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Review

A Strategy for Cheese Starter Culture Management in Australia

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The efficient manufacture of fermented dairy products on an industrial scale requires a supply of reliable starter cultures with properties suited to desired product specifications. These cultures must be backed by relevant research and development activities. This article describes the issues involved in establishing a centre to provide starter culture R & D for a group of independent cheese manufacturing companies, and discusses a strategic approach to the management of starter cultures.

Lactic acid bacteria (LAB) and other fermentative bacteria have been used by most human societies to produce traditional fermented food products with various flavours and textures from both animal and plant-derived substrates. These bacteria have been used in food fermentations for centuries, and so are generally recognized as safe (GRAS) for use in the food industry.

Modern foodstuffs still rely very heavily on LAB fermentations for their manufacture. The manufacture of dairy products such as cheese and yoghurt constitutes a world-wide industry, and the bacterial cultures (generally known as "starter cultures") used to start these fermentations occupy a position of central importance to the industry. Because of their importance in human food, LAB strain selection and starter production have been subjected to intense research activity in North America and Europe, and in some Asian and South Pacific countries including Japan, Australia and New Zealand.

In Europe, many traditional cheeses are still made using starter cultures of undefined microbial composition handed down by artisan cheesemakers. The economic importance of these products has prompted several European governments to initiate research programs to preserve the LAB and other microflora associated with specific traditional food types. Notable examples are the regional "Appellation Controllée" French and Italian cheeses. The respective national governments have enacted legislation to ensure that these products are made using the traditional microflora.

For Cheddar cheese manufacture in the USA, United Kingdom, Ireland, Canada, Australia and New Zealand, a different approach has been taken. The technology associated with starter propagation and preparation has reached a high degree of sophistication backed by strong basic science. This technological approach, using selected strains of LAB, has been one of the main factors allowing manufacture of consistent Cheddar cheese on a large industrial scale (7, 8).

Cheese production around the world is tending towards larger manufacturing sites to gain the benefit of economies of scale, and factories making more than 30,000 tonnes of cheese per year are now common. In response to this trend, technical support for the provision of reliable starters for Cheddar manufacture has been different in various countries. Commercial starter suppliers have taken the majority share of the North American and European markets, while in Australia and New Zealand the dairy companies themselves have taken a leading role in controlling starter research and supply.

Cheese manufacture in Australia is dominated by a few companies (most based on farmer co-operatives) that have traditionally competed with each other to produce distinctive products and gain market share. This paper describes the issues involved in establishing a centre to provide starter culture research and development (R & D) support for these companies and for the broader dairy industry, and discusses a strategic approach to the management of starter cultures. This centre, Australian Starter Culture Research Centre (ASCRC) Limited, has been in operation since 1992.

Historical Background

Prior to the 1970's, most of the Cheddar-type cheese made in the world used mixed-strain starter cultures, with each culture consisting of an unknown mixture of strains from several LAB genera. In Australia and New Zealand, defined isolates of LAB were being used. Strains were selected on the basis of their fermentative attributes, such as lactic acid production rate, flavour

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characteristics (based on past cheese-making experience) and, above all, resistance to bacteriophages. These defined strains were mainly used in pairs or other multiples (*i.e.* as defined multiple-strain starters). Different strains were commonly used in rotation, in order to minimize the effects of strain-specific bacteriophage infections. Strains that were found to be sensitive to phages were replaced with new strains which had been shown to be resistant to those phages. In Australia, this strain selection exercise was carried out in the private laboratories of each factory, and its success was dependent upon the standard of laboratory conditions and the technical skill of the laboratory personnel (2). With the advent of larger production units (with higher throughputs and tighter production schedules) and facing difficulties in retaining experienced personnel in rural factories, consistent cheese manufacture was not being achieved. This resulted in significant downgrading of the products and increased production costs.

