Chapter 4

Yeast Ecological Interactions. Yeast–Yeast, Yeast–Bacteria, Yeast–Fungi Interactions and Yeasts as Biocontrol Agents

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4.1 Introduction

When the domains of individual microorganisms overlap, it is likely that interactions will occur (Boddy and Wimpenny 1992). The outcome of these interactions is evaluated on the basis of the effect they have on population size (Odum 1953) regardless of whether the interactions are detrimental, neutral or beneficial. The types of interaction found in mixed populations of microorganisms are classified on the basis of these effects as direct or indirect interactions (Bull and Slater 1982). Indirect interaction refers to competition, commensalism, mutualism, amensalism or antagonism and neutralism (Linton and Drozd 1982), and direct interaction to predation and parasitism (Frederickson 1977; Bull and Slater 1982). However, fermented foods and beverages develop their nutritional and organoleptic qualities as a result of the metabolic activity of a succession of different microorganisms and it is unlikely that the interactions will separate into these discrete groups since more than one type of interaction occurs simultaneously (Verachtert et al. 1990).

Present understanding of the positive, negative or neutral role of interactions between yeasts, bacteria and fungi has its origins the first time fermentation was employed. The fermentation of many products includes interaction both within and between different microbial groups (e.g. yeast–yeast, yeast–bacteria, yeast–moulds), the physiological activity of which brings about desirable changes which decisively determine the character of a product and stabilise the population in a specific ecological niche (Wood and Hodge 1985; Leroi and Pidoux 1993; Geisen et al. 1992; Rossi 1978; Challinor and Rose 1954). However, interaction does not necessarily only imply the positive or negative attributes within fermentation but it also involves the antagonistic activity of yeasts against other microorganisms by means of the production of microcins (Baquero and Moreno 1984; Golubev and Boekhout 1992), secretion of antibacterial and antifungal compounds, co-fermentation, and their role as in biological control.

The Yeast Handbook
Amparo Querol, Graham H. Fleet (Eds.):
Yeasts in Food and Beverages

4.2 Ecological Interaction Between Microorganisms

4.2.1 The Secretion of Antifungal or Antibacterial Compounds

It is well known that certain fungi (Punja and Utkhede 2003) and members of the bacterial groups (Williams and Vickers 1986) possess the ability to synthesise and secrete secondary metabolites that exhibit antagonistic activities against other microorganisms. However, little attention has been given to yeasts as possible producers of similar substances despite positive indications already published early in the twentieth century (Hayduck 1909; Fernbach 1909).

Hayduck (1909) obtained a volatile thermolabile toxic extract from yeast which was confirmed by Fernbach (1909) to be an amine that inhibited the growth of *Escherichia coli* and staphylococci. Schiller (1924) demonstrated the presence of an inhibitory enzyme active against the staphylococci, while Bachmann and Ogait (1935) argued that the main reason for the inhibitory action of baker's yeast was due to the production of acetaldehyde. Barglowski (1938) found that *Saccharomyces cerevisiae* and *Mycotorula albicans* strains suppressed the growth of *Mycobacterium tuberculosis*, while Cook et al. (1941) prepared an antibiotic from baker's yeast which inhibited the growth of *Aspergillus niger* and *Penicillium glabrum*. Baker's yeast grown in rye decoction is also reported to exhibit strongly bactericidal activities against *Aerobacter aerogenes* owing to thermolabile enzymes (Tikka and Itkonen 1941). Owing to the development of acid, *Torulopsis utilis* showed antibiotic action against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas pyocyaneus* (Carpentier 1945), while Sartory and Meyer (1946) obtained an inhibitor from baker's yeast active against *Escherichia coli* and *Proteus vulgaris*. Florey et al. (1949) noted that unsaturated fatty acids from baker's and brewer's yeasts, *Debaryomyces mucosus* and *T. utilis*, as well as succinic acid from *T. utilis* var. *major* possess antibacterial properties inhibiting a variety of bacterial organisms. Complete inhibition of *Penicillium glaucum* and *Salmonella typhosa* by yeasts was reported by Toda (1950), while similar inhibiting effects were noted by Motzel (1956) due to cyclic peptides. Despite the inhibition of *Bacillus subtilis* and pediococci obtained by substances produced by the yeasts *Brettanomyces bruxellensis*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*, no attempts were made to isolate or identify the substances. Parfentjef (1953) isolated a fraction containing protein possessing anti-infectious properties, malucidin, from baker's and brewer's yeast. This protein protected animals against infection by a number of microorganisms, which included the yeast *Candida albicans*, pathogens like *Proteus* and *Shigella endotoxins* and many species of gram-negative and gram-positive bacteria (Parfentjev 1958).

Robinson et al. (1958) in studies on the decrease of the bacterial population in preferments, isolated two antibiotic substances designated as I1 and I2 from yeasts which possessed inhibitory properties for *Micrococcus pyogenes* and *Escherichia coli*. In a survey of the antibiotic powers of yeasts, MacWilliam (1959) examined 150 yeast strains for their antibiotic powers against bacteria and moulds. Strong inhibition against *Fusarium*, *Mucor* and *Penicillium* was achieved with the yeast strain *Candida pulcherrima* producing pulcherriminic acid, a derivative of the red pigment

pulcherrimin. Robinson et al. (1962) continued their research on the two antibiotic substances they had previously isolated from *Saccharomyces cerevisiae* which they identified as polypeptides, capable of surviving baking and showing antibacterial activity against *Staphyllococcus aureus*.

Despite ongoing arguments that the possible role of yeasts as a source for antimicrobial compounds is merely attributed to the natural effect of competition for nutrients; Fatichenti et al. (1983), in a study on the antagonistic activity of *D. hansenii* against bacteria, found that the yeast species produced extracellular and intracellular antimicrobial compounds that inhibited the growth of *Clostridium tyrobutyricum* and *Clostridium butyricum*. Antibacterial activity was also detected in *Kloeckera apiculata* and *Kluyveromyces thermotolerans*, secreting substances that inhibited the growth of beer-spoilage bacteria (Bilinski et al. 1985). The expression of antibacterial activity by these two yeasts against the gram positive bacteria *Bacillus megaterium* and *Lactobacillus plantarum* involves transformation of methylene blue into a pharmacologically active form. Antibacterial activity against *Staphyllococcus aureus* was noted by the production of extracellular glycolipids, called sophorosides, by *T. bombicola* (Cavalero and Cooper 2003). The sophorosides also proved to be active against *Candida albicans*.

Probably the most significant and well-known antagonistic action by yeasts in recent years comprises the production of killer toxins (Young 1987; Rosini and Cantini 1987; Shimizu 1993; Walker et al. 1995; Suzuki et al. 2001; Marquina et al. 2002). These toxins are extracellular proteins or glycoproteins that disrupt cell membrane function in susceptible yeasts. Although these killer toxins were originally considered species-specific, clear evidence indicated that they occur across species in different yeast genera (Palpacelli et al. 1991; Llorente et al. 1997; Suzuki et al. 2001), and they can kill various filamentous fungi (Walker et al. 1995).

