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International Journal of Food Microbiology 78 (2002) 119–131

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

# Commercial bacterial starter cultures for fermented foods of the future

Egon Bech Hansen *

*Bio Science, Danisco A/S, Langebrogade 1, DK-1001, Copenhagen K, Denmark*

Accepted 26 May 2002

**Abstract**

Starter cultures for fermented foods are today developed mainly by design rather than by screening. The design principles are based on knowledge of bacterial metabolism and physiology as well as on the interaction with the food product. In the genomics era, we will obtain a wealth of data making design on a rational basis even simpler. The design tools available are food grade tools for genetic, metabolic and protein engineering and an increased use of laboratory automation and high throughput screening methods. The large body of new data will influence the future patterns of regulation. It is currently difficult to predict in what direction the future regulatory requirements will influence innovation in the food industry. It can either become a promoting force for the practical use of biotechnology to make better and safer products, or it can be limiting the use of starter cultures to a few strains with official approval. Successful cultures based on modern technology is expected to be launched in the areas of: probiotics, bioprotection, general improvement of yield and performance for the existing culture market and probably the introduction of cultures for fermenting other food products. A scientific basis for dramatic innovations that could transform the culture industry is currently being established.
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*Keywords:* Starter cultures; Lactic acid bacteria; Food grade genetic engineering; Probiotic cultures; Biopreservation

## 1. Introduction

Food fermentation has been used for centuries as a method to preserve perishable food products. The raw materials traditionally used for fermentation are as diverse as: fruits, cereals, honey, vegetables, milk, meat and fish. It is possible to obtain a large variety of different food products by selecting different raw materials, starter cultures and fermentation conditions. The diversity covers, but is not limited to products as: wine, beer, vinegar, bread, soy sauce, kochujang, sauerkraut, kimchi, pickled olives, fermented milk products as buttermilk and yoghurt, a variety of cheeses and sausages. Fermentation was invented long before microorganisms were discovered, and therefore the process seemed mysterious. The magic of a fermentation process is, amongst others, reflected in the common origin of the words for yeast and ghost. The need for an inoculum was understood and usually satisfied by keeping a sample from the previous production. This procedure is still in use for propagation of sourdough for private use, and also for the production of some artisanal cheeses. For other

* Tel.: +45-32-66-22-01; fax: +45-32-66-21-67.
*E-mail address:* egon.bech.hansen@danisco.com (E.B. Hansen).

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PII: S0168-1605(02)00238-6

processes, inoculation was not necessary as naturally occurring microorganisms in the raw materials could, under proper conditions, be a reliable source of the microbial flora. This is the case for the production of raw milk cheeses, wine, sauerkraut and some fermented sausages. With the discovery of microorganisms, it became possible to improve the products and the fermentation processes by using isolated and well-characterised cultures. This became the norm already in the 19th century in the breweries for beer production, for the production of alcohol, vinegar and bakers yeast. It took another century before the dairy and the meat industry changed the procedures towards well-characterised and defined starter cultures. Main food products produced by fermentation are listed in Table 1 together with the raw materials used and the type of culture employed. Lactic acid bacteria are widely used in the production of fermented food, and they constitute the majority of the volume and the value of the commercial starter cultures. The primary activity of the culture in a food fermentation is to convert carbohydrates to desired metabolites as alcohol, acetic acid, lactic acid or $CO_2$. Alcohol and organic acids are good natural preservatives, but also appreciated in their own right in the fermented product. The $CO_2$ produced by some cultures contributes the gas needed to rise the dough, form eyes in the cheese or to make the foam of beer and buttermilk. In the production of wine, a secondary fermentation by lactic acid bacteria is responsible for the reduction of the acidity by converting malic acid to lactic acid. The cultures used in food fermentations are, however, also contributing by "secondary" reactions to the formation of flavour and texture. This secondary contribution can often be responsible for the difference between products of different brands, and thereby contribute significantly to the value of the product. In the special category of probiotic cultures, the primary activity is the effect on the health of the consumer, and not the effect on the fermented food. Several authors have made comprehensive reviews about fermented foods (recent reviews are: Wood, 1998; Caplice and Fitzgerald, 1999; Lee, 2001).

