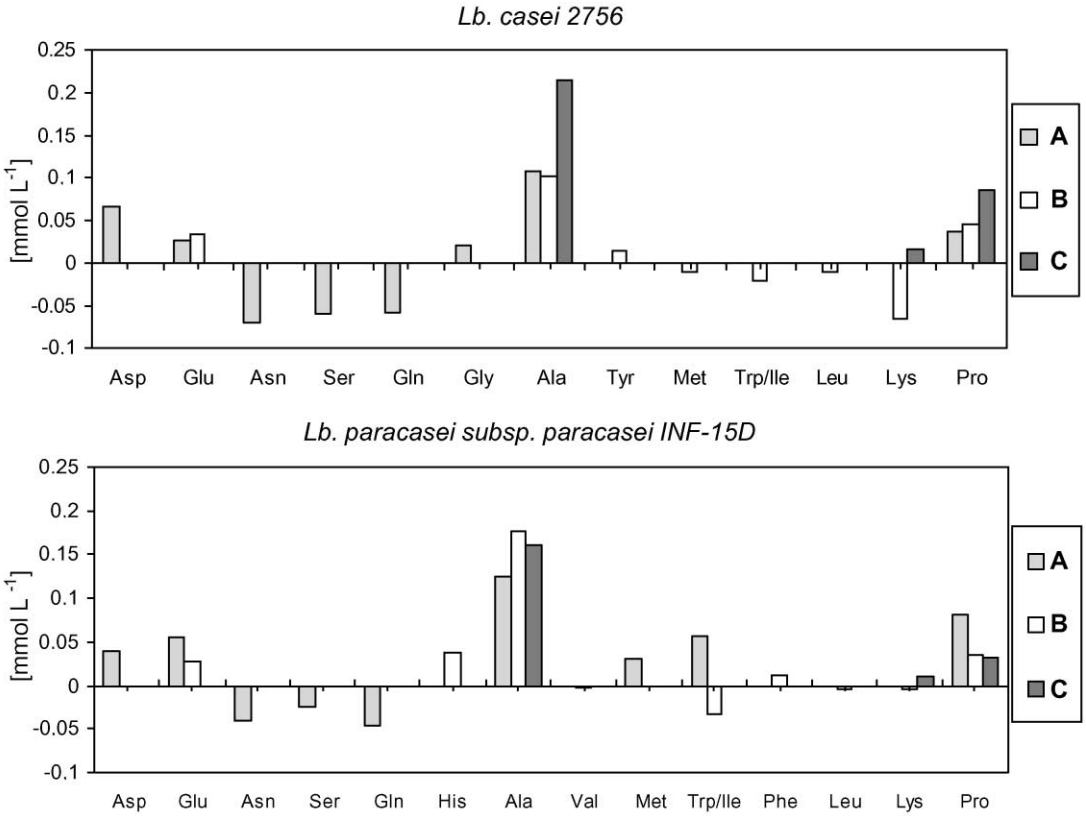

Fig. 1. The difference in amino acid content between time 0 and 144 h in resting cell suspension of *L. casei* 2756 and *L. paracasei* subsp. *paracasei* INF-15D in solutions A, B and C. The values are given only for the amino acids, whose concentration was significantly changed during the experimental time. The data represent the means for two replicates with analysis performed in duplicate. The relative standard deviation was $<21\%$.