By 1990, several major Australian dairy companies had recognized that starter cultures and their management could be regarded as "pre-competitive" (*i.e.* part of an industry infrastructure that can be used for mutual benefit), and so pooled their resources to establish a common starter research centre (ASCRC). The companies support the centre on a regular subscription basis, control the direction of the R & D undertaken and benefit directly from its outcomes. ASCRC operates with a full-time staff of only nine people, and so relies heavily on collaboration in research and industry involvement in development of concepts and technologies.

Challenge of the New Organization

The objectives of ASCRC are to raise industry awareness of the importance of starters and to provide the required R & D and technical support that will allow the production of consistent good-quality cheese. Consistent performance of starter is of paramount importance, especially during highly seasonal milk production in the Australian states of Victoria, Tasmania and South Australia. A new starter system has evolved as a result of a strong partnership between ASCRC and each of its subscriber dairy companies.

Strategy of the Starter System

The basic philosophy adopted by ASCRC is to use proven strains in planned rotations, changing the strains in use only when bacteriophage attacks have become too virulent for a long run of cheese manufacture (typically thirty-six 24,000-litre vats in a row) to progress without a noticeable drop in acid production rate (*i.e.* the rate of lactose fermentation by starter bacteria). The system uses modern technology to minimize the spread of phage within the manufacturing area and, especially, to keep phage out of the bulk starter preparation area. The system has five basic elements: (1) product-oriented characterization of a collection of starters, (2) a national phage monitoring system, (3) use of selected strains in rotations and selection of phage-resistant strains, (4) a centralized supply of frozen starter and (5) a UHT-based bulk starter preparation system. The inter-relationships of various aspects of these elements are illustrated in Fig. 1.

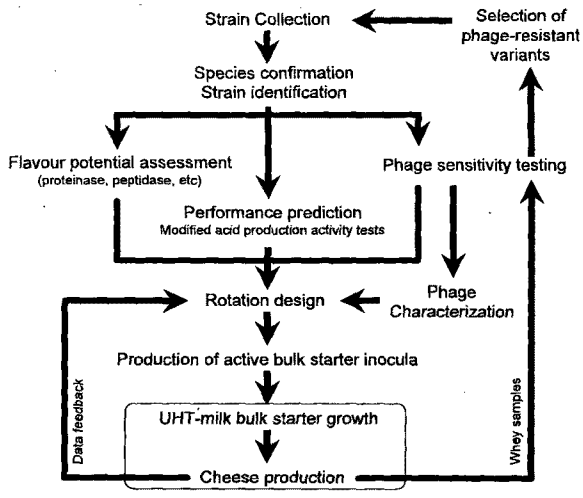

Fig. 1. Overview of the Cheddar starter system.
Boxed events occur in the cheese factory; other activities take place at ASCRC.

Strain Collection

The ASCRC approach to starter culture management depends on maintaining a large collection of well-characterized bacterial strains. Initially, subscriber cheese companies provided strains from historical collections held in their own factories. Most of these strains had been used in cheese manufacture at some time in the past fifty years, but they were generally not well characterized. Additional strains have since been obtained from local and overseas sources (including many from traditional cheeses, yoghurts and other strain collections), and the total is now over 1000 strains. The main product of ASCRC's subscriber manufacturers is Cheddar cheese, and this is reflected in ASCRC's strain collection. Most of the strains are *Lactococcus lactis* strains suitable for use in manufacture of Cheddar, but there is also a growing number of strains of other bacterial species for making other cheese types, notably including Mozzarella. This collection is the basic source of genetic diversity from which strains can be chosen for particular manufacturing applications (see Table 1 for examples).

Strain Characterization-Technological Properties

With the collection established, priority shifted away from simple accumulation of strains towards developing improved methods for laboratory characterization of strains, concentrating on properties crucial to cheese manufacture so that strains can be better understood and more effectively exploited by the industry.

Table 1. Typical starter species used for some cheeses mass-produced with defined-strain staters.