Table 1
Types of fermented foods with a long history of use in large geographical areas of the world

| Product | Raw material | Starter culture |
|---|---|---|
| Beer | Cereals | Yeast |
| Wine | Grape juice | Yeast, lactic acid bacteria |
| Bread | Grains | Yeast, lactic acid bacteria |
| Soy sauce | Soybeans | Mould (Aspergillus), lactic acid bacteria |
| Sauerkraut, Kimchi | Cabbage | Lactic acid bacteria |
| Fermented Sausages | Meat | Lactic acid bacteria |
| Pickled vegetables | Cucumbers, olives a.o. | Lactic acid bacteria |
| Fermented milks | Milk | Lactic acid bacteria |
| Cheese | Milk | Lactic acid bacteria, yeast, mould |

The manufacturers of fermented foods have the choice of either acquiring the starter culture in a ready-to-use, highly concentrated form, or to make a propagation of the culture in the factory. The choice between the two types of process will be influenced by a number of factors as: the number of different products produced, degree of automation, presence of expertise in microbiology and finally the economy. The highest level of safety and flexibility is achieved by using a commercial starter culture for direct inoculation. Such cultures are supplied either as frozen or freeze-dried highly concentrated and highly active cultures. Typical production processes for starter cultures have been described by Høier et al. (1999) and by Buckenhüskes (1993). All steps in the production of starter cultures are important for obtaining the desired identity, purity and quality of the culture product. The culture producers are applying the principles of hazard analysis critical control point (HACCP) in the production in order to assure stable high quality production procedures (European food and feed cultures association,, http://www.effca.com).

The purpose of the present paper is to describe the factors influencing the innovation potential within the area of fermented foods. In particular, the innovations requiring novel types of starter cultures are dependent on the delicate balance between several factors arising from: science and technology, safety and legislation, market needs and consumer attitudes and finally economics. My treatment of the subject might seem biased towards fermented dairy product. This is, however, a reflection of the distribution of value and volume of commercial starter cultures, and as the culture industry will be one of the key actors in the development of practical applications, I think this bias is justified.

## 2. Science and technology for screening, selection and construction of starter cultures

In order to make the ideal culture for any particular food application, it is necessary to understand the function we demand of the culture, and to have tools to improve the function of the culture. Both aspects have been advanced considerably through scientific achievements during the last few years. The search for a starter culture has until recently been relying on the screening of a large number of isolates in small-scale food fermentations. The starter culture finally selected would be the one giving a satisfactory performance in the process and also giving an acceptable organoleptic evaluation of the food product. Excellent cultures have been isolated this way, and the method will certainly also in the future be used to expand the pool of microorganisms to be used as starter cultures. The last two decades of research have, however, generated tools allowing us to specifically target the individual genes and metabolic pathways responsible for desired performance parameters of a starter culture. Specific targeting makes screening by high throughput methods possible, and it opens the possibility to use mutant selection and genetic engineering to construct starters that are superior to the ones found in nature.

### 2.1. Genetics of lactic acid bacteria

The era of molecular genetics of lactic acid bacteria was opened in 1982 by the development of a protocol for transforming DNA into *Streptococcus lactis* (Kondo and McKay, 1982). The taxonomy of *S. lactis* was subsequently changed as the genus *Lactococcus* was formed and the name changed to *Lactococcus lactis* (Schleifer et al., 1985). The DNA transformation protocols was refined (Kondo and McKay, 1984; Holo and Nes, 1989), and a number of versatile plasmid cloning vectors developed (Kok et al., 1984; Gasson and Anderson, 1985). Genetic engineering in *L. lactis* became routine during the eighties and similar procedures and vectors became available also for other industrially important lactic acid bacteria (Mercenier, 1990; Pouwels and Leer, 1993; Mercenier et al., 1994).