4.2.2 Yeast Co-Interrelationships with Other Microorganisms

Other than the antagonism exhibited by yeasts as just described, ecological theory describes a wide variety of interactions between yeasts and other microorganisms. Yeasts are added to foods and feeds as a source of proteins and vitamins, are represented in waste-treatment facilities, and are used for industrial purposes. These processes frequently rely on a variety of microorganisms (Linton and Drozd 1982; Kuenen and Harder 1982; Frederickson 1977; Hesseltine 1965).

The use of mixed cultures resulted in a higher growth rate, better biotransformations and higher yields in products (Verachtert et al. 1990). Although it has been stressed that the main interaction between the different microorganisms relied on microbial competition for the growth-limiting substrate (Bull and Slater 1982; Alexander 1971), various additional interactions occur simultaneously (Meyer et al. 1975; Yoon et al. 1977; Bungay and Bungay 1968). The consequence of other interactions often results in the interrelationship or co-existence of different species growing on a single growth-limiting substrate (Kuenen and Harder 1982). If physiochemical intrinsic and extrinsic conditions are within specified limits and the environment contains sufficient available energy and required nutrient sources for microbial growth, microbial communities will develop (Meers 1973). Interrelationships between and

within the communities develop, and as a result the stability of the environment is altered (Nakamura and Hartman 1961) by one species to stimulate the growth of other species because of changes in pH, growth factors, oxygen depletion, etc. For example, the growth of lactic acid bacteria reduces the pH value of media to encourage yeast growth, the removal of substances (osmophilic yeasts metabolise high sugar concentrations) that would otherwise prevent the growth of a second species (Mossel and Ingram 1955) or the excretion of relevant enzymes for the breakdown of complex carbohydrates (Antuna and Martinez-Anaya 1993). Owing to the change in the abiotic environmental conditions, the nature of the interactions between the populations may also change (Megee et al. 1972).

4.2.2.1 Yeast–Bacteria Interactions

When bacterial strains grow, environmental alterations may inhibit the growth of other species owing to the removal of essential nutrients or by the production of organic and inorganic toxic compounds (Meers 1973). Bacteria, predominantly lactic acid bacteria, commonly excrete organic acids which lead to a lowering in the pH, which either inhibits the growth of undesired pathogens or promotes yeast growth. Therefore, the interrelationship between lactic acid bacteria and yeasts, as applied in many fermented foods and beverages, plays an essential role in product preservation. In these ecosystems, they may compete for the same substrates (Bull and Slater 1982; Fleet 1990) or synergistically promote the growth of each other. Moreover, the antagonistic and synergistic effects exhibited by using the microorganisms in co-culture, may also be applied in converting wastes into feeds and in industrial processes.

Yeasts (*Trichosporon cutaneum*, *Candida krusei*, *C. valida* and *Pichia membranaefaciens*) and lactic acid bacteria (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Lactobacillus delbrueckii*), grown in co-culture during the fermentation of animal waste and corn were responsible for an increase in the total amino acid content, total nitrogen and protein content of the final product (Hrubant 1985). Moreover, indigenous enteric bacteria, coliforms and faecal streptococci were destroyed and even selected faecal coliforms and *Mycobacterium paratuberculosis* strains added to the media died within 9 th. In addition, the yeast *Saccharomyces boulardii* may be applied as a probioticum in feeds, preventing the development of the toxigenic *Clostridium difficile* (Elmer and McFarland 1987; Castex et al. 1990; Kimmey et al. 1990) and the consequent diarrhoea, leading to an improvement in the performance of steers (Mir and Mir 1994), lactating dairy cows (Swartz et al. 1994), sheep (Jouany et al. 1998) and poultry (Bradley et al. 1994). The pharmacological protective action of yeasts against pathogenic organisms has even been applied in aquaculture. Other than serving as sources of vitamins and proteins, yeasts increase the non-specific local immunity by changing the production and activity of bacterial toxins (Isayev and Nagornaya 1992). The interaction between bacteria and yeasts in aquaculture, however, remains very vague and needs attention.

Megee et al. (1972) described the symbiosis between *Saccharomyces cerevisiae* and *Lactobacillus casei* and indicated that by varying the concentration of the substrate's different types of symbioses like commensalism + competition, competition, and

mutualism and competition were present. When no riboflavin was present in the medium, the bacteria were dependent upon the yeast for supplying the riboflavin, but competed for limited supplies of glucose in the medium when sufficient riboflavin was present. "True commensalism" was reported by Shindala et al. (1965) on the symbiosis between *Saccharomyces cerevisiae* and *Proteus vulgaris* based on an essential niacin-like factor, and between *Saccharomyces cerevisiae* and *Proteus vulgaris* based on riboflavin deficiency both elaborated by the yeasts and required by the bacterium. Challinor and Rose (1954) observed 13 interrelationships between yeasts, mainly *Saccharomyces cerevisiae*, and *Lactobacillus* spp., and in each of them the yeast appears to be the active organism, synthesising the missing substances, like vitamins, amino acids or purines, essential for the growth of *Lactobacillus*. Symbiotic growth in a chemostat between *Acetobacter suboxydans* and *Saccharomyces carlsbergensis* was reported by Chao and Reilly (1972) based on the inability of the yeast to utilise mannitol which was added as the only carbon source, but actively ferments the fructose once it has been oxidised by the bacterium. On the other hand, during alcoholic fermentation of molasses worts, increasing yeast inocula enhanced the lactobacilli growth and contributed to the consumption of monosaccharides liberated during hydrolysis of sucrose by yeasts (Ngang et al. 1992). In a similar way, when *Lactobacillus plantarum* and *Saccharomyces cerevisiae* were grown in co-culture in a glucose–citrate medium under acid conditions, *Saccharomyces* reduced the lactic acid produced by *Lactobacillus* and thereby stabilised the pH, encouraging the fermentation of citrate by the *Lactobacillus* (Kennes et al. 1991a).

4.2.2.2 Yeast–Yeast Interactions

Mixed microbial populations are intentionally applied in industry to improve flavour and yield (Verachtert et al. 1990), to lower pH to inhibit undesired species and to create stability or to obtain desired physiological properties (Harrison 1978). Yeasts are an integral part of these populations and help to secure quality by a range of mechanisms and activities. Detailed yeast–yeast interactions, however, are not studied systematically as observed with bacterial interactions. Other than the most commonly found interrelationship between yeasts, namely the competition for nutrients to survive (Nissen et al. 2004), significant contributions similar to those for bacteria based on symbiosis between yeasts comprise typical mutualism, commensalism, amensalism and predation. These interrelationships have been successfully applied in industry.