Desirable properties	Bacterial species	Comments
Fermentation of lactose to lactic acid; proteolysis	*Lactococcus lactis* subsp. *lactis* *Lactococcus lactis* subsp. *cremoris*	Mesophilic species; primary component of starters used for many soft, semi-hard and hard cheese types *e.g.* Cheddar, Gouda, Brie, Camembert, Stilton, cottage cheese.
	Streptococcus thermophilus *Lactobacillus helveticus*	Relatively thermophilic species; used for cheeses made using higher temperatures *e.g.* Mozzarella, Emmental, Gruyere.
Metabolism of citrate to diacetyl (aroma and flavour) and CO_2 ("eye" gas pocket formation)	*Lactococcus lactis* subsp. *lactis* biovar diacetylactis *Leuconostoc* sp.	[a]In cultures for Gouda-style cheese, cottage cheese; less common as a minor component of Cheddar starters.
Metabolism of lactic acid to propionic acid (important for flavour), acetic acid and CO_2 (eye formation)	*Propionibacterium* sp.	[a]In cultures for Emmental-style cheese. *Propionibacterium* is not a LAB.

[a]These species are usually only a minor component of the starter culture, and are often used as adjuncts, added to the cheese vat at low level after the addition of the primary starter species.

Tests for bacterial growth and lactic acid production rate in milk under cheesemaking conditions (based on the activity test proposed by L. E. Pearce, 1969. N. Z. J. Dairy Sci. Technol. 4: 246-247) are fundamental predictors of strain performance during cheese manufacture. Tests to investigate the specific responses of each strain to changes in temperature during and after manufacture ("cooking" and cooling) and to increases in salt concentration (salting of the curd) are under continual development. The goal is to collate a body of data for each strain that describes how rapidly it grows and lowers milk pH during manufacture, what bacterial cell numbers are likely in the pressed curd, and what further pH changes can be expected during the first days of cool storage of the cheese.

Strain Characterization-Flavour Potential

There is growing evidence that the lactococcal cell-surface proteinase, various peptidases and other enzymes involved in amino acid metabolism are major influences on the flavour and aroma of matured Cheddar cheese (reviewed by Visser [14]). The lactococcal proteolytic system plays an essential part in cheese ripening by producing small peptides and free amino acids that are precursors of cheese aroma compounds and, importantly, by degrading bitter peptides responsible for cheese flavour defects. Lack of flavour and development of bitterness remain major potential problems in industrial Cheddar cheese manufacturing. Therefore, as part of the strain selection program, lactococcal strains are tested for enzymes known to be involved in the formation and breakdown of bitter peptides.

Two major proteinase types (PI and PIII) have been distinguished on the basis of their cleavage specificities towards milk caseins (14). Bitter flavour development in cheese has been reported to be due to accumulation of bitter peptides formed mainly by the action of PI-type proteinases. In a survey of lactococcal strains used in the Australian cheese industry (1), strains were identified with PI-type proteinase activity, PIII-type, and some strains with an intermediate type.

Recent studies involving cheese experiments have suggested a debittering role for the aminopeptidase PepN (14). The PepN activity levels of typical lactococcal starter strains in use in Australia vary 10-fold (1), indicating that these strains vary in their ability to remove bitterness.

A savoury, brothy type of flavour appears to be associated with small peptides and free amino acids; other volatile products of cheese ripening are considered to be responsible for desirable characteristic cheese flavours. It is now believed that amino acid metabolism, mediated by enzymes originating from LAB, may play a major role in the formation of desirable flavour components during cheese ripening. In particular, enzymatic degradation of the amino acid methionine produces methanethiol, a volatile sulphur compound apparently important in development of Cheddar flavour.

Recent research at ASCRC has identified the lactococcal enzyme cystathionine γ-lyase, which is likely to be involved in cheese flavour formation by producing methanethiol from methionine during cheese maturation (P. G. Bruinenberg *et al.*, submitted for publication). Identification of enzymes producing specific cheese flavour compounds creates the possibility of detailed characterization of the flavour potential of starter strains, leading to improved control over cheese flavour in both existing cheese varieties and in novel dairy products.