The new molecular tools were very early used to characterise the genes involved in carbohydrate uptake and utilisation (de Vos and Gasson, 1989; de Vos et al., 1990; van Rooijen et al., 1992) and to dissect the proteolytic systems of the lactic acid bacteria (Kok, 1990; Pritchard and Coolbear, 1993; Siezen, 1999; Christensen et al., 1999). The proteolytic system consists of proteinases anchored on the outside of the cell wall and a large number of peptidases. The proteolytic system is important for the growth of lactic acid bacteria, as they are auxotrophic for about half of the total amino acids. It is therefore of practical importance to understand proteolysis, as fast and optimal growth in food fermentations is an important parameter for the process economy in the food industry. A probably even larger interest for the proteolytic enzymes derives from the involvement of the bacterial enzymes in the cheese ripening process. The taste and flavour of a cheese depends on a delicate balance between a large number of volatile and non-volatile flavour compounds. The compounds are either already present in the milk or generated either in the cheese vat or during the ripening period (Law, 1999; Bockelmann, 1999). The length of the ripening period differs considerably from no ripening for products like kvarg, cottage cheese and Mozzarella, over a few weeks of ripening as used for continental European cheeses to the long ripening periods of several months or even years used for cheddar and hard Italian cheeses. It is very important to identify to what extent the starter culture is directly responsible for the generation of flavours and off-flavours, and which genes and enzymes are contributing to the ripening process. The genetic dissection of the proteolytic system has opened a method to study this complex network of reactions; but it has by no means eliminated complexity and a large number of trials will still be needed to elucidate which enzymes contribute to the process. One example of cheese ripening analysed by bacterial genetics was the analysis of the contribution of four amino peptidases and lysis of the starter culture published by Guldfeldt et al. (2001).

Another area for early application of molecular genetics of lactic acid bacteria was the analysis of bacteriophages and the defence systems used by the bacterial host to combat phages. Bacteriophage infections of the starter culture are causing the dairy industry severe losses due to failed fermentations. Cheese factories have been particularly vulnerable as contamination of a cheese vat with whey from the

previous batch can lead to a rapid accumulation of phages, which in the end can cause a complete stop of the acidification (Heap and Lawrence, 1976; Jarvis, 1989). Starter cultures containing *L. lactis* are used for the majority of the cheese volume produced, and therefore practical methods to control the phages attacking *L. lactis* are in high demand. Bacteriophages attacking *L. lactis* have been characterised and divided into 12 species based on a combination of molecular methods (Jarvis et al., 1991). In 1994, the complete genome of a lactic bacteriophage had, for the first time, been established (Schouler et al., 1994); but it was soon followed by sequences of several other lactic bacteriophages (Lubbers et al., 1995; van Sinderen et al., 1996; Mikkonen et al., 1996; Chandry et al., 1997; Kodaira et al., 1997; Stanley et al., 1997; Brønsted et al., 2001). With the establishment of complete genome sequences also for bacterial genomes (Bolotin et al., 1999, 2001), silent prophages residing in the bacterial genome became available for study. The bacterial genome of *L. lactis* IL1403 was found to harbour six prophages (Chopin et al., 2001). It was through another study of phage evolution by comparative genomics that the authors could conclude that genetic material had been exchanged between pathogens and non-pathogens (Desiere et al., 2001).

Molecular genetics opened new possibilities in the battle against bacteriophages. Identification, characterisation and combination of phage defence mechanisms opened new possibilities (Hill et al., 1989; Klaenhammer, 1989; Coffey et al., 1991). The defence mechanisms employed by the lactic acid bacteria have been divided into three modes of actions: interference with phage adsorption, abortive infection and restriction/modification systems (Allison and Klaenhammer, 1998). Each of the three groups contain numerous individual systems which are not necessarily related. The natural defence systems have been stacked and rotated in new intelligent combinations and put to practical use in the dairy industry (Durmaz and Klaenhammer, 1995; Daly et al., 1996). It has also been possible to construct entirely new phage defence mechanisms based on components from phage genomes (Allison and Klaenhammer, 1998; Walker and Klaenhammer, 2000).