Yeast–yeast co-fermentation of glucose and xylose, as obtained after the breakdown of polymers in agricultural waste streams, with immobilised *Pichia stipitis* and *Saccharomyces cerevisiae* resulted in higher ethanol yields from the mixed substrates (Grootjen et al. 1991). The treatment of the effluent of waste starch with *Endomycopsis fibuliger* and *Candida utilis* yielded high concentrations of single-cell protein (Jarl 1969, 1971) when the former hydrolysed the starch to dextrins and low molecular weight sugars, enabling *Candida utilis* to assimilate the soluble products released. The use of mixed yeast cultures for single-cell protein production from *n*-alkanes was used to overcome vitamin requirements. By culturing the biotin-requiring yeasts *Candida novellus*, *Candida tropicalis* or *Pichia sake* with B_1-requiring yeast species, such as *Trichosporon pululans* or *Candida lipolytica*, good growth was

obtained without any added vitamins as the yeasts supply each other's vitamin requirements. Another application of yeasts (*Candida utilis* and a *Mycotorula* sp.) in co-culture, grown on sulphite waste liquor for the production of single-cell protein, contributed to high yields when *Candida utilis* enhanced the growth of the *Mycotorula* sp. The invaluable role of autolysis of yeasts, as a means of indirect interaction between yeasts, should not be overlooked, as the amino acids and vitamins released may encourage the growth of other yeasts (Fleet 2001).

Direct interaction between yeasts mainly relies on the antagonistic interaction involving yeasts capable of producing soluble killer toxins. The secreted proteinaceous killer toxins are lethal to a wide variety of susceptible yeasts and have many potential applications in environmental, medical and industrial biotechnology (Young 1987; Rosini and Cantini 1987; Suzuki et al. 2001; Marquina et al. 2002). Recently, it was observed that zygocin, a protein toxin produced and secreted by the yeast *Zygosaccharomyces bailii* effectively kills pathogenic yeasts like *Candida albicans*, *Candida krusei and Candida glabrata* (Weiler and Schmitt 2003). In the late 1990s, predacious yeasts based on haustorium-mediated predation were also observed (Lachance and Pang 1997) as another means of direct interaction between yeasts. More information on predation between yeasts other than in laboratory situations, however, is needed.

4.2.2.3 Yeast–Filamentous Fungi Interactions

The most prominent interactive relationships between yeasts and filamentous fungi definitely comprise the antagonistic application of yeasts as biocontrol agents against fungi, and the mutualistic relationship with fungi during the processing of predominantly Asian fermented foods. Both topics will be dealt with later in this chapter. Commensalism and mutualism rely on the co-culture of yeasts and filamentous fungi and the latter provide the necessary enzymes to break down complicated substrates like cellulose. A typical example is when *Candida utilis* species contribute to high single-cell protein content when grown in co-culture with the cellulotic *Aspergillus niger* on apple promace (Bhalla and Joshi 1994). The higher yield of protein from the yeast–fungi co-culture relies on the hydrolysis of lignocelluloses by the fungi releasing hexoses and pentoses which the *Candida utilis* can efficiently metabolise.

On the other hand, yeasts exudates may also stimulate hyphal growth like *Rhodotorula mucilaginosa* enhancing the growth of the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea* (Fracchia et al. 2003).

4.3 Yeast Interactions in Foods and Beverages

Microbial communities with their combined physiology, interactions and enzymatic activities are responsible for the major biochemical and nutritional changes that occur in the substrates of fermented foods and beverages (Steinkraus 1982; Hesseltine and Wang 1967; Wood and Hodge 1985; Wood 1981). Antimicrobial effects present in fermented foods and beverages are attributed to organic acids, antibiotic factors, volatile acids, hydrogen peroxide and to a number of substrates

excreted in the products. These antimicrobial effects are the result of the presence of several kinds of microorganisms involved in the fermentation and putrefaction of foods which inevitably lead to beneficial or detrimental interaction among the populations (Noda et al. 1980; Frederickson 1977; Bull and Slater 1982; Slater and Bull 1978).

Microbial interactions involving yeasts, bacteria and/or fungi have been indicated from a number of examinations of food products like bread (Lues et al. 1993), meat, fish, fruit, vegetables, protein foods, dairy products and cereals. The metabolic interactions are governed by the inherent technological characteristics and biochemical activities of yeasts providing essential growth metabolites, such as amino acids, vitamins, removing toxic end products of metabolism, inhibiting the growth of undesired microorganisms by lowering the pH, secreting alcohol, producing CO_2, or encouraging the growth of the starter cultures by increasing the pH owing to the utilisation of organic acids.

These properties have been applied successfully in the processing of foods and beverages as a means of biological control to enhance food safety and shelf life by destroying, retarding or preventing the growth of pathogenic and spoilage microorganisms (Ray and Daeschel 1992; Campbell-Platt 1994). The most successful application of interactions in foods and beverages comprises the presence of yeasts and lactic acid bacteria in a product. The interactions rely on several modes of action; however, despite the many references to the occurrences of yeasts in co-culture with lactic acid bacteria (Wood and Hodge 1985; Steinkraus 1982), only a few researchers have studied the interactions systematically in defined media (Gobbetti et al. 1994a, b; Kennes et al. 1991b). Except for the studies in wine and to an extent in bread making, none of the other fermented foods or beverages have been studied in detail.

Yeast–bacteria associations are by far the most prominent interactions occurring in food and beverage production determining the flavour and other qualities by a range of mechanisms and activities. While lactic acid bacteria, comprising *Pediococcus*, *Leuconostoc*, *Lactobacillus*, *Lactococcus* and *Bacillus*, are the main species associated with fermented products, other species have significant roles. Yeast–yeast associations, on the other hand, are frequently indicated in foods and beverages, but few studies have reported the interactions between the yeasts in detail other than referring to the presence of them and their association with bacteria or moulds. Interactions between the different species occur at various stages throughout production, sometimes as multicultures, unimulticultures or as polycultures. In addition, these interactions may be initiated spontaneously, when the organisms originate from the environment or are inoculated as mixed cultures. These interactions again may appear simultaneously or sequentially to achieve a specific goal. A few typical yeast–bacteria interactions as encountered in foods and beverages are highlighted. As these interactions vary between different products, only the major groups will be discussed.

4.3.1 Microbial Interactions in Fermented Starch-rich Materials

Various fermentations of starch-rich raw materials utilising yeast–lactic acid bacteria associations or mixed cultures including fungi are evident in the literature. The processing involves acid fermentation or alcohol fermentation. Both exhibit distinct

advantages like prevention of spoilage, flavour development, preservation and creation of stability within the products. The fermenting processes relying on alcoholic production will be dealt with later under the heading alcoholic fermentation.

It is important to maintain an equilibrium between the yeasts and the lactic acid bacteria during acid fermentation (Wood 1985; Nout 1991). Excessive acid production by the lactic acid bacteria will result in a decline in the number of surviving yeasts, which consequently leads to a deficiency of growth factors. As a result of such deficiencies, the lactic acid bacteria would produce less acid, and in turn allow an increase in yeast numbers (Nout 1991; Nout et al. 1989). The interactive behaviour between yeasts and lactic acid bacteria creates environmental conditions that protect the products from spoilage by fungi and pathogens owing to the low pH and high compositions of acetic and lactic acids.