Potential lactococcal starter strains are also tested to see whether they metabolize citrate (producing carbon dioxide, acetoin and diacetyl). Citrate-metabolizing strains (*Lactococcus lactis* subsp. *lactis* biovar diacetylactis) are

particularly suited for inclusion (with other lactococcal strains) in starter blends for Gouda manufacture, but are also growing in popularity as flavour-enhancers for Cheddar-style cheeses.

Strain Characterization-Strain and Species Identification

Many different species and strains of bacteria are used in making fermented dairy products. Starters must be properly identified, not only to ensure reproducibility of starter performance but also for maximum protection of manufacturers under "product liability" laws. Traditional species classification on the basis of microscopic analysis and phenotypic characteristics (especially sugar fermentation abilities and other biochemical properties) is very useful and remains the most widely recognized approach, but it is increasingly being augmented by molecular methods of bacterial identification (reviewed in [9]).

At ASCRC, protein profiles (the separation patterns obtained by electrophoresis of the constituent proteins of a bacterium) are being used for comparative classification of isolates at the genus and species level. This method is broadly applicable, and is used by some major international culture collections. Identification of individual strains requires DNA analysis, by comparing electrophoretic patterns of bacterial plasmids or pulsed-field electrophoresis patterns of genomic DNA restriction fragments.

These methods are in use at ASCRC for identifying starter strains and, as research tools, are being extended to non-starter bacteria isolated from cheeses and to dairy factory environmental isolates (P. J. Williams *et al.* 1995. *Proc. Eighth Aust. Food Microbiol. Conf.*, Melbourne, Australia, P8). ASCRC is also equipped for other methods of DNA analysis (*e.g.* PCR, DNA hybridization) that have great potential to be applied within the food industry to follow the changing composition of the mixed bacterial populations in fermented foods or to trace the sources of specific bacterial contaminants.

Bacteriophages

Bacteriophage infection of starter cultures can be a major problem. Continual use of the same starter strain (or the same multiple-strain culture) can allow phage numbers in a cheese factory to rise to levels that cause a significant reduction in bacterial cell numbers. Reduced cell numbers lead to a reduced overall rate of acid production, longer manufacturing times and also poor flavour development owing to the fermentation of excess milk lactose by adventitious microflora. The commercial consequences of phage infection include disruption of production schedules, reduction in product quality (and reduction in commercial value) and, in the most severe cases, abandonment of production.

Strategies for reducing the impact of bacteriophage infections in cheesemaking have been very successful in diminishing the overall production and financial losses associated with this problem (8). Improved factory design, aseptic propagation of starter cultures and better selection of starter strains have been major advances. However, phages continue to be introduced into dairy factories (pasteurization of milk does not eliminate phages), and there is always a chance that these phages will infect starter bacteria. Therefore, successful management of the phage problem continues to demand constant monitoring of the factory environment for phages.

Starter strains are not all infected by the same phages; *i.e.* strains differ in their phage sensitivity and phages differ in their host range. The use of starter rotations is a crucial phage-control measure. Strains are used in defined multiple-strain cultures (two, three or more strains per culture), using different cultures in a planned sequence. For example, a simple three-day rotation would use three different multiple-strain starter cultures, using a different starter culture each day and repeating the cycle every three days. The strains in each culture differ in their phage-sensitivity from each other and from the strains in the other cultures. Strains infected by highly-virulent phages should not be used. Reliable data on the phage population of each factory is essential for this strategy to succeed.

ASCRC regularly receives whey samples from cheese factories in many parts of Australia, and these are tested for the presence of phages using a large panel of potential host strains and methods based on detection of inhibition of acid production in starter activity tests or formation of phage plaques in agar overlay plates (11). This provides phage host-range information that is crucial for designing multiple-strain starters and strain rotations, and also gives data on the multiplication rate of phages and the extent of starter inhibition that they cause. ASCRC-sponsored research is developing more rapid and sensitive phage detection techniques based on specific antibodies and PCR technology.

