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Biotechnology and food fermentations

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INTRODUCTION

Drying, salting, smoking and fermentation have been used by man for many centuries as methods of food preservation. Fermentation is probably the oldest form of biotechnology and fermented foods make a significant contribution to the human diet in many regions of the world.

Food fermentations have developed in many parts of the world because they have definite advantages over other preservation methods. The low-cost of food preservation by fermentation is particularly important in developing countries where canning, refrigeration and freezing may be limited or unavailable. Food fermentations usually have lower energy requirements than other processing methods and this may be particularly important where fuel is limited.

Fermentation often leads to an improvement in the nutritional value of foods by bio-enrichment with microbial protein, amino

acids, lipids and vitamins. Platt (1964) has introduced the term biological ennoblement to describe foods where a significant nutritional improvement occurs through the action of fermentation. Fermentation processes also have a role in improving nutritional value and acceptability by contributing to the degradation of toxins and antinutritional factors present in many plant foods (Reddy et al. 1986).

The organoleptic properties of fermented foods often differ from the unfermented substrate. For example, yoghurt cannot be produced simply by adding lactic acid to milk. New aroma and flavour components may be produced by fermentation including acids, carbonyl compounds, esters, alcohols and pyrazines. In addition to flavour and aroma, the physical form, texture and colour may change. Solid foods may become liquefied (eg. soy sauce from soybean) or liquid substrates converted into gels or solids (eg. milk into yoghurt and cheese).

Fermented foods comprise a diverse range of products from many regions of the world. Campbell-Platt (1987) has listed some 250 categories of fermented foods with more than 3500 individual products. Table 1 shows the geographical distribution of fermented foods based on the substrates used. Those fermented foods based on starch crops, legumes and various mixtures of fish, shellfish and crustaceans are produced mostly in developing countries where they make an important contribution to the diet

Fermentation processes usually involve either an alkaline (proteolytic), lactic acid, acetic acid or alcoholic fermentation or a combination of these processes. In some South East Asian fish fermented foods autolytic enzymes such as proteases play an important role in the fermentation with minimal bacterial involvement. Ingredients other than the primary substrate are often added to fermentations and may be important sources of microorganisms, enzymes, catalysts, nutrients and selective inhibitors. For example, in Zambia, amylases in the roots of Rhynchosia spp. (Leguminosae) reduce the viscosity of maize meal and release reducing sugars which are fermented in the production of munkoyo beverage. In Mexico various plant ingredients appear to act as catalysts which accelerate the fermentation of maize to produce the beverage tesgüino (Lappe & Ulloa 1989).

Application of molecular techniques to the improvement of food fermentative organisms is already under way but only in areas (beer, bread, dairy, meat, soy sauce) where a large industrial base already exists. For small scale, indigenous, non-industrialised food fermentations the transition to large scale industrial production may be a necessary prerequisite to the application of recombinant DNA technology. Much progress could be achieved by the use of basic biotechnological techniques such as the design of simple fermenters both for liquid and solid-state fermentations. Simple selection techniques can be used to 'improve' strain properties and even the development of mixed culture systems may offer advantages over the use of single strains.

FERMENTATION TECHNOLOGY

Although the majority of Western fermentation technology utilises aseptic liquid fermentations solid substrate systems are widely used in food fermentations and have been employed in the Orient for many centuries for the production of tempe (moulded legume cake) and koji for soy sauce and sake production. Solid substrate fermentations have advantages over liquid fermentations particularly with the current interest in 'clean technologies' which generates minimal waste disposal problems.

Advantages of solid-substrate fermentations are:

1. The capital investment in fermentation technology is lower.
2. Energy and water requirements are less.
3. Fermentation is usually non aseptic.
4. Fewer problems with downstream processing and disposal of large volumes of liquid waste.
5. In some food fermentations there is a high affinity between the microorganism(s) and the substrate as developed through domestication.