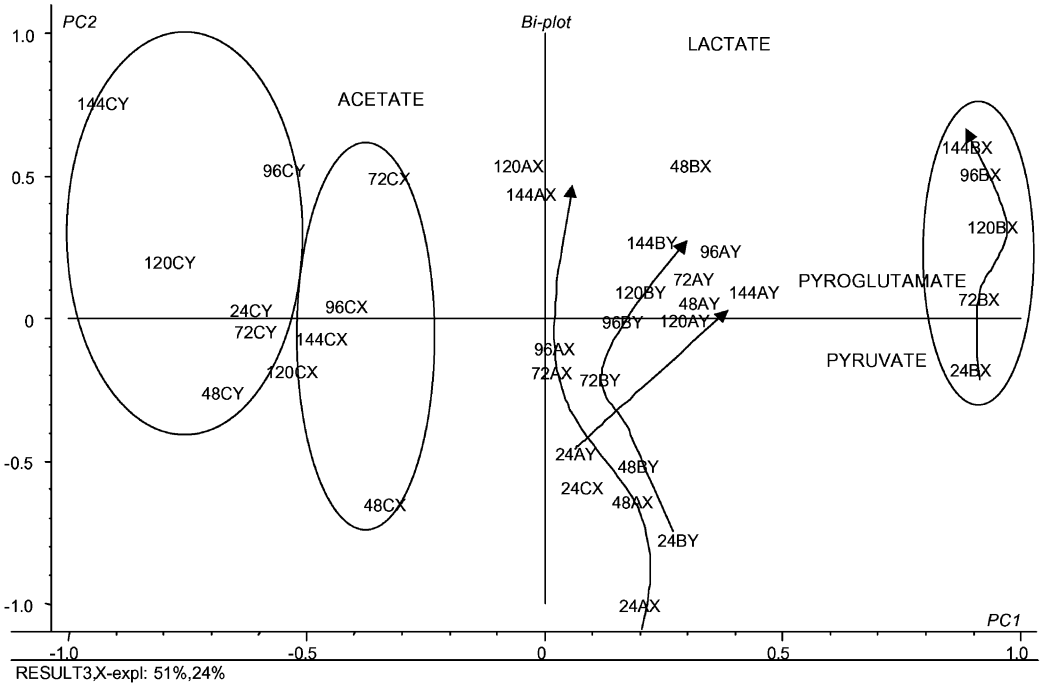

Fig. 2. The score plot from principal component analysis (PCA) of organic acids production during the experimental time (24, 48, 72, 96, 120, 144 h) by *L. casei* 2756 (X) and *L. paracasei* subsp. *paracasei* INF-15D (Y) in solutions A, B, and C.

from leucine and isoleucine, respectively, were determined. The production of volatile compounds after 144 h is shown in Figs. 4 and 5.

High amounts of acetoin were detected in the solution supplemented with α-ketoglutarate (B). The average value determined for acetoin was 40 and 48 mg L^{-1} for *L. casei* and *L. paracasei,* respectively. The acetoin production in suspension A was also higher in comparison to the blank.

Low amount of acetone was produced in solutions A and C (blank), while remarkably high concentrations (0.8 to 0.9 mg L^{-1}) were detected in solution B. The blank suspension (C) contained more acetaldehyde than the solution A and B. Ethanol production varied

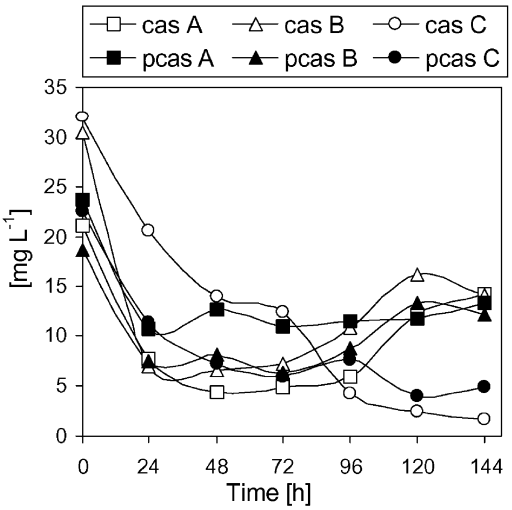

Fig. 3. The production of pyruvic acid by *L. casei* 2756 (CAS) and *L. paracasei* subsp. *paracasei* INF-15D (PCAS) during the experimental time in solutions A, B and C. The relative standard deviation was <18%.

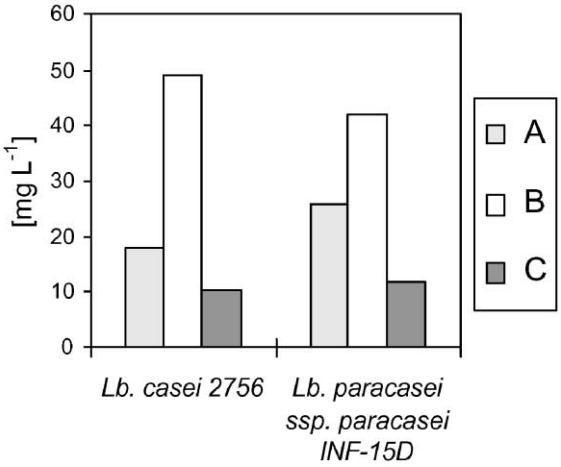

Fig. 4. Production of acetoin by *L. casei* 2756 and *L. paracasei* subsp. *paracasei* INF-15D in solutions A, B and C. The relative standard deviation was <20%.