Many studies have now been done to determine how many different phage types infect lactococcal starters (5). Relatively few studies have been done on phages of other LAB. At ASCRC, DNA analysis techniques (especially restriction fragment comparisons) are being applied to questions of phage epidemiology, determining the genetic relationships (and, by inference, historical relationships) between phages isolated in different cheese factories, at different times, or infecting different host strains. Such issues of phage ecology are largely unaddressed, especially in the context of an industry dispersed over a large country like Australia.

Phage-resistant Starter Strains

Phage-resistant variants (*i.e.* resistant to particular phages) can be selected from an infected strain (2, 6, 8). Variants with high resistance to a phage (apparently-com-

plete resistance to a phage, rather than merely reduced sensitivity) are sometimes difficult to obtain, and many phage-resistant isolates are either less active in milk than their parent strain or are subsequently found to be sensitive to other phages in the factory environment (2, 6). Aseptic laboratory conditions are essential in order to minimize the probability of isolating adventitious contaminants (rather than authentic variants of a strain) in this selection. At ASCRC, variants are subjected to the same testing as other starter strains to ensure their suitability for use.

Some bacterial strains carry natural conjugally-transferable genetic factors (usually genes carried on plasmids) that diminish their sensitivity to phage infection by "restriction" of phage DNA or by interfering with intracellular phage replication (4). The molecular mechanisms of this interference (often referred to as abortive infection) are not known. Strains with these genetic factors are increasingly being used world-wide as a source of phage-resistance genes for deliberate construction (via natural conjugation) of starter strains with enhanced resistance to bacteriophage infection. Starter strains carrying phage-resistance plasmids pMU1311 (10) and pNP40 (3) are in use as part of ASCRC strain rotations, and their performance is being monitored to evaluate the effectiveness of their phage resistance. Starter strains that have been in extensive industry use but have not suffered from phage infection are being considered as possible sources of other phage-resistance plasmids.

Recombinant DNA technology (*in vitro* DNA manipulation to generate genetically modified organisms) has great potential for diminishing the phage sensitivity of starters, but the food industry is understandably cautious about the use of such strains. ASCRC has not yet made use of this technology. To our knowledge, no starter suppliers anywhere in the world have introduced manipulated strains into commercial use. Ethical and safety concerns about this issue seem likely to be satisfied (at least in principle) in coming years (13). However, unless public support for recombinant DNA technology increases and food regulations are harmonized internationally, marketing and free trade could be unacceptably difficult for cheese manufacturers if they choose to use genetically-manipulated starters.

Choosing Strains for Industry Use

All relevant criteria are taken into account when strains are selected for industry use. Collating all the available data is facilitated by a computerized database. Importantly, strains are evaluated not only on their individual merits but also on the combined properties of all the strains used in a multiple-strain starter. The main goals are reliable acid production, consistent flavour (especially absence of flavour defects) and minimal inhibition by phage.

Within the range of appropriate strains available, ASCRC does not dictate the final choice of strains. Each subscriber factory is able to make its own decisions about strain use (based on advice from ASCRC) within the framework of its own product specifications. This independence of action is a major part of the operating philosophy of these manufacturers.

Production of Frozen Cultures for Factory Use

Consistent starter performance in the cheese factory cannot be achieved unless a reliable supply of starter with reproducible characteristics is available. A facility has been set up at ASCRC to produce frozen cultures of starter strains developed and characterized by ASCRC for use by the factories. These frozen cultures are used to inoculate "bulk starter" fermenters in the cheese factory. The methodology is adapted from that published by Turner *et al.* (12). Cultures are grown in 20-litre fermenters under pH control (using ammonium hydroxide as neutralizer) for 15 h at constant temperature in a medium based on hydrolysed cheese-whey powder. Cell densities of about 5×10^{10} cfu/ml are routinely obtained. The grown culture is cooled, mixed with a cryoprotectant (7% sterile lactose, final concentration), and then aseptically packaged into pre-sterilized 200 ml plastic containers ("pottles"). Starter pottles are frozen in a blast freezer capable of chilling to -40°C within 3 h, and stored long-term at -40°C. Under these conditions, no loss of starter activity has been detected after three years of storage.