### *2.2. Food grade genetic engineering and engineering for safety*

In order to use molecular genetics for the construction of starter cultures for practical applications, it is obvious that the strains constructed should be safe and acceptable ingredients in our food. This excludes some of the most convenient tools of the molecular biologist from being used in the final products. The presence of antibiotic resistance markers, promiscuous recombinant plasmids and virulence genes from pathogenic bacteria are obvious examples of undesirable traits. It was therefore necessary to construct special food grade cloning vectors to be used for the construction of recombinant *L. lactis* starter cultures. Replicons isolated from natural plasmids isolated from *L. lactis* was used for the construction of these vectors. The $\theta$ – replicating plasmids are best suited as vectors due to the greater structural and segregational stability (Kiewiet et al., 1993). A few different selection principles were developed for use in the plasmid vectors of *L. lactis* (reviewed by de Vos, 1999). Selective markers based on complementation or suppression of chromosomal mutations like the lacF system (de Vos et al., 1990) or the use of nonsense suppressors (Dickely et al., 1995) might seem inconvenient, as they can be moved between different strains only after the necessary host mutation has been selected or introduced. They have, however, one big advantage, that the selective pressure can be maintained in milk without addition of any supplements (Dickely et al., 1995). The food grade plasmid vectors are useful for the overexpression of native enzymes and for the introduction of heterologous genes. It is, however, also necessary to be able to engineer the bacterial chromosome. Efficient food grade tools for the introduction or inactivation of markers on the bacterial chromosome was developed based on homologous recombination (Biswas et al., 1993), insertion elements (Maguin et al., 1996) or based on the site specific recombination systems from temperate bacteriophages (Lillehaug et al., 1997; Brøndsted and Hammer, 1999). In the food grade tool-box, we finally also have elements to control the expression and secretion of homologous and heterologous proteins (Israelsen et al., 1995; Jensen and Hammer, 1998; de Vos, 1999; Bredmose et al., 2001).

The engineering of recombinant starter cultures by the aid of food grade techniques can now be done on a safety level matching or even exceeding the safety level of ''natural'' screening and selection. Being natural does not guarantee safety, and we may need to put efforts into applying molecular genetics on the engineering of safety into the microorganisms for food and feed. One example of engineering for safety was described by Mollet (1999) where the removal of the D-lactate dehydrogenase (*ldhD*) gene from *Lactobacillus johnsonii* La1 eliminated the accumulation of the undesired D-isomer of lactate and leaving only the desired L-lactate. It is likely that the accumulation of genome sequence data will result in the discovery of numerous problematic or even unacceptable genes in our familiar ''friendly'' microorganisms. Fortunately, the technology to cure this emerging problem is already at hand.

### 2.3. Metabolism and metabolic engineering of starter cultures

Metabolic analysis of lactic acid bacteria is hampered by the complex nutritional requirements of lactic acid bacteria, which often forced researchers to conduct their analysis of growth physiology in rich laboratory media or in milk. The development of a well-defined minimal medium for *L. lactis* allowed for more fundamental physiological studies (Jensen and Hammer, 1993). Due to the initial difficulties in designing defined media, a number of metabolic pathways in *L. lactis* were analysed by cloning, sequencing and characterising the genes coding for the key enzymes. The nucleotide metabolism including the salvage pathways was characterised this way (Nilsson and Lauridsen, 1992; Martinussen et al., 1994; Martinussen and Hammer, 1994; Andersen et al., 1996; Wadskov-Hansen et al., 2000; Martinussen et al., 2001). With the establishment of entire genome sequences of lactic acid bacteria, the future approach to metabolic analysis will be an initial computer analysis of the metabolic potential followed by experimental verification.

The currently known metabolic pathways of immediate practical importance are the metabolic pathways for the conversion of sugars via pyruvate to acids and metabolites with distinct flavours. These pathways are also the ones used to generate energy for bacterial growth. The main intermediates and enzymes are shown in Fig. 1. The possibility to use metabolic engineering to alter or optimise various aspects of this metabolic network has been described and reviewed by several authors (Hugenholtz, 1993; Swindell et al., 1996; de Vos et al., 1998; Renault et al., 1998; Daly et al., 1998). Metabolic engineering of the pyruvate metabolism has been used to develop an optimal culture for the production of sour cream (Curic et al., 1999; Curic, 1999). A high level of diacetyl