Despite the economic and environmental attractiveness of solid-substrate processes they have not been widely exploited in biotechnology principally because of difficulties in a) modelling heat and mass transfer, b) quantifying microbial biomass and c) scaling up production. There have been few advances in the provision of instrumentation such as pH, temperature, O_2, a_w and

ion selective probes for monitoring solid-substrate systems. Consequently, there are many challenges for food technologists, microbiologists and chemical engineers to model growth, behaviour and product formation in these systems. Some progress has been made in a few areas and we (Cook et al. 1991a) have developed a relatively simple low cost method of quantifying fungal growth in fermented rice. Similar approaches may have value in quantifying and modelling fungal growth in other solid substrate food fermentations.

Despite the drawbacks of solid substrate systems the Japanese have very successfully exploited the solid-substrate koji fermentation on a large scale. Opportunities must exist for the application of similar approaches to other fermented foods. Glenn & Rogers (1989) have provided a more detailed discussion of the advantages and disadvantages of solid-substrate fermentations.

Raw starch hydrolysis

Most starchy substrates are cooked before they can effectively be hydrolysed by amylolytic enzymes and considerable energy inputs may be required for this purpose. A number of fungi are known to produce amylases which are capable of acting on raw non-gelatinised forms of starch and interest has recently focused on selecting strains of Bacillus capable of raw starch hydrolysis.

Examples of raw starch utilisation occur in a number of fermented foods and include the ragi starter cake fermentation which is used as a traditional sources of amylolytic organisms for alcoholic fermentations in Asia. In Africa a dark moulded cassava flour is made using uncooked cassava (Essers & Nout 1989) and there are probably many other traditional fermentations which use raw starchy substrates.

The ability to hydrolyse raw starch at acid pH has been reported from relatively few organisms but includes species of Rhizopus (Yamazaki & Ueda 1951), Aspergillus (Ueda et al. 1984) and the filamentous yeast Saccharomycopsis fibuligera (Ueda & Saha 1983). One of the attractive features of using raw starch substrates is the low energy input required. In the case of ethanol production from cooked sweet potato, some 30-40% of the energy input comes from cooking the starch prior to hydrolysis. Dalmia & Nikolov (1991) have shown that starch binding domains on Aspergillus niger glucoamylase I are responsible for the specific interaction with starch granules. If these could be characterised then this might enable the development of increased specificty for raw starch and the application of molecular techniques to open up much wider exploitation. Clearly if fermentation processes could be developed which utilised raw rather than cooked starches then there would be a significant saving in energy costs for producing food.

Upgrading of waste products

Several indigenous food fermentations use waste products from other industries. In Indonesia for example, press-cakes from groundnut oil extraction or tofu manufacture are fermented using the moulds Neurospora intermedia or Rhizopus oligosporus to produce oncom a meat substitute. A press-cake residue from coconut oil extraction is also fermented by Rhizopus oligosporus to produce tempe bonkrek. Many of these processes have developed following the transfer of technology from similar food fermentations such as tempe fermentation of soybeans.

The Scandinavian Symba process was developed to convert starchy wastes to an edible product consisting principally of Candida utilis biomass. As Candida utilis is unable to grow on starchy substrates a mixed culture fermentation is used and the amylolytic yeast Saccharomycopsis fibuligera hydrolyses starch to a mixture of sugars which are used by Candida utilis for growth. Fellows and Worgan (1987) have shown that a Symba type process can also be produced using pectic materials or starch pectin combinations. Saccharomycopsis fibuligera is also used in the production of Indonesian tapé using cassava or glutinous rice and to produce Thumba an alcoholic beverage from Bangladesh. Clearly such organisms may have considerable flexibility both for producing traditional fermented foods and for the upgrading of waste products to foods or feeds. Novel fermentations should always be assessed for safety since there may be altered

metabolism of the fermentative organisms or the new system may favour growth of food borne pathogens or toxigenic microorganisms as in the case of Pseudomonas cocovenenans in coconut presscake.