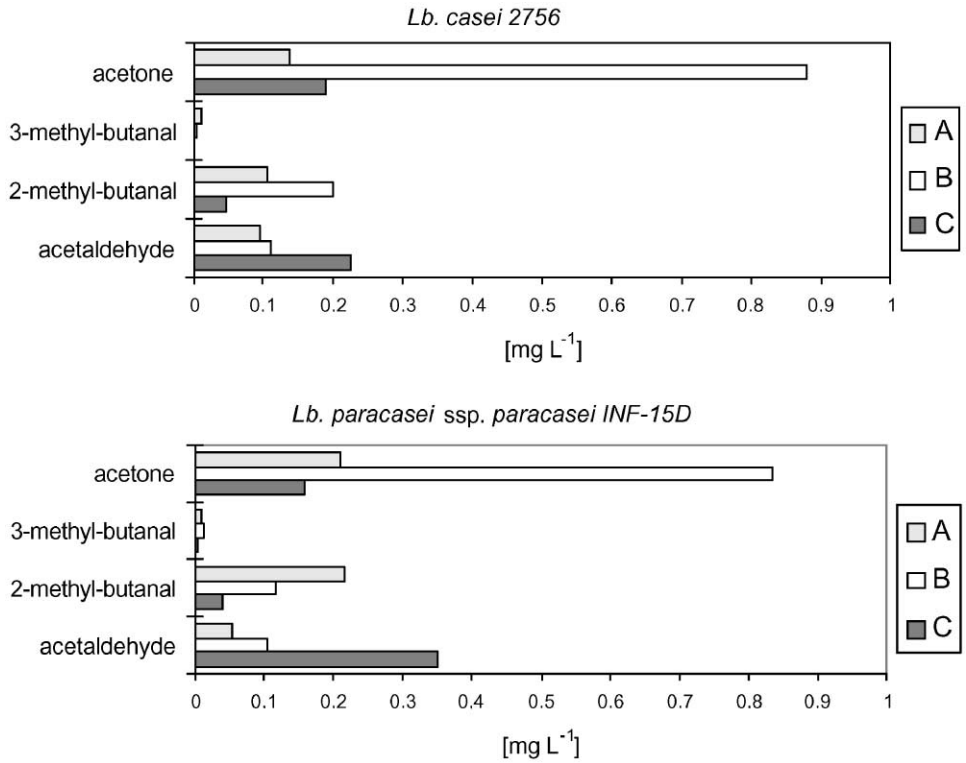

Fig. 5. Production of acetone, 3-methylbutanal, 2-methylbutanal and acetaldehyde by *L. casei* 2756 and *L. paracasei* subsp. *paracasei* INF-15D in solutions A, B and C. The relative standard deviation was <19%.

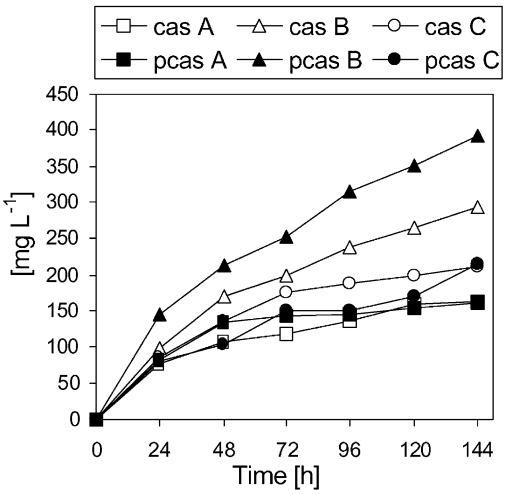

Fig. 6. Carbon dioxide production by *L. casei* 2756 (CAS) and *L. paracasei* subsp. *paracasei* INF-15D (PCAS) in solutions A, B and C. The relative standard deviation was $<14\%$.

between samples, but no correlation was found in relation to type of solution or bacterial strain (data not shown).

L. paracasei produced higher amounts of 2-methylbutanal in solution A, which in contrast to *L. casei* produced higher amounts in solution B. The amount of 3-methylbutanal produced by the two strains was below $0.1\,mg\,L^{-1}$ in suspensions A and B. The corresponding alcohols of 3-methyl-1-butanol and 2-methyl-1-butanol were present but in concentration below limit allowing quantification.

