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Role of Lactobacilli in Flavour Development of Cheddar Cheese

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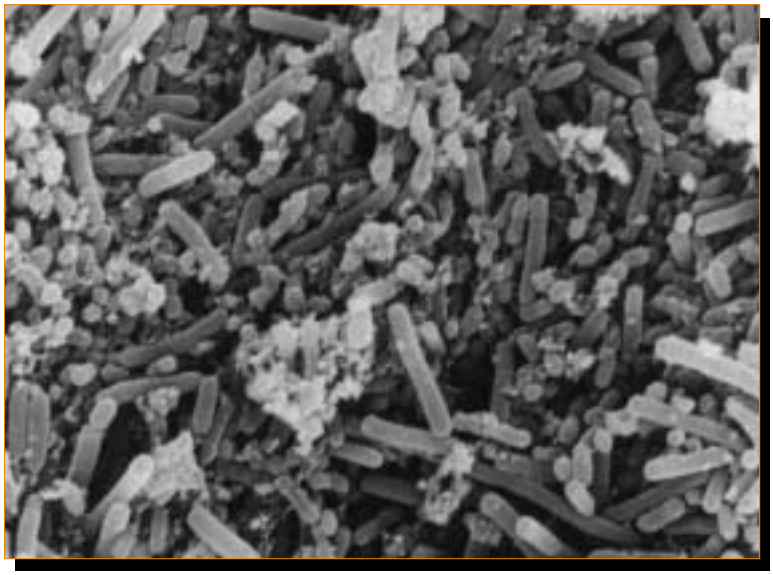
*A number of non-starter lactic acid bacteria (NSLAB) strains used in pilot scale Cheddar cheese manufacture were judged to improve cheese flavour. However, improved flavour was not obtained in follow-up commercial trials. Commercial Cheddar cheese trials with a thermophilic strain, (**Lb. helveticus DPC 4571**, see End of Project Report No. 2), as starter adjunct, also improved flavour.*



Role of Lactobacilli in Flavour Development of Cheddar Cheese

This report is based on two complementary projects:

* *The role of lactobacilli in flavour development in Cheddar cheese (ARMIS 4538)*
* *Control, acceleration and diversification of cheese flavour formation by enzymatic conversion of amino acids (ARMIS 4539)*

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Background

Cheddar cheese is a complex microbial ecosystem. The internal cheese environment, in particular of hard and semi-hard cheeses, is not conducive to the growth of many microorganisms.

At the beginning of ripening the dominant microorganisms are the starter bacteria which are present at high levels (~10^9/g). However, during ripening, non-starter lactic acid bacteria (NSLAB) grow from relatively low levels (<10^3/g) at the beginning of ripening, to 10^8/g within 6 - 8 weeks. Other bacteria, e.g. enterococci and staphylococci, may also be present but in much lower numbers.

*In a previous study of mature and extra mature Cheddar cheeses from different manufacturers (*see End of Project Report No. 1*), it was found that the NSLAB population was dominated by strains of* ***Lb. paracasei****. However, their contribution to cheese flavour and their source(s) are still unclear, nor is it known if the NSLAB flora is unique to each plant. Hence, understanding the growth of this group of organisms in cheese is a key to defining their role in flavour development.*

The biochemistry of flavour development in cheese is poorly understood. For most cheese varieties, including Cheddar, proteolysis, which results in the accumulation of free amino acids, is of vital importance for flavour development. Increasing evidence suggests that the main contribution of amino acids is as substrates for the development of more complex flavour and aroma compounds. The manner by which such compounds are generated in cheese is currently the focus of much research.

Starter bacteria have been shown to contain a range of enzymes capable of facilitating the conversion of amino acids to potential flavour compounds. However, the potential of lactobacilli (NSLAB) to produce similar enzymes has only recently been investigated.

Hence, although, it is generally accepted that the cheese starter flora is the primary defining influence on flavour development, the contribution of NSLAB is also considered significant.

The objectives of these studies were:

- to develop a greater understanding of the behaviour of NSLAB in cheese, and

- to identify suitable strains, and other cheese bacteria, to be used as starter adjuncts for flavour improvement.