ROLE OF FOOD FERMENTATIONS IN THE DETOXIFICATION OF FOODS

Plant components

Plant materials such as cereals, green vegetables, legumes and root crops are widely used in food fermentations although many of these contain significant levels of antinutritional and toxic components (Table 2). A number of these components can reduce the nutritional value of foods by interfering with the digestability of proteins and carbohydrates (eg. lectins, tannins) or reducing the biological availability of certain minerals (eg. phytate). Stages in the preparation of foods prior to fermentation such as washing, soaking, boiling, steaming, roasting and grinding can result in a significant reduction in the levels of some of these components. In some cases fermentation is an important stage in degrading some of the toxic and antinutritional components in foods (Reddy et al. 1986).

Many food fermentative organisms have enzymes capable of degrading toxic and antinutritional factors (Campbell-Platt & Cook 1991) and strain selection or recombinant DNA technology could open up a powerful route to detoxifying plant substrates using microorganisms.

Mycotoxins

In the field and during storage, cereals and legumes may frequently become contaminated with aflatoxins and other mycotoxins. The highly toxic and carcinogenic aflatoxins produced by Aspergillus flavus and A.parasiticus are usually resistant to degradation except by strong oxidising and alkali treatments. Certain cereals and legumes used in fermented foods production (eg. maize, ground nuts) are particularly prone to aflatoxin contamination. Although aflatoxins are heat stable, some cooking treatments such nixtamalisation of maize (boiling with lime) for Mexican pozol (fermented maize dough) does reduce levels of aflatoxins (Ulloa & Herrera 1970). Although washing, soaking grinding and steaming probably have a only minimal effects on aflatoxin levels in foods there is evidence that biotransformation of aflatoxins can occur in some traditional food fermentations (Campbell-Platt & Cook 1991). Nakazato et al. (1990) have shown that Rhizopus arrhizus and Rhizopus oligosporus are capable of reducing aflatoxin B_1 and recently, Bol and Smith (1989) have demonstrated that certain Rhizopus spp. are capable of degrading more than 80% of aflatoxin B_1 to non-fluorescent compounds, although these are of unknown toxicity. In some lactic acid fermentations the low pH may contribute to the conversion of aflatoxin B_1 to the less toxic aflatoxin B_{2a} (Nout 1991). Enzymic processes may be involved in aflatoxin degradation and characterisation of these processes may reveal strategies for a more widespread decontamination of food commodities.

FERMENTED FOODS AND FOOD SAFETY

Many fermentation products such as organic acids, alcohols, diacetyl, acetoin and esters kill or suppress the growth of food-borne pathogenic bacteria. This may be particularly important in developing countries and outway any nutritional benefits from consuming these foods. Fermentations involving lactic acid bacteria probably have potential for more widespread applications, particularly for the preservation of cereals, root crops and legumes to provide safe, low-cost weaning foods. Lactic acid bacteria found in fermented foods are a rich source of antimicrobial compounds including organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins (Klaenhammer 1988; Daeschel 1989). Bacteriocins are relatively narrow spectrum antimicrobials and many are only active against related bacteria. Table 3 presents a selection of bacteriocins which have been characterised from lactic acid bacteria. Nisin a bacteriocin produced by Lactococcus lactis is active against Gram +ve bacteria, is relatively stable to cooking and low pH, has received GRAS (Generally Regarded As Safe) status and is now approved for use as a food preservative in more than 40 countries (Delves-Broughton 1990). With increasing interest in natural minimally processed foods other bacteriocins produced by lactic acid bacteria may eventually get approval for use as preservatives both in fermented and non fermented foods. Although many fermented foods are regarded as safe products, under certain circumstances food borne pathogens may be capable of growing. For example, Bacillus cereus is able to grow and

survive during Indonesian tapé fermentation (Cook et al. 1991b) and during Indonesian tempe production (Ashenafi & Busse 1991). Bacteriocins such as nisin may have applications for controlling undesirable organisms such as Bacillus cereus in fermented products although the stability of bacteriocins in the fermentation environment is unknown.