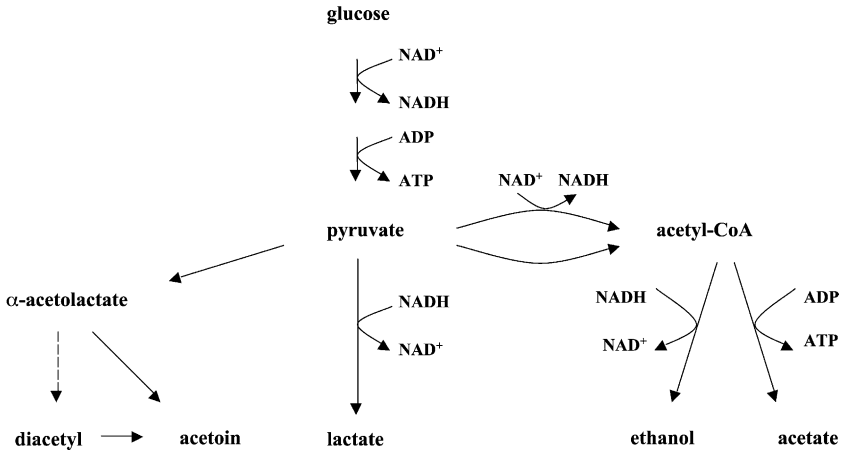


Fig. 1. The main metabolic pathways of for the generation of acid and flavour by lactic starter cultures in fermented foods. Under normal anaerobic conditions, the main flux is from sugar to lactate. By altering the enzyme levels or by aerating the culture, it has been possible to increase fluxes through the other paths of the network.

production was obtained from an *ald*$^-$ mutant of *L. lactis* and stability of the diacetyl was achieved by combining the *Lactococcus* strain with a *Leuconostoc* strain mutated in two genes each coding for a diacetyl reductase (Curic, 1999).

The metabolism of *L. lactis* under aerobic conditions has recently been identified as an overlooked and potentially very useful research area (Lopez de Felipe et al., 1997; Lopez de Felipe et al., 1998; Duwat et al., 2001). The negative effects of oxygen on the growth of *L. lactis* have been easily recognised and have drawn attention away from the positive effects, and the general wisdom has been to avoid aerobic conditions (Duwat et al., 2001). *L. lactis* does, however, possess a number of enzymes and metabolisms which allow the bacteria to tolerate and to use oxygen. A key enzyme is the NADH oxydase, which allows the bacterium to use $O_2$ to regenerate $NAD^+$ (Lopez de Felipe et al., 1997, 1998). The bacterium can hereby be relieved from the limitation imposed from the necessity to maintain redox neutrality in the metabolic network. As a consequence of this, *L. lactis* showed higher yield and altered product formation during aerobic growth (Jensen et al., 2001). It was, however, a surprise that *L. lactis* is able to produce a cytochrome and grow by respiration if a source of heme is supplied in the medium (Duwat et al., 2001). The potential applications of the aerobic metabolic pathways of *L. lactis* is primarily higher yields in the fermentations and the redirection of carbon fluxes towards various desired flavour compounds.

### 2.4. Probiotics

Metchnikoff (1908) described the beneficial effect of lactic acid bacteria on human health almost a century ago. Although numerous studies have substantiated the findings of Metchnikoff, it has been a difficult scientific discipline to identify and prove the mode of action for probiotics (Mattila-Sandholm et al., 1999; Pathmakanthan et al., 2000). The pre-existing flora of the digestive tract is complex and ill-defined, which makes it very difficult to determine how probiotics influence the intestinal ecosystem (Tannock, 1998; Kleessen et al., 2000; Macfarlane et al., 2000). The presence of microbial flora is necessary for the normal function of the digestive system. Elimination or severe perturbations of the flora leads to diarrhoea or constipation, and the maintenance of a healthy bacterial flora is therefore desirable (Tannock, 1998; Pathmakanthan et al., 2000). In the absence of precise models for the mode of action, a number of practical criteria for selecting probiotic strains have been formulated (Collins et al., 1998; Salminen et al., 2000; Klaenhammer and Kullen, 1999; Mattila-Sandholm et al., 1999).

The large increase in the occurrence of allergy in the populations of the industrialised world is still largely unexplained. One of several hypothesises is the ''hygiene hypothesis'', which explains the increase by our modern environment being too aseptic (Martinez and Holt, 1999; van den Biggelaar et al., 2000). If this is, indeed, a part of the problem, we will need to design our future fermented food or the probiotic products to contain safe microorganisms counteracting this unwanted distortion of the immune system.