Main Conclusions and Achievements

** Using a common starter culture, Cheddar cheese was manufactured at pilot scale using 24 independent NSLAB strains as starter adjuncts.*

** A number of the NSLAB adjuncts were judged by commercial cheese graders to improve the cheese flavour relative to the control.*

** Three of the most promising strains were selected for further pilot scale evaluation. Sensory analysis of these cheeses indicated that the experimental cheeses were similar to the controls, but with some subtle variations.*

** However, significant improvement in flavour, using these three strains, was not confirmed in follow-up commercial trials.*

** Based on studies of Cheddar cheese manufactured in commercial cheese plants, the dominant (NSLAB) strains were found to be uniformly distributed throughout the cheese, but some of the less dominant strains appeared and disappeared in the cheese during ripening. The initial levels in cheese increased throughout the manufacturing run.*

** An average of 7 strains of NSLAB were present in each cheese and the majority of strains from the same factory clustered together. This suggests that there is a 'unique' NSLAB flora associated with each factory.*

** The sugar in the milk fat globule membrane was identified as a possible energy source for NSLAB in cheese.*

** Enzymes critical to the breakdown of amino acids were found in NSLAB, providing further evidence of their role in cheese flavour development.*

** Commercial cheese trials with a thermophilic strain,* ***Lb. helveticus DPC 4571*** (see End of Project Report No. 2)*, as starter adjunct, indicated that this strain improved the flavour of Cheddar cheese.*

** In an effort to identify more of these strains, a bank of 71 thermophilic lactobacilli was assembled. Genetic typing using RAPD indicated that the* ***Lb. helveticus*** *strains clustered into 8 groups at the 80% similarity level. Four of the isolates were genetically identical to strain DPC 4571.*

Research and Results

Behaviour of NSLAB in Cheese

The RAPD technique developed during a previous study (*see End of Project Report No. 1*) was further refined during the current project and a computer-based method was applied to analyse and compare the complex data generated from the large numbers of strains. This molecular technique is based on the fact that a random primer will generate species or strain specific DNA fragments in a PCR. These DNA fragments are then separated by agarose gel electrophoresis and

the resulting fingerprints are used to type the isolates to species/strain level. The original technique was based on the use of one random primer. However, it was felt that at least two primers should be used to determine whether isolates are the same or different. Using this approach it was possible to separate 3 different strains of *Lb. paracasei* at the 80% level of similarity. In addition, species specific bands were generated by one of the primers used which allowed differentiation between *Lb. paracasei*, *Lb. rhamnosus*, *Lb. plantarum* and *Lb. curvatus* *(Fig. 1)*.

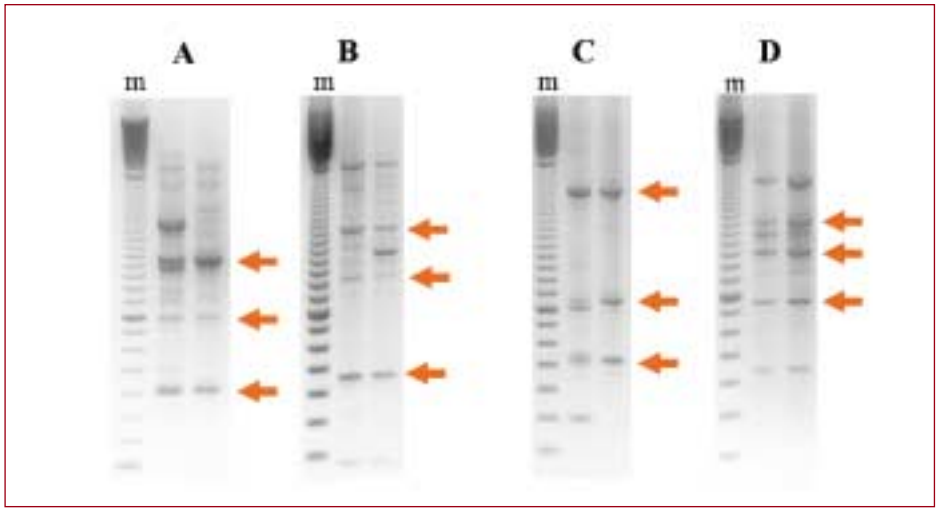

Fig. 1: RAPD profiles of strains of A: ***Lb. paracasei****, B:* ***Lb. rhamnosus****, C:* ***Lb. plantarum*** *and D:* ***Lb. curvatus****. Molecular weight markers are denoted by m. Species specific bands are indicated by arrows.*