A relatively new area of antimicrobials is that of enzymes produced by bacteria which can be used as food preservatives. Lotz et al. (1990) have described a process for preserving raw meat using a bacterial lysing enzyme from Streptomyces. Nielson (1991) has described some novel bacteria lysing enzymes and Proctor and Cunningham (1988) have reviewed the chemistry of lysozyme and its use as a food preservative. Bacteria or fungal lysing enzymes have not been reported from fermentations although it seems likely that such interactions probably occur between some bacteria in fermented foods.

BIOTECHNOLOGICAL RESOURCES

Cook (1991a b) has drawn attention to the diverse range of products which can be derived directly from food fermentative microorganisms or produced as a result of their activity. These organisms may be sources of useful components such as enzymes (eg. amylases, proteases and lipases) uncommon lipids, pigments and flavour compounds (eg. pyrazines). Gamma linolenic acid is traditionally obtained from plant sources but is now being commercially produced from food fermentative fungi

(Campbell-Platt & Cook 1989). Those food fermentations which use mucoraceous moulds (eg. tempe, tapé, sufu) may also be valuable 'natural'sources of this polyunsaturated fatty acid.

Pigments of Monascus spp. have traditionally been used as natural food colorants for wines, soybean curd, fish and candy. Monascus pigments are regarded as safe natural alternatives to many coal tar dyes and between 1971 and 1990 some 39 patents were filed on food colorant applications using Monascus.

Chitosan a natural polymer of n-acetyl glucosamine is abundant in mucoraceous fungi used in food fermentations and offers an alternative source to that produced by alkaline hydrolysis of crustacean chitin. Sandford (1989) gives examples of the diverse range of applications for the chitosan polymer.

Fermented foods are potentially a rich source of antimicrobial agents both from fungi and bacteria. The bacteriocins produced by lactic acid bacteria (Table 3) have already been mentioned. Bacillus spp. also occur in a number of fermented foods (Iru/Dawadawa in West Africa, Natto in Japan, Kinima in India and Nepal and Thu-nao in Thailand) and the genus is well known as a source of more than 30 broad and narrow spectrum antimicrobials. Clearly more work is need to establish potential applications for antimicrobials produced by food fermentative microorganisms.

SELECTION AND GENETIC MANIPULATION OF FOOD FERMENTATIVE ORGANISMS

Traditional mutation and selection methods and genetic manipulations using recombinant DNA technology are now being used to optimise food fermentative bacteria and fungi. These approaches should eventually enable the selection of desirable starter organisms which are non-toxigenic and have desirable enzymic profiles for food production. Other characteristics which may be suitable for genetic manipulation include increased protein production, the ability to detoxify plant and microbial toxins, resistance to bacteriophages and the stable expression of bacteriocins in bacterial starter cultures for fermented foods. The controlled production of enzymes such as amylases, lipases and proteases should enable more predictable fermentations and the development of novel organoleptic properties.

Currently, there is considerable interest in the extracellular enzymes of fungi and both protoplast fusion techniques and DNA transformation systems using drug resistance markers are being developed for a range of species (Table 4). It is only a matter of time before this technology is applied for the improvement of food fermentative fungi such as the mucoraceous moulds used in tempe and tapé production. Glenn & Rogers (1989) have drawn attention to the potential of strain selection for the development of temperature sensitive and asporogenous mutants. In the case of fungi these would be particularly valuable since it is usually undesirable for a fungus to sporulate on a fermented product.