4.3.1.1 Cereal Fermentations

Sourdough bread leaven relies on various associative interactions whereby the lactic acid bacteria (*Lactobacillus sanfrancisco*) and yeasts (*Saccharomyces cerevisiae* and *Saccharomyces exiguus*) survive in co-existence (Gobbetti and Corsetii 1997; Gobbetti et al. 1995). The *Lactobacillus* sp. utilises the carbohydrate, maltose, made available owing to amylase action, providing the yeasts with glucose, a stage that may be best described as commensalism, since *Saccharomyces exiguus* strains lack the ability to utilise maltose. However, *Saccharomyces cerevisiae* strains may consume maltose competitively, leading to a decrease in bacterial metabolism (Gobbetti et al. 1994a). Under normal fermentation conditions the yeasts utilise the glucose liberated from the breakdown of maltose and in return produce CO_2 for leavening (Sugihara 1985; Steinkraus 1979). *Lactobacillus sanfrancisco* has a positive influence on yeast leavening and gas production (Gobbetti et al. 1995). A similar positive tendency in yeast fermentation and gas production was observed in the Corleywood baking process by Viljoen and Lues (1993) and Lues et al. (1993). The yeasts secrete compounds like amino acids (Gobbetti et al. 1994b; Spicher and Schroder 1979), peptides (Berg et al. 1981) and vitamins (Spicher and Schroder 1979; Spicher and Nierle 1984) that stimulate the growth of the lactic acid bacteria (Spicher et al. 1981, 1982). Moreover, the yeasts produce phenolic compounds, glycerol (Yong and Wood 1976), etc., which are specific for the aroma (Noda et al. 1980), while the synthesis of antimicrobial compounds by the lactic acid bacteria inhibits spoilage organisms like *Bacillus subtilis*, coliforms and others (Corsetti et al. 1994). The increased protective association in co-culture is expanded in bread by the inclusion of *Propionibacterium freudenreichii* in order to prevent ropy bread induced by *Bacillus subtilis* (Odame-Darkwah and Marshall 1993). Killer activity, however, may cause a serious decrease in the quality of the product if the inoculated yeasts are killed, as indicated in the Turkish baking industry.

This associative interaction between lactic acid bacteria and yeasts, as applied in the processing of sourdough bread (Sugihara et al. 1971; Kline and Sugihara 1971; Wood et al. 1975; Martinez-Anaya et al. 1990; Boraam et al. 1993; Gobbetti et al. 1994a, b, 1995; Oura et al. 1982), is also applied during the production of Pannettone, rye sourdough (Spicher et al. 1981) and soda crackers. For more details, Sugihara (1985) reviewed these processing methods.

Various mixed-culture fermentations are initiated spontaneously in cereal ferments from organisms present in the natural environment, equipment, substrates or through the repeated use of inocula originating from a previous fermentation (Hesseltine 1965, 1983; Verachtert et al. 1990). These mixed inocula may be added simultaneously or sequentially. Unfortunately, most of the cereal fermented foods have been inadequately studied, and contribute little to the modes of interaction between yeasts, bacteria and fungi. It is therefore very difficult to refer to precise interactions as they occur. Most of the literature only refers to the microorganisms present or the biochemical changes, with no indications of interaction.

References to the aspects of the microbiology of ogi preparation are abundant (Akinrele 1970; Odunfa 1999; Banigo and Muller 1972; Odunfa and Adeyele 1987; Banigo et al. 1974). Ogi is a natural fermentation, the microbial flora originate from the maize, sorghum or millet grains (Odunfa 1999; Steinkraus 1982). The grain fungal flora *Aspergillus*, *Penicillium*, *Cephalosporium* and *Fusarium* spp. are eliminated early during the steeping period (Akinrele 1970) and their contribution to the product or other organisms is not clear. The cause for their early elimination is probably due to their inability to compete under the acidity and low oxygen conditions prevailing in the fermenting dough-like mass. *Corynebacterium* hydrolyses the starch and initiates acidification owing to the production of organic acids. *Lactobacillus plantarum* and *Aerobacter cloacae* are also involved in the acidification. The *Lactobacillus* utilises the dextrins from the corn following depletion of the fermentable sugars and contributes most to the acidification by producing lactic acid, while *Aerobacter* increases the niacin and riboflavin content of the mash (Akinrele 1970). The lowering of the pH encourages the yeasts *Saccharomyces cerevisiae* and *Candida mycoderma* to grow, contributing to the flavour and enrichment of vitamins. The lactic acid is a good growth substrate for *Candida*, and the species is therefore considered to play an important role in the preparation of ogi involving the partial destruction of organic acids (Akinrele 1970). Consequently, this will increase the pH and may allow the growth of undesired bacteria. The associative action between the yeasts and the bacteria may therefore be explained as mutualism, since the bacteria create growing conditions for the yeasts by hydrolysing the starch and lowering the pH. The yeasts, in return, provide growth stimulants such as vitamins needed by *Lactobacillus plantarum* (Akinrele 1970) and increase the pH. This was shown earlier with lactic acid bacteria from sourdoughs which required vitamins (Spicher and Schroder 1979) and amino acids to be supplied by yeasts.

Similar associative interactions were observed by Nout (1991) studying the ecology of natural lactic acid fermentation of sorghum-based infant food formulas during repetitive fermentation cycles. During the early fermentation stages, *Leuconostoc* and *Lactococcus* spp. dominated, inhibiting yeast growth owing to excessive production of organic acids. When the nutrients became deficient, *Lactobacillus plantarum* and *Candida* spp. succeeded, which consequently led to an interactive equilibrium. The pH was regulated by the lactic acid bacteria producing organic acids, which allowed adequate yeast growth, and the yeasts supplying the micronutrients enabled the growth of the lactic acid bacteria. Moreover, the fermented mixtures of cereals exhibited a strong antimicrobial effect towards a range of pathogenic bacteria (Nout et al. 1989).

Other cereal fermented foods relying on spontaneous fermentation such as kenkey, koko, banku, panjabi waries, papadams, jalebies, pozol, etc., are prepared in much the same way as described for ogi, although with a different microbial composition. Despite inadequate information on the associative interaction among the microorganisms, the same mutualistic relationships as discussed earlier might be possible. Kenkey fermentation is dominated by *Aspergillus*, *Rhizopus* and *Penicillium* in the initial fermenting stages. The acid-producing *Leuconostoc* spp. soon decrease in numbers during the fermentation, succeeded by *Lactobacillus brevis* and *Acetobacter* spp. in the fermenting dough. Wild types of yeasts, including *Saccharomyces cerevisiae*, are present at all stages of the fermentation, contributing to the flavour by producing esters and ethanol (Muller and Nyarko-Mensah 1972). Koko fermentation comprises the lactic acid bacteria (*Pediococcus cerevisiae*, *Leuconostoc mesenteroides* and *Leuconostoc fermenti*) and yeasts. Panjabi waries and papadams include the yeasts *Saccharomyces cerevisiae* and *Candida* spp., while jalebies are prepared with *Saccharomyces bayanus* (Batra and Millner 1974). Pozol includes fungi (*Geotrichum* and *Mucor*), *Trichosporon* and *Agrobacterium* (Verachtert and Dawoud 1990).