Lactobacilli require a fermentable carbohydrate for energy production and growth but there is no obvious one in cheese. *Lb. paracasei* failed to grow on the water-soluble or insoluble fractions of Cheddar cheese unless it was supplemented with glucose. However, the strain did grow in sterilised cheese slurry, suggesting that a component of the fat phase contained growth substrates for this microorganism. The strain also grew in a basal medium containing the glycoprotein fraction of the milk fat globule membrane, implying that it can use the sugar portion of the glycoprotein as an energy source.

NSLAB levels in a commercial Cheddar plant throughout a typical day of manufacture were measured using selective and non-selective growth media. The results showed that the initial levels of NSLAB increased throughout the day and that the selective medium used, LBS, underestimated the NSLAB level. Spatial and temporal distribution of the dominant strains of NSLAB in cheese were investigated using the RAPD technique. The dominant strains were uniformly distributed throughout the cheese. However, some of the less dominant strains were not always detected suggesting that they may not be evenly distributed. The data obtained on cheese during ripening indicated that a number of species of lactobacilli were present immediately post manufacture.

*However, by two weeks of ripening **Lb. plantarum** and **Lb. paracasei** predominated. Thereafter **Lb. plantarum** decreased as a proportion of the NSLAB population while **Lb. paracasei** increased. Within 6 - 8 weeks of ripening only **Lb. paracasei** were isolated from the cheese. Strain dynamics during ripening was observed (Fig. 2).*

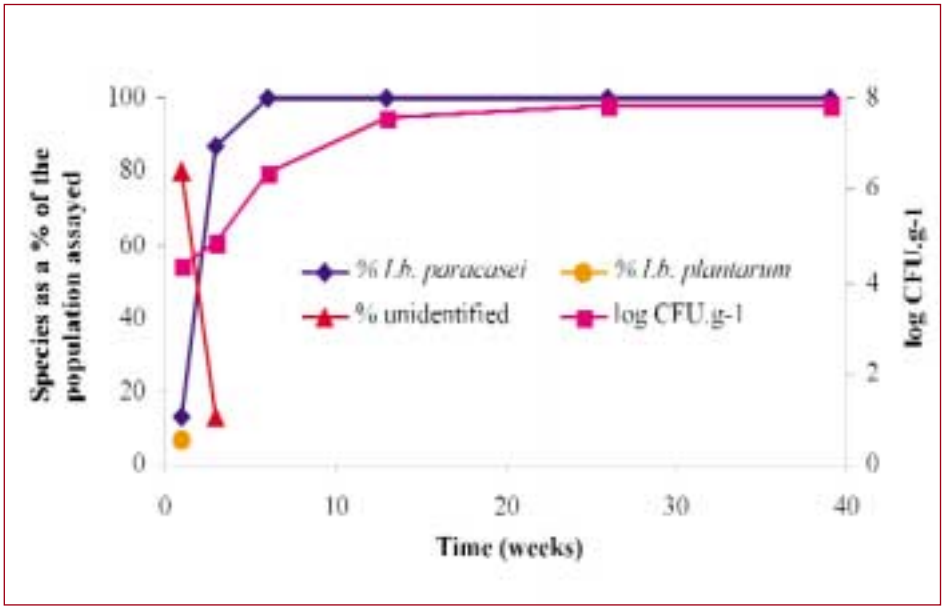

Fig. 2: Counts of NSLAB and evolution of species during the 39-week ripening period of a commercial Cheddar.