Although there is considerable interest in the use of bacteriocins from lactic acid bacteria, applications have been hampered by the instability of bacteriocin production in pure cultures. The application of genetics to lactic acid bacteria is relatively recent and most interest has focused on dairy starters particularly Lactococcus lactis with genera such as Lactobacillus and Leuconostoc being relatively little studied. Because many bacteriocins appear to be plasmid encoded their production can be readily lost by a particular strain. Molecular techniques are being used to characterise production of bacteriocins and will hopefully lead to more stable expression by transfer of genes to stable chromosomes, both in producer strains and other fermentative bacteria.

Although there is considerable appeal in improving starter cultures using molecular techniques it must be remembered that many fermentation processes developed over many centuries through a process of selection by domestication. In many instances organisms which are 'improved' by genetic modification may offer little advantage over existing naturally selected mixtures of strains. There are several reasons for this including:

1) Bacterial cultures of a single strain are more prone to bacteriophage infection which can seriously affect food production through product loss, spoilage and wasted operating costs. This problem has most often been seen in the dairy industry where bacteriophages survive the pasteurisation of milk.

As shown in Table 5 bacteriophage infection is probably not restricted to the dairy industry. In some fermentations, food safety might be compromised by bacteriophage infections, since in non-aseptic processes, reduced acid production could enable the growth of food-borne pathogens. The incidence of bacteriophage infections in non industrialised fermented foods is unknown but it may be important factors in the often reported variability of fermented products.

There is currently considerable interest in the use of recombinant DNA techniques with the aim of developing bacteriophage resistant strains of lactic-acid bacteria for the dairy industry. Molecular techniques are being used to establish the mechanism of naturally occurring bacteriophage resistance including the inhibition of bacteriophage development and its adsorption to the host cell.

2) Genetic manipulation may lead to a loss of competitive ability. Unless fermentations with genetically modified organisms are carried out under aseptic conditions, genetically modified organisms may show poor competitive ability compared to 'wild' type strains. Genes for the over-production of large molecular weight metabolically expensive components such as enzymes and bacteriocins might render such strains competitively inferior to wild type or domesticated strains. Any benefit from the over-production of metabolites must be balanced against the

ability of genetically modified organisms to support increased uptake of fermentation products so that growth rate and production of other metabolites is not affected.

3) Genetically modified organisms must offer economic advantages over the use of existing pure or undefined cultures of microorganisms. The costs of using genetically modified strains must not exceed the economic advantages gained such as faster processing times and reduced product variability, energy and manpower costs.

4) Mixed culture systems may offer a simpler, low cost alternative to the use of genetically engineered strains. Mixed culture systems have not been widely used in biotechnology although there are a number of industrial processes employing more than one organism including yoghurt production and the Symba process. Whereas single strains under aseptic conditions are used in the production of pharmaceuticals, organic acids and enzymes many traditional fermented food processes involve mixtures of microorganisms (consortia) under non-aseptic conditions. Simplification of a fermentation process by using single strains modified by selection or recombinant DNA-techniques may remove the ability of fermentations to sustain environmental (temperature, pH, gases) and biological (competitors, bacteriophages, food borne pathogens) purtebations. Such factors can only be controlled effectively in closed aseptic systems which may not be economic for the production of many traditional fermented foods.

FUTURE OUTLOOK

Fermented foods have been used by man over several millennia and are likely to remain an important part of our food supply. Interest in food fermentations is likely to increase, particularly with the attractions of minimal processing and efficient use of food and fuel resources. Modern biotechnology has an important role to play in the development of new starter cultures for the efficient large scale production of fermented foods. However, increasing the efficiency of many food fermentation processes should not be at the expense of 'simple' low cost technology which will continue to have an important role to play in food production.

It is important that scientists keep in touch with developments in food fermentations and modern biotechnology and this is probably best achieved through the formation of a network of interested workers in different countries. Regular contact through newsletters, workshops, conferences and exchange programmes will enable the benefits of modern biotechnology to be effectively applied to food fermentations. Indeed, there is much potential for the application of food fermentations and biotechnology in developing countries. For instance, the biological upgrading of waste products, low energy starch conversion, detoxification of foods and the exploitation of microbial products such as natural antimicrobials could all make significant contributions to improvements in the availability, processing, quality and safety of foods.