4.3.1.2 Cassava

Cassava is considered a major source of starch-rich food, but with low levels of protein (Steinkraus 1982; Odunfa 1999; Akinrele et al. 1975). The fermentation of gari, the most important fermented cassava product, is anaerobic and follows a two-stage process. In the first stage, *Corynebacterium manihot* and *Bacillus* spp. break down the starch owing to the production of pectinolytic enzymes (Okafor et al. 1984) and release organic acids, which consequently lowers the pH (Collard and Levi 1959; Akinrele 1970). *Bacillus* spp. cause hydrolysis of starch by disintegrating the cell components (Ejiofor and Okafor 1981). According to Okafor (1977), the lactic acid bacteria (*Leuconostoc* and *Lactobacillus*) and *Alcaligenes* are also present during the first stages of fermentation, utilising the free fermentable sugars originating from the tuber and adding to the acidity. Abe and Lindsay (1978) supported by Ngaba and Lee (1979) reported the presence of *Streptococcus faecalis* and claimed that the species is the primary fermentative organism in acidic cassava fermentation. In the second stage, the acid condition stimulates the growth of the fungus *Geotrichum candidum* and presumably also the yeast *Candida*. Collard and Levi (1959) and Akinrele (1970) reported that the fungus added to the acidification, and for the production of aldehydes and esters that are responsible for the taste and aroma. The yeast species quickly proliferates and appeared to be essential as part of the microbiota present during gari fermentation (Okafor 1977). However, no indication of the contributions of the yeasts was reported by any of the authors, despite their growing to numbers as high as 10^6 cfu/g. Moreover, no reference to the associative interaction between the yeasts and the other microorganisms was reported.

During the fermentation of foo-foo, similar microbial populations and interactions were evident, with the exception of *Klebsiella* and the absence of the fungi (Okafor et al. 1984). The *Bacillus*, *Klebsiella* and *Corynebacterium* spp. develop early and contribute to acid formation and the hydrolysis of starch, but are overgrown

by the lactic acid bacteria that further increase the acidic conditions. At the same time, the yeast *Candida* develops in large numbers, and contributes to the lowering of the pH. The prevailing acidified environment permits only these organisms to grow.

4.3.1.3 Fermented Flavouring Products

The production of soy sauce represents a typical sequential inoculation method making use of a two-stage process. The first stage is an aerobic process growing *Aspergillus oryzae* or *Aspergillus sojae* on soybeans and wheat which amylolytically hydrolyses the starch (Yokotsuka 1985; Hesseltine and Wang 1967; Verachtert and Dawoud 1990). As predominant in most mixed-culture fermentations, the fermentation relies on the development of yeasts and lactic acid bacteria. This association is only visible during the second stage. After relying on simple sugars liberated from the first stage, an anaerobic fermentation with *Lactobacillus delbrueckii*, *Pediococcus halophilus* and *Zygosaccharomyces rouxii* takes place. The lactic acid bacteria proceed to grow and produce lactic acid, which decreases the pH, encouraging the growth of *Zygosaccharomyces rouxii*, which results in vigorous alcoholic fermentation (Yong and Wood 1976). Excessive lactic acid fermentation by *Pediococcus halophilus*, however, results in the depression of alcoholic fermentation (Noda et al. 1980). Other osmophilic yeasts such as *Candida etchelsii* and *Candida versatilis* present produce phenolic compounds and furfural, which are desirable flavour enhancers (Morimoto and Matsutani 1969; Yokotsuka 1985; Noda et al. 1980; Wood and Hodge 1985). Similar processes occur in the preparation of miso, except for the use of barley or rice and soybeans, kaffir beer, merissa brewing in Sudan, etc.

4.3.2 Microbial Interactions in Dairy Products

4.3.2.1 Milk-Based Beverages

The commensalistic interaction between *Lactobacillus acidophilus* and the lactose fermenting yeast *Kluyveromyces fragilis* in acidophilus-yeast milk (Subramanian and Shankar 1985) relies on the co-existence of both organisms to secure a good product. Although the lactic acid fermentation originally relied on the fermentation of *Lactobacillus acidophilus* either alone or in mixed cultures with other lactic acid bacteria, the overgrowth of these organisms resulted in fewer viable cells of *Lactobacillus acidophilus*, which consequently reduced the species contribution to gastrointestinal disorders (Lang and Lang 1975). The co-culture of *Lactobacillus acidophilus* with lactose-fermenting yeasts reduces the time of coagulation of the milk owing to acid production by the yeasts, elevates the number of viable lactic acid bacteria cells attributed to stimulating influences of yeasts, and inhibits the growth of *Escherichia coli* and *Bacillus cereus* (Subramanian and Shankar 1985).

Mutualism (synergism) occurs between yeasts and lactic acid bacteria during the fermentation of milky kefir (Rossi 1978) and sugary kefir (Leroi and Pidoux 1993). The predominant species isolated from milky kefir are *Saccharomyces kefir*, *Candida kefyr*, *Lactobacillus caucasicus*, *Lactobacillus casei* and *Leuconostoc* spp. (Oberman 1985; Loretan et al. 2003). The yeasts provide growth factors like amino acids,

vitamins and other compounds for bacterial growth, which consequently lead to elevated acid production, while the bacterial end products are used by the yeasts as an energy source (Challinor and Rose 1954; Wood and Hodge 1985). This phenomenon creates stability in the products. However, a decrease in alcohol production by the yeasts might occur owing to excessive lactic and acetic acid production by osmophilic lactic acid bacteria (Noda et al. 1980; Essia Ngang et al. 1990; Tani et al. 1963), competition for the carbon source or lysis of the yeast cell walls by bacterial enzymes (Lonvaud-Funel et al. 1988; Borregaard and Arneborg 1998).

Similar symbiotic relationships based on acid or alcohol fermentation occur when lactic acid bacteria are responsible for lowering of the pH as a result of the secretion of organic acids (Wood 1981) allowing the yeast population to be competitive in the immediate environment, followed by yeast fermentation as in various milk-based fermentations like Leben, Dahi, Koumiss, etc. (Wood 1981; Bankole and Okagbue 1992; Steinkraus 1982). Oberman (1985) and Vedamuthu (1982) reviewed the fermented milks, whereas Narvhus and Gadaga (2003) reviewed the role of interactions in African fermented milks. The combination of conditions (acidic, saturated with CO_2 and alcohol), is inhibitory to many spoilage bacteria and filamentous fungi and thereby substantially increases the shelf life and safety of the products (Wood and Hodge 1985).