A comparison of the strains of NSLAB present in defective cheeses with those in mature and extra mature cheeses from the same manufacturer could give an important lead in elucidating the role of lactobacilli in cheese flavour development. Therefore, the refined RAPD technique was applied to studying the NSLAB flora from 9 cheeses from 3 different manufacturers. The cheeses studied included 3 mature/extra mature cheeses (including a prize-winning cheese) and 3 defective cheeses from one manufacturer.

A typical example of the data obtained for one cheese is presented in *Fig. 3*. The NSLAB flora in this cheese was composed of 4 strains present in the ratio 10:7:2:1. A total of 181 isolates from the 9 cheeses and 35 type/collection strains were separated into 43 clusters by the RAPD technique. 96% were *Lb. paracasei*, 2% *Lb. plantarum*, 0.3% *Lb. curvatus*, 0.3% *Lb. brevis* and 0.9% could not be identified. The biggest cluster contained 32 strains from the defective cheeses, one strain from a mature cheese from a different manufacturer and 6 type strains. Another cluster contained 27 strains from the defective cheeses, the prize-winning and a mature cheese from the same manufacturer and a mature cheese from a different manufacturer. Thus the same strains of lactobacilli are found in defective and mature cheeses, implying that, if they are involved in causing the defect, their role is not a direct one. Another cluster contained 23 strains, all of which were isolated from two cheeses from a different manufacturer. *Lb. plantarum* and *Lb. curvatus* strains were also found in the latter cheeses. There was an average of 7 strains of NSLAB present in each cheese and the majority of strains from the same factory clustered together. This suggests that there is a 'unique' NSLAB flora associated with each factory.

Variations in initial NSLAB numbers in commercial cheese and dynamics in populations during ripening may explain the variation in flavour, which is found within cheese made on the same day using the same starter. The same strains of lactobacilli are found in defective and mature cheeses, implying that, if they are involved in causing the defect, their role is not a direct one. Evidence that a 'unique' NSLAB flora is associated with each factory was obtained.

Amino Acid Catabolism by NSLAB

Production of flavour active compounds from amino acids is considered to play a role in cheese flavour development. The potential of NSLAB to catabolise amino acids and thus contribute to flavour development through this route is poorly understood.

Lactobacillus strains, isolated from mature Cheddar cheese, suspended in a mixture of 20 amino acids in buffer at different pH values varied in their ability to degrade amino acids; methionine in