The production unit is run under an ISO9002-certified quality system with a comprehensive set of quality control tests. Two percent of all production is tested for microbial purity, with checks for other LAB types, coliforms, *Salmonella*, *Staphylococcus*, enterococci, *Bacillus cereus*, *Listeria*, *Campylobacter*, *Vibrio*, anaerobic and aerobic spores, clostridial vegetative cells, yeast and moulds. In addition, starter performance is tested in small-scale simulated bulk starter preparation.

ASCRC serves factories spread over a large geographical area. Starter pottles are packed in dry ice (CO_2 ice) in cardboard boxes lined with polystyrene foam and transported by road. For transport to more distant factories (at present, the most distant factory supplied by ASCRC is nearly 2,000 km away), the boxes are sent as refrigerated cargo. Starters remain frozen in transit for more than four days when sent in this way.

The production unit currently supplies frozen starter to the main Australian bulk cheese manufacturers, contributing to an overall 80% of all Cheddar (120,000 tonnes) and 70% of all Mozzarella (13,000 tonnes) cheese produced in the country. A factory producing 25,000 tonnes of cheese per year needs less than 1000 starter pottles, equivalent to less than one month's production by the ASCRC unit.

The Bulk Starter Fermenter

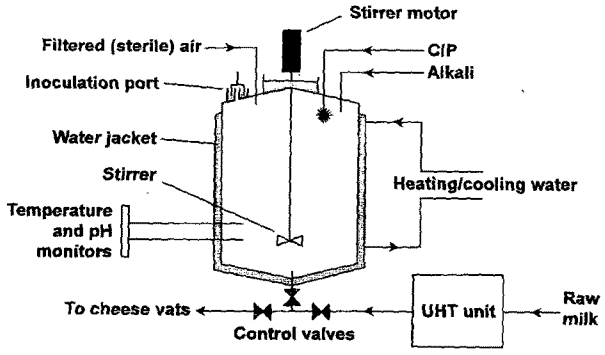

Fig. 2. Schematic of a pH-controlled bulk starter fermenter unit.

The vessel is sterilized chemically by a cleaning-in-place system. Milk is sterilized in the UHT unit and fed into the vessel at the appropriate temperature. Sterile air from a compressor is fed into the fermenter via a HEPA (High Efficiency Particulate Air) filter and is allowed to bubble out gently through the water seals around the stirrer shaft and inoculation port. Frozen pottle cultures are added through the inoculation port. A constant pH is maintained using a pH electrode connected via control circuitry to an alkali (ammonium hydroxide) dosing pump. The stirrer is kept on throughout the whole incubation period to ensure thorough mixing of the intermittent alkali additions and stirrer speed is deliberately kept low to prevent excessive air incorporation into the starter medium. At the end of the fermentation, chilled water is used to cool the bulk starter to 4°C. At this temperature, satisfactory starter activity is maintained for up to 48 h.

The factory bulk starter system supported by ASCRC (Fig. 2) is a significant refinement of earlier bulk starter technology, based on use of a stainless-steel bulk starter vessel (typically 5,000 to 15,000 liters) with an inbuilt cleaning and sterilization system (cleaning-in-place, C.I.P.), using chemical sterilization with H_2O_2/peracetic acid at room temperature. The medium used for growth of the bulk starter is UHT milk (milk sterilized by a short high-heat treatment, typically 137°C for 2 sec). Frozen starter pottles from ASCRC are used as the inoculum. A positive flow of filter-sterilized air is fed to the vessel to help exclude contaminants. During the last five years, six major factories (representing about 80% of all Australian Cheddar manufacture) have installed similar bulk starter preparation systems.