4.3.2.2 Cheese

The production of cheeses involves a maturation stage characterised by a complex ecology of yeasts, bacteria and filamentous fungi (Devoyod and Desmazeaud 1971; Fleet 1990; Jakobsen and Narvhus 1996; Viljoen 2001). The microbial interaction between this microbiota determines the quality, safety and acceptability of the final product.

Several yeasts assist the starter cultures in cheeses by proteolytic activity (Besançon et al. 1992), lipolytic activity (Siewert 1986), the formation of aroma components and participation in the maturation (Welthagen and Viljoen 1999). The positive interaction of yeasts with the starter cultures in surface-ripened cheeses has been well reviewed (Fleet 1990; Jakobsen and Narvhus 1996; Corsetti et al. 2001; Addis et al. 2001). The yeasts, by utilising the accumulated lactic acid in the cheeses, increase the pH and secrete growth factors which promote the growth of *Brevibacterium linens*, which is essential for cheese ripening (Marth 1978). Yeasts also assist the development of fungi in blue-veined and Camembert cheeses (Kaminarides and Laskos 1992; Schlesser et al. 1992) by gas production leading to curd openness (Coghill 1979). In contrast, however, strain-specific interactions between *Yarrowia lipolytica* and *Penicillium roqueforti* may result in the inhibition of mycelial growth and sporulation of the mould mainly owing to competition for nutrients (Van den Tempel and Jakobsen 1998).

Similar yeast–lactic acid bacteria associations were detected in harder cheeses like cheddar (Fleet and Mian 1987; Welthagen and Viljoen 1998, 1999), Parmesan (Romano et al. 1989) and Gouda (Welthagen and Viljoen 1999). On the basis of these associations, Guerzoni et al. (1996) proposed the inclusion of *D. hansenii* and *Y. lipolytica* as adjunct starter cultures during the making of cheese to support the

starter cultures during ripening based on proteolytic and lipolytic activity. In addition, the ability of *D. hansenii* to inhibit *Clostridium* species further adds to the justification (Deiana et al. 1984). Ferreira and Viljoen (2003) applied these yeast species as adjunct starter cultures in cheddar cheese and clearly indicated the mutualistic interaction not only between the yeasts and the lactic acid bacteria, but also between the two yeast strains. When the yeast strains were inoculated individually, a much lower survival was evidenced. The exact mutualistic association between the yeast strains, however, was not clarified other than the indication that both strains survived better when co-inoculated and enhanced flavour development was, detected. Addis et al. (2001), however indicated the yeast–yeast interaction between the two strains in blue-veined cheeses, evidenced by an enhancement in the growth of *Y. lipolytica* caused by *D. hansenii*.

4.3.3 Microbial Interactions in Meat Products

The low initial numbers and reduced growth rates at low temperatures of yeasts in meat products are constraints that prevent them from effectively competing with psychrotrophic bacteria (Walker and Ayres 1970; Dillon and Board 1991; Fleet 1990). However, storage and processing conditions that reduce bacterial competition favour the growth of yeasts (Fleet 1990) and they may cause spoilage or add to the flavour. In fermented meat sausages, when *D. hansenii* species is added as an adjunct starter culture, the species adds a yeast flavour and stabilises the reddening reaction (Hammes and Knauf 1994; Geisen et al. 1992). The sulphite-tolerant species (Banks 1983) *D. hansenii* and *Candida* spp. are responsible for encouraging the growth of pseudomonads and members of the *Entobacteriaceae*, which is usually inhibited by sulphite (Banks et al. 1985). Owing to acetaldehyde production and thereby sulphite binding (Dillon and Board 1991) the yeast species reduce the antibacterial activity of the preservative. Similarly, yeasts utilise organic acids playing a preservative role in processing, and thereby increase the pH, favouring the growth of spoilage bacteria (Walker 1977). No specialised studies on the interaction between these microorganisms, however, have been attempted, and therefore data regarding microbial associations remain very vague.

4.3.4 Microbial Interactions During Vegetable Fermentations

The fermentation and the subsequent storage of olives rely on various interactions between a developing yeast flora and bacteria. During the first phase, when active lactic acid fermentation occurs, fermentable sugars are present in the brine under anaerobic conditions. The strong fermentative yeasts predominate when bacteria are inhibited, outcompeting the other yeasts for the available sugars or they may disrupt the lactic acid fermentation under normal conditions causing “stuck” fermentations (Vaughn et al. 1972). When the available sugars are depleted, oxidative yeasts like *Pichia membranaefaciens* and *Candida mycoderma* develop, utilising the desirable organic acids in the brines and thereby increasing the pH, which allows spoilage bacteria to grow (Mrak et al. 1956). The commonest spoilage incurred by fermenting pectolytic or cellulolytic yeasts during this period is gas formation and softening

(Garcia et al. 1992; Vaughn et al. 1972). Similar results were reported for cucumber fermentation (Vaughn 1983). In contrast to the detrimental effects of yeasts in olive brines, Marquina et al. (1992) reported on significant contributions of yeasts which utilise the lipids present in olives or produce lipases, which resulted in the formation of compounds that stimulate the growth of desirable lactic acid bacteria. Moreover, yeasts utilise the bitter oleuropein, an olive component with antibacterial action, which consequently stimulates bacterial growth (Marquina et al. 1992). Halotolerant yeasts contribute to the flavour (Suzuki et al. 1989), and the occurrence of killer activity might be used to avoid the growth of undesired yeast contaminants.

4.3.5 Microbial Interactions During Alcoholic Fermentations

Several types of fermented beverages which include alcoholic production are evident in the literature derived from fruit, sorghum, rice, barley, plants, etc. Many of the beverages rely on mixed-culture fermentations, reviewed by Wood and Hodge (1985), Steinkraus (1982), Verachtert and Dawoud (1990), Wood (1981), and others.

4.3.5.1 Wine

The interaction between the microorganisms associated with wine fermentation relies on a series of inter-relationships: yeasts–fungi, yeasts–yeasts, yeasts–acetic acid bacteria and yeasts–lactic acid bacteria (Fleet 1992). The existing interactions have been reviewed in detail by Fleet (2003). A typical yeast–filamentous fungi interaction occurs when infection is incurred by *Botrytis cinerea* favouring the presence of non-*Saccharomyces* yeasts and causes a slower fermentation and an increase in glycerol and acetic acid production. The extracts of grapes infected with *Botrytis cinerea* will inhibit (Ribéreau-Gayon 1985) or activate (Reed and Nagodawithana 1988) alcoholic fermentation.