An increased production of ammonia occurred in solutions supplemented with amino acids. In solution A, *L. casei* produced $0.81\,mmol\,L^{-1}$ of ammonia while the *L. paracasei* strain produced $0.65\,mmol\,L^{-1}$. In solutions B, production of ammonia increased slightly and reached the concentration of $0.86\,mmol\,L^{-1}$ for the *L. casei* and $0.90\,mmol\,L^{-1}$ for the *L. paracasei* strain. The amount of ammonia determined in solution C was below $0.40\,mmol\,L^{-1}$.

3.4. Carbon dioxide

For both *L. casei* and *L. paracasei* considerably higher amounts of carbon dioxide were produced in solution B with α-ketoglutaric acid (Fig. 6). No significant difference ($p<0.05$) in carbon dioxide production was found between suspension C (blank) and suspension A. In solution B, *L. paracasei* produced higher amounts of carbon dioxide than the *L. casei*.

3.5. Autolysis and growth of bacteria

Only a slight decrease in numbers of lactobacilli was observed in the suspensions after 144 h. Furthermore, the extent of autolysis of bacterial cells due to the unfavourable physiological condition (no source of carbon in the medium) was determined to be in the range of 8–17% for both strains in solutions A, B and C.

4. Discussion

Direct examination of the influence of different species and strains of lactic acid bacteria on cheese ripening and flavour development is difficult due to the high complexity of the cheese interior. We therefore investigated amino acid catabolism in a model system using resting cell suspensions. The experiment was performed under conditions similar to those in cheese in terms of pH, temperature and carbohydrate starvation.

For the two strains tested, a decrease in serine, asparagine and glutamine content was found in the suspension containing a mixture of amino acids without α-ketoglutarate. As reported by Kristoffersen (1956), *L. casei* could deaminate serine and asparagine at pH levels similar to those of cheese ripening, releasing ammonia in considerable amounts. In the same study, addition of free DL-serine to the cheese-curd increased the number of lactobacilli and this implicated the use of serine as an energy source. In the work of Crow, Liu, and Holland (1998), serine and arginine were utilised by some of non-starter lactic acid bacteria. However, none of the two strains in our experiment was found to degrade arginine in the solutions tested. *L. brevis* has been shown to utilise glutamine and asparagine during the fermentation of wheat dough (Collar, Mascaros, Prieto, & Debarber, 1991).

The addition of α-ketoglutarate (solution B) changed the pattern of amino acids utilisation, and bacterial use of both leucine and lysine was indicated. In addition, either tryptophan or isoleucine was used, but these two amino acids co-eluted and further experiments are needed for any conclusions to be drawn. Transamination was suggested to be the first step in the conversion of amino acids to flavour compounds by starter lactoccoci in cheese (Yvon et al., 1997). Successive transamination of tryptophan under cheese like conditions has earlier been reported for *L. casei* by Gummalla and Broadbent (1999). The decrease in content of some amino acids in a solution supplemented with α-ketoglutarate indicates that transamination might also be active in the investigated lactobacilli strains. The aldehydes 3-methylbutanal and 2-methylbutanal that originate from catabolism of isoleucine and leucine, respectively, were found in the samples, but not exclusively in those with decreased amounts of the two amino acids indicating a more complicated picture.

The results of our study indicated that *L. casei* 2756 could degrade methionine in the presence of α-ketoglutarate showing a potential importance in the generation of sulphur flavour in cheese.

The amino acids alanine, lysine and proline were detected in the control suspension (C) and increased more than any other amino acid in the other two suspensions (A and B). They could have been released to the solution due to cell lysis or leakage, as reported in a similar experiment by Brendehaug and Langsrud (1985). Bacterial cells exposed to osmotic stress tend to accumulate several amino acids to high intracellular concentrations (Kempf & Bremer, 1998). In *Lactobacillus plantarum*, an increase in glutamate, proline, alanine and glycine has been observed (Glaasker, Konings, & Poolman, 1996). Exposure of *L. acidophilus* to osmotic stress also resulted in significant increase in the concentration of the intracellular proline (Jewell & Kashket, 1991). Therefore, the higher quantities of proline and alanine found in this experiment may possibly be caused by osmotic stress and their subsequent release to the solution by cell lysis or leakage.