### 2.5. Bioprotection

A general preservation effect is obtained by most food fermentations due to the accumulation of organic acids and alcohols concomitantly with the reduction of the level of free sugars, depletion of oxygen and lowering of the pH (Lindgren and Dobrogosz, 1990). Cultures with much stronger preservation effects have been identified and, in most cases, found to produce antimicrobial bacteriocins (Cleveland et al., 2001). *Lactobacillus reuteri* constitutes an interesting exception as the antimicrobial substance, reuterin, is a low molecular weight metabolite 3-hydroxypropionaldehyde (Chung et al., 1989). Nisin was the first bacteriocin to be discovered about 70 years ago. Nisin is produced by strains of *L. lactis,* and the molecule is a small peptide containing unusual amino acids due to posttranslational modifications (Gross and Morell, 1971). Nisin has been in practical use as a food preservative for more than 50 years and its use is approved in most countries. A large number of bacteriocins have been characterised from lactic acid bacteria and classified into three groups based on their structural differences (Cleveland et al., 2001). Bacteriocins share a common mode of action in their ability to form pores in the membrane of the target bacteria, and the molecular aspects of the formation of pores have particularly, for nisin, been well charac-

terised (Moll et al., 1999; van Kraaij, 1999). The ability to produce bacteriocins is quite common among microorganisms isolated from fermented foods, and the consensus among all studies is that this property is beneficial and safe (Wessels et al., 1998; Hugas, 1998; Cleveland et al., 2001). It is therefore surprising that nisin is still the only bacteriocin in practical use. It is even more surprising as the need for food preservation is high, and consumers demand gentle and natural ways of preserving food. Bacteriocins are well suited for application in combination with other preservative factors in the so-called hurdle technology (Leistner, 2000). The paradox can probably be explained by a generally negative attitude towards food additives and E-numbers in Europe combined with a poor understanding of the beneficial effects of microorganisms. In addition to this, regulation of the area does not facilitate the introduction of novel bacteriocins in food applications. The practical use of bioprotection might therefore be focused on systems where cultures can be applied instead of purified bacteriocins.

### *2.6. Bacterial genomics and high throughput technologies*

The current era of biological sciences is the era of genomics. Apart from the availability of complete genome sequences, we also have a whole new approach in acquiring and analysing biological data. As biological systems are very complicated, researchers usually have to simplify and reduce the complexity of systems. This reductionistic approach has now been followed to an end and has hereby paradoxically resulted in the generation of a number of holistic methods. Today, we can obtain a complete overview by collecting and analysing each and every detail. Technological possibility has been established by combining laboratory automation with IT systems able to handle and analyse very large data sets. Sequence data are used for the construction of biochips and DNA arrays allowing complete analysis of the transcriptome and the proteome.

The first complete bacterial genome to be fully sequenced was the *Haemophilus influenzae* genome (Fleischmann et al., 1995), and the first published lactic acid bacterial genome sequence was for *L. lactis* IL1403 (Bolotin et al., 1999, 2001). Currently, the number of sequenced prokaryotic genomes is approaching 100 and more than 50 are publicly available (Ussery, 2001;, http://www.cbs.dtu.dk). Already, the *L. lactis* genome sequence revealed a number of unexpected genetic and metabolic potentials (Bolotin et al., 2001). With the establishment of a larger collection of microbial genome sequences, we will obtain a rich source of inspiration to guide physiological studies, mutant selections and the use of genetic and protein engineering for starter culture construction. We are, however, also going to find genes and potential capabilities which we dislike. We will find antibiotic resistance genes, potential virulence genes, metabolic pathways which could lead to unwanted metabolites, etc., we may find some of these properties to be present on potential mobile genetic elements. These findings will probably require actions to be taken, and depending on the size of the problem, it could lead to a general requirement for establishment of genome sequences of all microorganisms used in food and feed.

### *2.7. Human genomics*

Two draft versions of the human genome sequence were published early last year (Venter et al., 2001; International Human Genome Sequence Consortium, 2001). Human genomics is already the key element in drug discovery, and also the food industry is expected to benefit from this new source of information. A complete overview of the normal human metabolism will emerge from the analysis, and it will lead to the discovery of the molecular basis for a large number of diseases such as allergy, obesity, cancer, rheumatic arthritis, and even the molecular basis for human behaviour. General sequence data is used for designing arrays and chips to detect individual variations, and a fast-growing fraction of the population will have individual knowledge about their own genotype. This information will be used to guide personal choices. The choice of diet will probably be an important area in this respect.