Except for killer yeast activity (Young 1987; Shimizu 1993; Guriérrez et al. 2001), the recognition of non-*Saccharomyces* yeasts as important contributors to wine fermentation (Fleet et al. 1984; Heard and Fleet 1987; Martinez-Anaya et al. 1990; Mora et al. 1992; Schutz and Gafner 1993) results in various yeast–yeast associations that can be exploited. Other than the production of ethanol, organic acids, sulphur, etc. (Fleet 1990, 2001; Bisson 1999, Soden et al. 2000; Mills et al. 2002) by some yeasts, inhibitory to the growth of competing yeasts, the medium-chain fatty acids, decanoic and octanoic acids (Lambrechts and Pretorius 2000), their corresponding ethyl esters (Lafon-Lafourcade et al. 1984; Ribéreau-Gayon 1985) and yeast ghosts (Edwards et al. 1990) produced all contribute to yeast–yeast interactions. Inhibitory effects by *Kloeckera apiculata* against *Saccharomyces cerevisiae* (Mortimer 2000) and *Metschnikowia pulcherrima* against a range of other yeasts have been reported (Nguyen and Panon 1998). The interaction between the non-*Saccharomyces* and *Saccharomyces* species based upon competition for carbohydrates, nitrogen, other compounds and dominance during the fermentation remains largely unexplored.

Yeast–bacteria interaction in wine production relies predominantly on the yeast association with the lactic acid bacteria and acetic acid bacteria. Detailed studies on

the combined growth of acetic acid bacteria (*Acetobacter aceti*, *Acetobacter pateurianus* and *Gluconobacter oxydans*) and wine yeasts (*Saccharomyces cerevisiae*, *Kloeckera apiculata* and *Candida* spp.) were performed (Lafon-Lafourcade et al. 1984; Drysdale and Fleet 1988, 1989). Antagonistic effects by *Acetobacter*, due to acetic acid excretion, result in decreased fermentation by *Saccharomyces cerevisiae* and may cause stuck fermentation (Ludovico et al. 2001).

Wine yeasts vary in their interaction with lactic acid bacteria (Fornachon 1968; Thornton 1991; Suzzi et al. 1995) as they may inhibit or stimulate the growth of lactic acid bacteria. The naturally present lactic acid bacteria occur at low numbers, and die during alcoholic fermentation (Fleet 1993, 2003; Fleet and Heard 1993) and exert little or no effect on yeast growth. If the alcoholic fermentation is restricted or retarded, multiple yeast–lactic acid bacteria interrelationships occur that play a substantial role during malolactic fermentation (King and Beelman 1986; Lemaresquier 1987; Markides 1993; Fleet 1990; Martineau and Henick-Kling 1995), which commenced after alcohol fermentation. The antagonism of the yeasts is related to alcohol production (Wibowo et al. 1985), SO_2 (Wibowo et al. 1988), proteins (Dick et al. 1992), fatty acids (Edwards et al. 1990; Edwards and Beelman 1987; Lonvaud-Funnel et al. 1988; Lafon-Lafourcade et al. 1984), antibacterial factors (Fornachon 1968) and the removal of substances important to bacterial growth (King and Beelman 1986). Growth stimulation of the lactic acid bacteria is encouraged by yeast autolysis (Fleet 1992; Charpentier and Feuillat 1993; Crouigneau et al. 2000), removal of inhibitory fatty acids (Edwards and Beelman 1987), yeast ghosts (Lafon-Lafourcade et al. 1984), amino acids (Lonvaud-Funel et al. 1988), vitamins (Lemaresquier 1987), sucrose hydrolysis (Ngang et al. 1992) and ethanol at low concentrations (King and Beelman 1986).

Although amensalism is indicated during wine fermentation, whereby the *Saccharomyces cerevisiae* strains prevent the growth of initially present non-conventional wine yeasts owing to elevated concentrations of ethanol, useful commensal relationships between yeasts occur when wine is allowed to become partially aerobic, leading to the formation of sherry (Amerine and Kunkee 1968; Carr et al. 1969). According to these authors, the interaction between the flor-filming yeasts relies on competitive, amensal and commensal relationships. In addition, neutralism is reported as *Saccharomyces diasticus* strains possess glucoamilase, which enables them to ferment polysaccharides which cannot be metabolised by other yeasts found during beer and wine fermentations.

4.3.5.2 Fruit Juices and Cider

The yeast–bacteria interrelationship between *Saccharomyces cerevisiae* and *Leuconostoc oenos*/*Lactobacillus plantarum* plays an important role in the degradation of glucose, malate and citrate, the major carbon sources in fruits and fruit juices like orange and cider, during fruit fermentations under acidic and anaerobic conditions (Kennes et al. 1991a, b). The microbial ecology follows the principles of wine fermentations. The microflora of the apples includes yeasts (*Saccharomyces cerevisiae*, *Kloeckera apiculata* and *Candida* spp.), lactic acid bacteria (*Lactobacillus brevis*, *Pediococcus* spp., *Leuconostoc mesenteroides* and *Leuconostoc oenos*) and

acetic acid bacteria (*Acetobacter* and *Gluconobacter* spp.). The non-proliferating population of yeasts initiates the fermentation, but is inhibited by a lack of nutritional growth factors and the toxic effect of ethanol owing to competition and amensalism from the ethanol-tolerant species *Saccharomyces cerevisiae*. Similarly, competition for nutrients between lactic acid bacteria and the yeast also exists as well as positive or negative contributions from the indigenous microflora present. The yeast outcompetes the lactic acid bacteria for the utilisation of the sugars, which results in the production of ethanol without changing the pH. The ethanol present in the media favours the subsequent conversion of citric acid (with oranges) or malic acid (with apple juice) to acetic acid by the lactic acid bacteria *Lactobacillus plantarum* and *Leuconostoc oenos*, respectively (Kennes et al. 1991a). *Leuconostoc oenos* usually fulfils a similar role during wine fermentation (Fleet et al. 1984), although it has been proposed that *Schizosaccharomyces pombe* can remove excess organic acids with inferior results. Although the lactic acid bacteria compete for carbohydrates, they also depend on essential stimulants excreted by the yeasts as reported earlier. Similar competitive/commensal interrelationships may occur between *Pediococcus cerevisiae* and the yeasts during beer production when the bacteria form diacetyl, which spoils the taste of the beer, or polysaccharides, which cause ropiness.

4.3.5.3 Beer

Beer is a product derived from malted barley, hydrolysed under controlled conditions by amylases to maltose and glucose to make it available to yeasts which produce ethanol (Rainbow 1981; Priest and Campbell 1996). Under normal conditions, the inoculated brewer's yeast *Saccharomyces cerevisiae* quickly dominates during fermentation and suppresses the growth of spoilage *Enterobacteriaceae*, lactic acid bacteria and other competitive microorganisms by elevated alcohol concentrations, low pH, CO_2 production, SO_2, co-sedimentation and organic acid secretion, while the anaerobic conditions that prevailed prevent the growth of aerobic acetic acid bacteria (Jespersen and Jakobsen 1996). Brewer's yeast inhibition of lactic acid bacteria is also attributed to competition for nutrients (Pfenninger et al. 1979), heat-labile yeast metabolites (Dolezil and Kirshop 1980), alanyl dipeptides and co-sedimentation of certain bacteria with brewing yeasts (White and Kidney 1979, 1981). The hop bitters present also inhibit the growth of lactic acid bacteria. Competition for nutrients, low pH conditions and hops, moreover, inhibits *Pediococcus damnoses* and related *Pediococcus* spp. However, if insufficient cleaning, heating, hops-resistant bacteria (Fernandez and Simpson 1995) and delays in pitching of the wort occur, beer contaminants derived from barley, wort and the equipment may cause spoilage (Flannigan 1996). Filamentous fungi affect the flavour of the beer and mycotoxins have a concentration-dependent effect on yeasts, resulting in reduced CO_2 evolution and ethanol production. Similarly, *Pediococcus* spp. and *Lactobacillus* spp. contribute to the flavour (Priest 1996) and may compete for the available nutrients. The role of "wild yeasts" such as *Pichia membranaefaciens*, *Pichiasubpelliculosa* and species of *Schizosaccharomyces*, *Brettanomyces*, *Kloeckera*, *Debaryomyces*, *Candida* and *Torulaspora* is well reviewed by Rainbow (1981) and Campbell (1996). Although these yeast contaminants are able to grow under anaerobic conditions (Campbell