The analysis of organic acids showed a significant difference between experimental suspensions. Higher amounts of pyroglutamic acid were produced in samples containing α-ketoglutarate since transamination with α-ketoglutarate as an amino group acceptor, leads to the formation of glutamic acid, which further may be enzymatically converted to pyroglutamic acid (Tschager & Jager, 1988). The slight increase of pyruvate in suspensions supplemented with amino acids after 96 h might be caused by amino acid deamination (Hemme, Bouillane, Metro, & Desmazeaud, 1982).

Increased amounts of acetoin were detected in samples supplemented with amino acids. In the work of Ott, Germond, and Chaintreau (2000) the depletion of free branched-chain amino acids by yoghurt starter cultures was shown to be linked to the production of acetoin and vicinal diketones. Aldehydes were mainly produced in suspensions supplemented with amino acids, however, acetaldehyde was found at the highest concentration in the blank. No difference in carbon dioxide production was found between samples C and samples A, and therefore, decarboxylation of amino acids could occur only to a very limited extent in our experiments. As described by McSweeney and Sousa (2000) transamination of free amino acids results in the formation of intermediate imides that are subsequently degraded by decarboxylation or by Strecker reaction, yielding aldehydes such as 3-methylbutanal and 2-methylbutanal. This process releases CO_2, which may explain its increased production in samples supplemented with α-ketoglutarate. Another explanation could be decarboxylation of acetolactate leading to high production of acetoin in suspension B.

The experiments were carried out at low temperature and acid pH resulting in low metabolic activity, but still a significant decrease in amino acid content was found for both lactobacilli strains tested. Besides, compounds important for cheese flavour development were detected. Although the experimental conditions differed from the cheese environment in terms of anaerobicity and water activity, the results indicate that non-starter lactobacilli might play a role in cheese flavour formation.

Acknowledgements

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References

Brendehaug, J., & Langsrud, T. (1985). Amino acids metabolism in propionibacteria: resting cells experiment with four strains. *Journal of Dairy Science*, *68*, 281–289.

Bütikofer, U., & Ardö, Y. (1999). Quantitative determination of free amino acids in cheese. *Bulletin of International Dairy Federation*, *337*, 24–32.

Christensen, J. E., Dudley, E. G., Pederson, J. A., & Steele, J. L. (1999). Peptidases and amino acid catabolism in lactic acid bacteria. *Antonie van Leeuwenhoek*, *76*, 217–246.

Collar, C., Mascaros, A. F., Prieto, J. A., & Debarber, C. B. (1991). Changes in free amino acids during the fermentation of wheat doughs started with pure cultures of lactic-acid bacteria. *Cereal Chemistry*, *68*(1), 66–72.

Crow, V. L., Liu, S-Q., & Holland, R. (1998). The diversity of amino acids utilisation and ethyl butyrate formation by dairy bacteria. *Australian Journal of Dairy Technology*, *53*, 118.

Engels, W. J. M., & Visser, S. (1996). Development of cheese flavour from peptides and amino acids by cell-free extracts of *Lactococcus lactis* subsp. *cremoris* B78 in a model system. *Netherlands Milk and Dairy Journal*, *50*, 3–1117.

Fernandez-Espla, M. D., & Fox, P. F. (1998). Effect of adding *Propionibacterium shermanii* NCDO 853 or *Lactobacillus casei* subsp. *casei* IFPL 731 on proteolysis and flavour development of Cheddar cheese. *Journal of Agriculture and Food Chemistry*, *46*, 1228–1234.

Fox, P. F., McSweeney, P. L. H., & Lynch, C. M. (1998). Significance of non-starter lactic acid bacteria in cheddar cheese. *Australian Journal of Dairy Technology*, *53*, 83–89.

Gao, S., Oh, D. H., Broadbent, J. R., Johnson, M. E., Weimer, B. C., & Steele, J. L. (1997). Aromatic amino acid catabolism by lactococci. *Lait*, *77*(3), 371–381.

Glaasker, E., Konings, W. N., & Poolman, B. (1996). Osmotic regulation of intracellular solute pools in *Lactobacillus plantarum*. *Journal of Bacteriology*, *178*(3), 575–582.

Gobbetti, M., Fox, P. F., & Stepaniak, L. (1996). Esterolytic and lipolytic activities of mesophilic and thermophilic lactobacilli. *Italian Journal of Food Science*, *2*, 127–135.