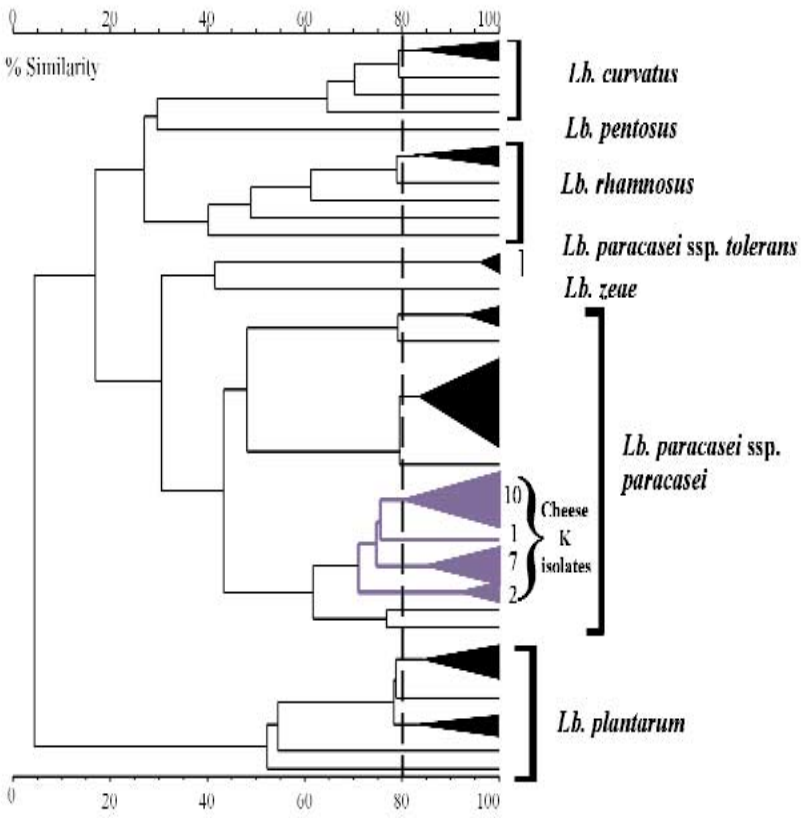

Fig. 3: Dendrogram obtained from the RAPD patterns from the NSLAB isolates from the prize-winning cheese and 35 type/collection strains. All strains over 80% limit are considered identical.

particular was degraded at pH 5.2, which is the normal pH of Cheddar cheese.

This demonstrates that NSLAB have the potential to degrade amino acids.

Amino acid catabolism involves a complex series of biochemical reactions and thus a wide range of enzymes is necessary to support such reactions. Amino transferase (AT) is thought to be the enzyme responsible for initiating amino acid conversion to flavour compounds during cheese ripening. A range of NSLAB, e.g. *Lb. paracasei* subsp. *paracasei*, *Lb. curvatus* and *Lb. plantarum* were screened for AT activity with valine, isoleucine, leucine, tyrosine, tryptophan, phenylalanine and methionine as substrates. All strains showed AT activity but there was interstrain variation in their capacity to do so. AT activity in one *Lb. paracasei* strain with valine, leucine and isoleucine as substrates showed highest activity close to neutral pH and activity was reduced in the presence of 5% salt.

Because the breakdown products of sulphur containing amino acids, e.g. methionine, are known to produce odour active compounds, factors affecting methionine amino transferase (MAT) activity were studied in a range of NSLAB strains *(Fig. 4). Lb. paracasei* strains showed better activity at pH 5.2, the normal pH of Cheddar cheese, than at higher pH values; the effect of pH was not as marked with *Lb. plantarum* strains. MAT activity of *Lb. paracasei* strains was reduced by more than 50% in the presence of 5% salt. *Lb. paracasei* 4076 contained high levels of MAT. When this strain was grown under conditions which simulate cheese ripening, i.e. pH 5.2 and 5% salt, MAT activity was found in both aerobically and anaerobically grown cells. However, when salt was also used in the assay, MAT activity was only found in anaerobically grown cells.

The presence of other enzymatic activities such as cystathionine- γ-lyase, deaminase or L-amino acid dehydrogenase activity from methionine or the branched chain amino acids as substrate, was not detected in any of the NSLAB tested over a range of pH values.

*These data demonstrate that NSLAB strains, and **Lb. paracasei** in particular, have the potential to initiate amino acid degradation under the environmental conditions of cheese ripening.*

NSLAB Strains as Starter Adjuncts

The potential of NSLAB strains to influence cheese flavour was investigated by adding individual strains to cheese milk at the beginning of manufacture and following the resulting cheese during ripening for key indices of ripening including sensory analysis or commercial grading.