and Msongo 1991), they do not compete well under the acid and ethanol concentrations of the beer. Access of air stimulates their growth, and competition for the nutrients. Killer strains of *Saccharomyces cerevisiae* may cause the severest competition. The killer strain kills sensitive culture yeast strains, and establish itself as the dominant yeast of the fermentation. Yeast autolysis, which occurs after lengthy secondary fermentation, and nitrogen released by *Saccharomyces carlsbergensis* may also encourage microbial growth in providing essential growth stimulants.

Verachtert et al. (1990) in unravelling the complex mixed-culture process during Lambic and Gueze beer fermentation identified a succession of different microbial species during four fermentation phases. The spontaneous fermentation starts with the development of *Enterobacteria* and low numbers of maltose non-fermenters such as *Kloeckera apiculata*, *Saccharomyces globosus* and *Saccharomyces diarensis*. When these yeasts disappear, a second group of fermenting yeasts, *Saccharomyces cerevisiae* and related *Saccharomyces* spp., are responsible for ethanol fermentation, followed by actidione-resistant yeasts belonging to *Brettanomyces* that metabolise the sugars not assimilated by *Saccharomyces*. A fourth group of oxidative yeasts remain in the yeast layer found on top of the fermenting wort with no significance to the fermentation. After the main ethanol fermentation, lactic acid bacteria, usually *Pediococcus* spp., are responsible for the synthesis of lactic acid, adding to the acid–vinous character of the beer. Despite clear indications of numerous yeast–yeast interactions present during the production of these beers, the data remain vague and need attention.

4.3.5.4 Distilled Beverages

Similar interesting interrelationships may occur in distilled alcoholic beverages, such as whiskey and rum. But, as with beer, the interactions between yeasts, fungi and bacteria have been inadequately studied, despite numerous references to spoilage (Barbour and Priest 1988) and advances in brewing and distilling yeasts. Whiskey fermentation relies on a mixed fermentation of added yeast (*Saccharomyces cerevisiae*) and indigenous bacteria (Barbour and Priest 1988). The fermentation comprises the bacterial species *Lactobacillus*, *Enterobacteriaceae*, *Peddiococcus* and *Leuconostoc*, which originate from the malted barley and equipment. *Enterobacteriaceae* spp. are eliminated in the early stages of the fermentation by the low pH and alcohol concentration, while the lactic acid bacteria proliferate and compete with the yeast, which reduces the ethanol yield. The yeast, however, encourages the growth of lactic acid bacteria by the excretion of glycerol and products due to yeast autolysis (Barbour and Priest 1988).

4.4 Yeast Antagonism Applied as Biocontrol Agents in Preventing Plant-Spoilage Fungi

Numerous yeasts capable of playing a significant role in interactions have been isolated from fruit, fermented products, soil and other natural environments over the last few decades (Fleet 2003). However, our knowledge of the ecological distribution of such yeasts is still very limited. The interest of biological control in the

ecology of representative yeasts species arises from the necessity to control their metabolic activity by factors that can be influenced by technological means. Ideally it should be possible to predict that a yeast which possesses certain characteristics regarding biocontrol occurs most frequently in a certain environment within a defined habitat.

There is little doubt that various yeasts afford some protection to post-harvest spoilage (Chalutz and Wilson 1990; Chalutz and Droby 1998); consequently there is renewed interest in the possibilities of harnessing and accentuating the mechanisms of biological control as awareness of the dangers and disadvantages and public resistance to chemical control by fungicides posing potential oncogenic risks increase (Wilson and Wisniewski 1989). Moreover, the stresses of modern concepts of quality assurance require products of a high standard in quality, and biological control contributes to an improvement in hygienic safety, constant levels of quality and shelf life.

During the last decade a steady flow of reports claimed that particular post-harvest diseases and temperate fruit can be controlled to some extent by the antagonistic interaction between yeasts and mycotoxic fungi (Guinebretiere et al. 2000). Some of these yeasts like *Candida oleophila* and *Pseudozyma flocculosa* have been commercialised, known as Aspire and Sporodex, respectively (Droby et al. 1998; Punja and Utkhede 2003). Yeast antagonistic efficiency is also successfully reproduced in the inhibition of spoilage or toxin-producing fungi in high-moisture wheat stored under airtight conditions (Petersson and Schnurer 1995; Bjornberg and Schnurer 1993).

The high efficiency of yeasts applied as biocontrol agents is related to their indigenous adaptation to the immediate environment, the nutritional conditions prevailing at the wound site (Chalutz and Wilson 1990), their resistance to fungicides, survival at varying temperatures and ability to colonise (Roberts 1990). To evaluate the usage of yeasts as biocontrol agents, an understanding of the antagonistic interaction between the yeasts and fungal pathogens is needed.

Since limited evidence related to the production of antimicrobial compounds is evident, alternative ways of inducing biocontrol activity have been claimed. According to Avis and Belanger (2001) the antifungal metabolites produced by *Pseudozyma flocculosa* are a mixture of fatty acid containing derivatives that affect membrane permeability of the target organisms, thereby inhibiting the growth of powdery mildews. Nutrient competition, however at the wound site is regarded as the principal mode of antagonism (Droby et al. 1989; Punja and Utkhede 2003), although other modes of action like induced resistance, pathogen and antagonist density, age of the cells (Droby et al. 2002), cell wall degradation by β-(1-3)-glucanase enzymes (Wisniewski et al. 1991), killer toxins produced by yeasts (Walker et al. 1995), antifungal toxins like zygocin (Weiler and Schmitt 2003), and attachment of the yeasts by cell-surface proteins or lectin (Wisniewski et al. 1991).

Naturally occurring yeast antagonists like *Metschnikowia pulcherrima*, *D. hansenii*, *Pichia anomala* and *Pichia guilliermondii* are continuously isolated and reapplied to the fruit or silaged grain as effective biocontrol agents. Scientists will continue to collect more species, as our current knowledge regarding yeasts as biocontrol agents has only just begun.

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