Gummalla, S., & Broadbent, J. R. (1999). Tryptophan catabolism by *Lactobacillus casei* and *Lactobacillus helveticus* cheese flavour adjuncts. *Journal of Dairy Science*, *82*(10), 2070–2077.

Hemme, D., Bouillane, C., Metro, F., & Desmazeaud, M. J. (1982). Microbial catabolism of amino acids during cheese ripening. *Sciences Aliments*, *2*, 113–123.

Jewell, J. B., & Kashket, E. R. (1991). Osmotically regulated transport of proline by *Lactobacillus acidophilus* IFO-3532. *Applied and Environmental Microbiology*, *57*(10), 2829–2833.

Kang, O. J., Vezinz, L. P., Laberge, S., & Simard, R. E. (1998). Some factors influencing the autolysis of *Lactobacillus bulgaricus* and *Lactobacillus casei*. *Journal of Dairy Science*, *81*, 639–646.

Kempf, B., & Bremer, E. (1998). Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Archives of Microbiology*, *170*(5), 319–330.

Kristoffersen, T. (1956). Degradation of amino acids by *Lactobacillus casei* in relation to flavour development in Cheddar cheese. *Iowa State Collage Journal of Science*, *30*, 399–400.

Marsili, R. T., Ostapenko, H., Simmons, R. E., & Green, D. E. (1981). High performance liquid chromatographic determination of organic acids in dairy products. *Journal of Food Science*, *46*, 52–57.

McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for production of flavour compounds in cheese during ripening: A review. *Lait*, *80*, 293–324.

Muir, D. D., Banks, J. M., & Hunter, E. A. (1996). Sensory properties of Cheddar cheese: Effect of starter type and adjunct. *International Dairy Journal*, *6*, 407–423.

Narvhus, J. A., Hulbaekdal, A., & Abrahamsen, R. (1993). Occurrence of lactobacilli and leuconostoc in Norwegian cheese of Gouda type and their possible influence on cheese ripening and quality. *International Dairy Journal*, *3*(4–6), 566.

Narvhus, J. A., Hulbaekdal, A., Baugeroed, H., & Abrahamsen, R. K. (1991). Measurement of CO_2 production and O_2 metabolism by pure and mixed cultures of lactic acid bacteria growing in milk. *Proceedings of the Symposium* "*Actes du Colloque Lactic 91*", *Caen*, p. 371.

Narvhus, J. A., Osteraas, K., Mutukumira, T., & Abrahamsen, R. K. (1998). Production of fermented milk using a malty compound-producing strain of *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, isolated from Zimbabwean naturally fermented milk. *International Journal of Food Microbiology*, *41*(1), 73–80.

Ott, A., Germond, J. E., & Chaintreau, A. (2000). Vicinal diketone formation in yogurt: ^{13}C precursors and effect on branched-chain amino acids. *Journal of Agriculture and Food Chemistry*, *48*, 724–731.

Roudot-Algaron, F., & Yvon, M. (1998). La catabolisme des acides amines aromatiques et des acides amines a chaîne ramigiée chez *Lactococcus lactis*. *Lait*, *78*, 23–30.

Trepanier, G., El Abboudi, M., Lee, B. H., & Simard, R. E. (1992). Accelerated maturation of Cheddar cheese: Influence of added lactobacilli and commercial protease on composition and texture. *Journal of Food Science*, *56*, 1238–1240.

Tschager, E., & Jager, H. (1988). Pyroglutamic acid in milk and milk products with specific consideration of cheese. *Milchwirtsch Berichte*, *95*, 79–83.

Williams, A. G., Withers, S. E., & Banks, J. M. (2000). Energy sources of non-starter lactic acid bacteria isolated from Cheddar cheese. *International Dairy Journal*, *10*, 17–23.

Yvon, M., Berthelot, S., & Gripon, J. C. (1998). Adding α-ketoglutarate to semi-hard cheese curds highly enhances the conversion of amino acids to aroma compounds. *International Dairy Journal*, *8*, 889–898.

Yvon, M., Thirouin, S., Rijnen, L., Fromentier, D., & Gripon, J. C. (1997). An aminotransferase from *Lactococcus lactis* initiates conversion of amino acids to cheese flavour compounds. *Applied and Environmental Microbiology*, *63*, 414–419.