## 3. Legislation and safety issues

The safety of food has received considerable public attention in Europe during the last decade. This has

led researchers to focus on the development of new systems for increasing food safety. The large attention on food safety has also created a demand for more legislation and regulation of food and feed products within Europe. Apart from the intended effect of increasing the safety (hopefully) of our food and feed products, increased legal requirements have an unintended side effect of reducing the diversity of products and delaying the innovation process. Increased regulatory demands can hereby become a bottleneck for the introduction of new solutions. The industry for bacterial cultures, which constitute a relatively small market, can easily become arrested and limited to the current range of products. The safety of starter cultures has been advanced through molecular methods for identification and taxonomy of bacteria (Axelsson, 1998). The food industry and the culture producers have been eager to use the modern techniques in their manufacturing processes (Høier et al., 1999, , http://www.effca.com).

The spread of antibiotic resistance among pathogens is currently one of the most important safety issues in clinical microbiology, and as some of the pathogens are food borne the issue is also important in food microbiology. The removal of antibiotics as growth promoters from animal feed is an important step towards reducing the problem. This step has opened the market for probiotic cultures as growth-promoting feed additives. In this context, it does of course become important that the bacteria used in the feed does not contribute to the problem. The European Union Scientific Committee on Animal Nutrition have analysed the potential risk and made a proposal for guidelines for the evaluation of the bacterial strains (Anonymous, 2001). In this recommendation, it was pointed out that the proposed guidelines would put a tighter regulation on feed cultures than on food cultures. Obviously, food for humans has to be at least as safe as feed, and the industry is already implementing the guidelines for all cultures. The accumulation of large sets of sequence data is going to reveal a large number of previously unrecognised risks. We will find undesirable genes and mobile genetic elements to constitute new potential risks. We will obviously have to use all available information for increasing the food safety. I would, however, recommend against excessive use of regulatory measures in this context, as excessive regulation would only limit our ability to exploit the benefits of the new knowledge. Regulatory measures are needed if a real problem has been identified, if the problem is hypothetical regulation will probably be harmful.

## 4. The targets for novel starter cultures

Having reviewed the new possibilities made available by science, it is now very relevant to try to answer the basic question: ‘‘What new starter cultures are needed?’’

It is important to realise a big difference between medicine and food science. In medicine, we still have a number of serious diseases where we need to find a cure, it is therefore generally known and accepted that we need science and technology to search for new treatments. In food science the problems seem less fundamental as the problem of preparing a nutritious and delicious meal has been solved numerous times using the art of gastronomics rather than the science of genomics. The requirement for making a good meal is to have ingredients of a good quality available, and to have sufficient variety to generate interesting taste and flavour. This is, however, not as simple as it sounds, as a large number of our basic food items are perishable, and quality is also not always easy to recognise. Furthermore, a large part of the food we eat today is not prepared in our homes from the basic raw materials. In the industrialised world, the fraction of our food prepared outside the private kitchen is exceeding 50% and this fraction is still rising.

The main problems to solve (or to improve) are still the old ones: reduce spoilage, avoid food borne diseases and finally preserve or develop an attractive flavour, taste and appearance of the food. Industrialised food manufacturing has generated new versions of these old problems, and has prompted the search for new solutions. It is, however, not only in the industrialised production we need improvements. We do experience big losses in all steps from the producer to the final consumer. In the less developed world, problems in preserving the food after harvest is limiting the availability of food for local consumption and also severely limiting the export potential from these countries.

Lactic acid bacteria have much to offer within food preservation and flavour generation. Lactic acid bac-

teria have a long history of safe use for these purposes, and the application of lactic acid bacteria in new areas does therefore in many cases seem straightforward. However, even in the "obvious" cases, one should not underestimate the time and cost associated with obtaining legal approval and subsequently develop a new market. With the knowledge about the genetics and the physiology of lactic acid bacteria we now possess, it is possible to engineer starters for a variety of purposes where a suitable starter culture cannot easily be found in nature. If we use genetic engineering we will, however, in Europe face a costly and time-consuming approval procedure. As the combined costs of research and approval need to be recovered, we will probably see only a few products targeted primarily for Europe. The major targets for this type of innovation will be set according to needs in the US and the rest of the world outside Europe.