Prior to the introduction of this system, it was common practice to heat milk at 85 to 95 for 30 to 60 min within the bulk starter vessel. Such treatments subjected the steel vessel to severe stresses of heating and cooling without always achieving sufficient inactivation of phages and spores. The new system ensures the production of a microbiologically pure starter culture within the factory environment and has lower long-term costs, thanks to an expected operational life of at least twenty years. This long life expectancy is due to the use of chemical sterilization of the vessel and external UHT treatment of the medium.

Why a Bulk Starter System?

Considerable debate has centred on the question of what starter preparation system is the most cost-effective for the cheese manufacturer: direct-to-the-vat culture concentrates (frozen or freeze-dried off-site by commercial starter suppliers for direct inoculation of the cheese vat) or bulk starter cultures grown in large fermenters on-site in the dairy factory.

At present market prices, direct-to-vat starters typically cost A$80 per tonne of cheese. Direct-to-vat starters are the most cost-effective option in specialty cheese manufacture and in smaller factories, for whom the cost of installing and operating a bulk starter facility would be too great.

The proportional cost of in-factory bulk starter preparation decreases with increasing factory size. The frozen pottles are produced at very low cost (A$1 to A$2 per tonne of cheese, depending on cheese factory capacity), and it is estimated that bulk starter prepared in a moderate-sized cheese factory (10,000 tonnes of cheese per year) using pottle cultures as the inoculum costs about A$20/tonne. These estimates take into account the capital cost of equipment, depreciation, and ASCRC membership subscription. It is likely that bulk starter preparation will in the future be used for the manufacture of more than 95% of all Australian Cheddar cheese.

Performance of the Starter Management System

By maintaining selected rotations, the total number of strains used by ASCRC subscriber companies decreased steadily from about 60 in 1992 to 46 strains during the 1995-96 manufacturing season. The actual number of strains (mesophilic and thermophilic) used to manufacture about 80% of the dairy companies' production was only 29. This co-ordinated reduction of strain numbers is part of the rational management of strains and rotations to minimize the effects of phage infection. One strain (ASCC47) showed remarkable phage resistance. This strain was widely used in ASCRC subscriber factories for over two years without phage being detected in any factory, and continues in extensive use without significant phage-associated inhibition over four years after its introduction.

Some strains that had been successfully used in the 1970's (but were withdrawn because of phage problems) have been re-introduced in a managed manner. Although phages eventually appeared for all of them, it is clear that these strains can be used safely in rotations provided the relevant phage host ranges are well known.

Cheese flavours have improved because of judicious strain selection. A significant proportion of Australian cheese exports goes to Asian countries where bitterness is considered to be an important defect. The use of PepN peptidase activity as a guide to selecting the best strain combinations appears to have worked well in minimizing bitterness.

The system relies on direct links and close communication between ASCRC and cheese manufacturers. Inspections and meetings with technical personnel from each factory are carried out regularly and important feedback information is obtained which leads to further R& D and innovations.

Continuing Development

ASCRC faces a continuing challenge to operate cost-effectively in research, starter development and starter production. These activities are separate but necessarily linked to each other. Indeed, ASCRC's past and future development is based on an integrated approach to the generation of new knowledge and its application in an industry-based context. With domestic and international demand for cheese increasing, management and improvement of the starter system for Cheddar is on-going. Similar principles are being applied to Mozzarella starters, and there is industry interest in a similar approach to starters for yoghurt manufacture and in adjunct bacteria for enhancement of cheese flavour and manufacture of novel types of cheese.

ASCRC provides a useful model of how competitors can co-operate in establishing and using a shared research and development resource, and shows how close industry involvement in a research centre can lead to commercial application of relevant knowledge and technologies.

Acknowledgements

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