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Table 1. The major producing regions for fermented foods based on substrate categories.

Fermented products	Major producing regions
Meats	Europe, North & South America
Seafoods (Fish, shellfish and crustacea)	East & South East Asia
Dairy	Europe, North & South America Middle East
Cereals	Worldwide
Root crops	Africa
Legumes	East & South East Asia Indian subcontinent
Vegetables & Fruits	Europe, North America, East Asia

Adapted from Campbell-Platt (1987)

Table 2. Antinutritional and toxic components present in plant and animal foods used for fermentation

Component	Principal occurrence
Plant origin	
1. cyanogenic glucosides	cassava, some legumes
2. enzyme inhibitiors	legumes
3. glucosinolates	Brassica vegetables
4. lectins (haemoglutanins)	legumes
5. oligosaccharides (eg. raffinose, stachyose)	cereals, legumes
6. phytate	cereals and legumes
7. oxalates	cereals, legumes, root crops, vegetables
8. tannins and other polyphenolics	cereals, legumes, tea
9. saponins	cereals, legumes
Microbial toxins	
1. aflatoxins	cereals, legumes
2. ochratoxins	meats
3. other mycotoxins	cereals, legumes, root crops
4. bacterial toxins	various
Miscellaneous	
1. biogenic amines	stored fish

Sources: Reddy et al. (1986); Campbell-Platt (1987)

Table 3. Bacteriocins produced by lactic acid bacteria

Name	Producer organism	Food use status
Acidolin	Lactobacillus acidophilus	
Acidophilin	Lactobacillus acidophilus	
Bulgaricin	Lactobacillus delbrueckii subsp. bulgaricus	
Brevicin	Lactobacillus brevis	
Caseicin	Lactobacillus casei	
Diplococcin	Lactococcus lactis subsp. cremoris	
Helveticin	Lactobacillus helveticus	
Lactacin	Lactobacillus acidophilus	
Lactocin	Lactobacillus sake	
Nisin	Lactococcus lactis	GRAS status, Used in foods
Pediocin	Pediococcus acidilactici Pediococcus pentosaceus	
Reutericin 6	Lactobacillus reuteri	
Sakacin	Lactobacillus sake	
Non peptides		
Reuterin	Lactobacillus reuteri	

Table 4. Examples where biotechnology is being used to improve fungi for food use or product formation.

Organism	Approach used	Reference
Penicillium caseicolum	protoplast fusion	Reymond et al.(1986)
Penicillium nalgiovense	DNA transformation	Geisen & Leistner (1989)
Penicillium roquefortii	DNA transformation	Durand et al. (1991)
Rhizomucor spp.	protoplast fusion	Goto-Hamamoto et al. (1986)
Rhizopus niveus	protoplast fusion & DNA transformation	Yanai et al. (1990 & 1991)
Saccharomyces diastaticus	protoplast fusion	Sakai et al. (1986)

Table 5. Examples of fermented foods where bacteriophage infections have been reported.

Fermented food	Infected microorganism	Reference
Dairy		
Cheese	Lactococcus lactis	Whitehead & Cox (1936)
	Lactococcus lactis subsp. cremoris	
Yakult	Lactobacillus casei	Shimizu-Kadota & Sakurai (1982)
Yoghurt	Lactobacillus delbrueckii subsp. bulgaricus	Peake & Stanley (1978)
	Streptococcus thermophilus	Accolas & Spillmann (1979)
Legume		
Natto	Bacillus subtilis var. natto	Yoshimoto et al. (1970)
Alcoholic		
Sake	Leuconostoc mesenteroides	Kaneko et al. (1955)
Australian wine	Leuconostoc oenos	Davis et al. (1985)

Adapted from Sanders (1987)