To identify the primary areas for development of new starter cultures, it is worthwhile to examine the economics of the lactic starter culture industry. The main companies involved in the production of lactic acid bacteria for the food industry are listed in Table 2. The starter culture industry has been in a phase of consolidation and restructuring. The consolidation phase is probably not yet complete. The largest part of the lactic culture market consists of the cultures for the dairy industry. The size of this market is currently approximately US$250 million. If commercial starter cultures for direct inoculation were used worldwide for the production of cheese and fermented dairy products, the size of the dairy culture market would be approximately US$1 billion. As the existing market is divided between a number of suppliers, each producing a large number of cultures for different applications, it is evident that individual culture products each have relatively limited global turn over. The financial resources available to develop and introduce a new culture into the existing market would therefore be modest, unless this new culture has the potential to entirely transform the market. The culture producers would look for improvements that can be applied on a general scale to improve the entire range of cultures. These types of improvements could yield developments in the fermentation step, better survival through the steps of freezing and freeze-drying or improvements in culture formulation. The culture producer would also look for improvements that could help expand the size of the total culture market. The untapped market within the existing food industry could be reached by increasing the benefit of cultures for direct inoculation. The known advantages of direct inoculation are reliability, performance and safety, as well as convenience of use.

Finally, the culture market can also be increased by expanding the application of cultures and by increasing the value of the products. In both cases, the cultures developed will contribute a larger part of the value of the final product compared to the current applications. If it is clearly recognised that the culture is not just producing acid, but also other characteristics of critical importance to the value of the food product, it will be possible to charge a price allowing to recover the investment in developing the new product. The only clearly successful culture category of this type has been the probiotic cultures. Since Metchnikoff discovered the beneficial effect of lactic acid bacteria on human health it has become general knowledge that yoghurts and fermented milks are promoting health. Based on this general knowledge, a number of products with especially well-documented bacterial strains have been introduced with success in different segments of the market. Probiotic cultures are now used for a number of different products as yoghurt drinks, yoghurt, infant formula, dietary supplements a.o. There is no reason to believe that the value of the total probiotic market has already reached the maximum. The market can

Table 2
Commercial suppliers of food starter cultures

| Company | Country |
|---|---|
| Alce | Italy |
| ASCRC | Australia |
| Centro Sperimentale del Latte | Italy |
| Chr. Hansen | Denmark |
| CSK | The Nederlands |
| Danisco | Denmark |
| Degussa | Germany |
| DSM | The Nederlands |
| Gewürzmüller | Germany |
| Lallemand | Canada |
| NZDRI | New Zealand |
| Quest International | The Nederlands |
| Rhodia | France |

still be expanded if probiotic strains that can alleviate a serious health problem are found or constructed. In order to maintain credibility in this area, it is very important that the documentation is based on clinical standards. The possible targets to be influenced by probiotics are: diarrhoea, the intestinal flora, vaginitis, colon cancer, immune system modulation, hypertension, lactose intolerance, cholesterol lowering, urinary tract infection (Sanders, 1998). The highest demand from the consumers appear to be for products reducing the risk of heart disease, stress, high blood pressure, obesity, cancer, osteoporosis, tooth decay and products which provide energy and increase the athletic performance (Hilliam, 1998). It is very likely that several of these consumer demands can be satisfied by the combined use of human genomics and bacterial genomics to understand and define the mode of action, and subsequently select or engineer the superior probiotic bacteria. If the health benefits are well documented and easy to understand, it is likely that even the European consumers will accept a genetically engineered probiotic strain.

In addition to probiotics, it would be highly desirable to establish other high value segments of the starter culture market. Biopreservation has, for the last decade, been the most likely emerging segment, and apparently the main delaying factor is now the regulatory issues. The general conclusion from scientific evaluations of bio-protective cultures producing bacteriocins is that it is a safe, natural and desirable way of preserving food (Wessels et al., 1998; Cleveland et al., 2001). Compared to probiotics, products for biopreservation suffer under a less-than-enthusiastic reception by consumers. The consumer feels he is entitled to safety, but does not necessarily want to hear about it, whereas longevity is a popular message. In order for bioprotection to become really popular, we would need examples where bioprotection is the determining factor for bringing a demanded but perishable product on the market.

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