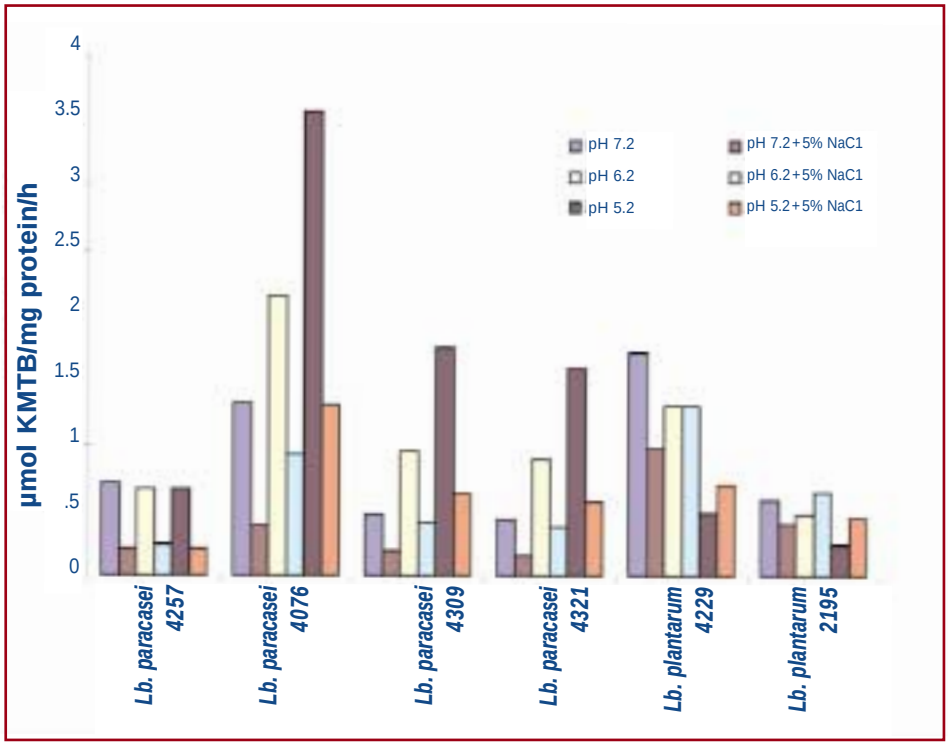

Fig 4: Influence of pH and NaCI on methionine amino transferase activity for a range of NSLAB isolated from cheese.

Twenty-four strains were used as starter adjuncts in pilot scale trials (one strain per cheese). After 9 months of ripening, 7 of these cheeses were considered superior to the control by a commercial grader from a local company, while a further 11 were judged to be equivalent. Graders from all the cheese manufacturers were invited to analyse the cheeses and this data was used to select 3 strains of *Lb. paracasei* (DPC 4076, DPC 4309 and DPC 4257) for further pilot scale trials.

Sensory analysis on the follow-on trials using DPC 4076, DPC 4309 and DPC 4257 as starter adjuncts indicated that the overall sensory attributes of the control and experimental cheeses were similar, however, subtle variations occurred. The cheese containing adjuncts scored higher than the control cheese for most attributes. At 4 months the cheese containing DPC 4076 scored highest for 10 of the 25 attributes assayed, while the cheese containing DPC 4309 scored highest for another 10 of the attributes. A similar result was obtained at 10 months of ripening with DPC 4076 containing cheese scoring highest for 10 attributes and DPC 4309 containing cheese scoring highest for 6 attributes. The adjuncts did not influence the level of proteolysis or the type of volatile compounds identified by GC/MS in the cheese during ripening; however, the NSLAB influenced the relative levels of the compounds. Commercial trials with these 3 strains did not result in any overall improvement in flavour.

Hence, NSLAB strains used as starter adjunct can influence flavour development. However, their use in commercial production needs further development where growth of endogenous flora needs to be controlled.

Thermophilic Lactobacilli as Starter Adjuncts

Previous research had indicated that *Lb. helveticus DPC 4571* autolysed in cheese and resulted in improved cheese flavour *(see End of Project Report No. 2)*. Commercial trials also resulted in

cheese of enhanced flavour and one of the cheese manufacturers has indicated that they wish to exploit this strain. A study on the peptidase system of DPC 4571 indicated that cell-free extract of this strain, grown in milk, hydrolysed 15 of 19 peptide substrates tested. In an effort to identify more strains of thermophilic lactobacilli with the potential to improve Cheddar cheese flavour, a bank of 71 strains of thermophilic lactobacilli was assembled. Genetic typing using RAPD indicated that the *Lb. helveticus* strains clustered into 8 groups at the 80% similarity level. Four of the isolates were genetically identical to DPC 4571.

These results indicate that DPC 4571 has a complex peptidase system that, through autolysis, will contribute to proteolysis during ripening and thus contribute to cheese flavour development. Identification of genetically similar strains will ensure that a bank of such strains will be available for exploitation by industry.

For further information please contact:
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