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Antagonistic activities of lactic acid bacteria in food and feed fermentations

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1. SUMMARY

Many factors contribute to a successful natural fermentation of carbohydrate-rich food and feed products. Metabolic activities of lactic acid bacteria (LAB) play a leading role. Their ability to rapidly produce copious amounts of acidic end products with a concomitant pH reduction is the major factor in these fermentations. Although their specific effects are difficult to quantitate, other LAB metabolic products such as hydrogen peroxide and diacetyl can also contribute to the overall antibiosis and preservative potential of these products. The contribution of bacteriocins is also difficult to evaluate. It is suggested that they may play a role in selecting the microflora which initiates the fermentation. Bacteriocins are believed to be important in the ability of LAB to compete in non-fermentative ecosystems such as the gastrointestinal tract. During the past few decades interest has arisen in the use of the varied antagonistic activies of LAB to extend the shelf-life of protein-rich products such as meats and fish. Recent findings indicate that the newly discovered *Lactobacillus reuteri* reuterin system may be used for this purpose.

2. INTRODUCTION

Lactic acid bacteria (LAB) have been used traditionally to improve the aroma and texture and to prevent a rapid spoilage of dairy and meat products as well as vegetables and silages [1,2]. Losses in nutritional value during the fermentation process are regarded as minimal [3], product shelf-life is extended, and acid foods are less likely to harbour pathogenic microorganisms [4]. In addition, properties such as antitumour and anticholesterol activity, chemical reactions associated with reduction of nitrite, improvements in immunological status, and decreased gastrointestinal disorders have been attributed to the consumption of fermented dairy products [5,6].

Historically, these food fermentations have been based on empirical processes involving the activities of the natural flora present on the raw material combined with technical manipulations or additions [7]. Among these manipulations are processes such as mincing, chopping or tight packing which enhances the system's anaerobicity and promotes an equal distribution of the fermentative flora and its access to nutrients. A more selective propagation of the fermentative flora can be ob-

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tained using different methods such as salt and sugar marination of the raw material or back slopping, a process wherein a former succesful fermentation is used as inoculum for a subsequent fermentation [8]. In some fermentations the raw material is enriched by adding substrates like cereals, which are also rich sources of LAB [9].

The complex nutritional requirements of LAB and the lack of suitable nutrients in the raw material used for some fermentations has called for the addition of stimulants such as carbohydrates, amino acids, fatty acids, nucleic acid derivates, vegetable extracts, minerals and vitamins [2,10]. Malt enzymes were recognized early as a means to increase production of fermentable sugars in fish and crop silages enriched with cereals [11]. Fungal cellulases and amylases today are included as additives for silage fermentation [12,13].

The introduction of inoculants into dairy products early in this century was an important step in the industrialization of lactic acid fermentations [14], and inoculants are used today for silages [15], meat products [16,17], dairy products [18,19], and vegetables [9,20]. The desired characteristics for these starter culture organisms include: aroma production, carbohydrate, protein and peptide utilization, homo- or heterofermentation, suitable growth rate at desired temperatures, phage resistance and antagonistic properties. These criteria have been used in the past as selection parameters, and are being used today in the construction of genetically designed organisms [21].

3. FERMENTATION END PRODUCTS

LAB fermention products are characterized by the accumulation of organic acids, primarily lactic and acetic acid, and the accompanying reduction in pH. Levels and proportions of fermentation end products which accumulate depend on the species of the organism(s) involved, the chemical composition of the culture environment [22,23], and the physical conditions encountered during the fermentation process [24]. The microorganisms associated with these LAB fermentations include species found primairly in the following genera: *Streptococcus*, *Lactococcus*, *Pediococcus*, *Leuconostoc* and *Lactobacillus*, the latter represented by the sub-groups *Betabacterium*, *Streptobacterium* and *Thermobacterium* [25,26]. Based on biochemical and molecular characteristics the lactic streptococci (i.e. type N streptococci) are nc longer members of the genus *Streptococcus*. They have been reclassified into a new genus designated *Lactococcus* [27].

Hexose fermentations carried out by LAB in excess of sugar have been characterized as involving either the homofermentative production of lactic acid or the heterofermentative production of equimolar amounts of lactate, acetate/ethanol and carbon dioxide [28]. The homofermentation involves splitting of a hexose moiety into trioses as catalysed by the classic aldolase reaction. The heterofermentation is characterized by hexose decarboxylation and subsequent splitting of the pentose moiety by phosphoketolase into glyceraldehyde-3-phosphate and acetyl-phosphate. Depending on hydrogen acceptors available, the acetyl-phosphate is either metabolized to acetic acid with concomitant ATP generation, or it is reduced to ethanol. The glyceraldehyde-3-phosphate is further metabolized and excreted as lactic acid [22]. It is well known that a distinction between homo- and heterofermentative LAB cannot be made solely on the basis of end products formed. For example, some homofermenters (i.e. facultative homofermenters) exhibit a heterofermentative end product pattern when grown in the presence of limited amounts of carbohydrates [29].

Pentoses are fermented through the phosphoketolase pathway by most heterofermentative LAB yielding lactate and acetate as the major end products. Some homofermentative species of lactococci, pediococci, and streptobacteria encode an inducible phosphoketolase and thus are able to ferment pentoses. These latter species are referred to as facultative heterofermentative LAB [25,26]. Facultative and strictly heterofermentative LAB can use an assortment of alternative electron acceptors. The most well-known reaction in this regard is the heterofermentative reduction of fructose to mannitol accompanied by accumulation of acetate rather than ethanol [30]. Citrate

and glycerol can also be used as alternate hydrogen acceptors [31,32]. Most species posessing heterofermentative activity also contain flavoprotein oxidases which catalyse reduction of oxygen resulting in accumulation of hydrogen peroxide [24]. This use of oxygen as an alternate hydrogen acceptor also promotes conversion of acetyl-phosphate into acetic acid and ATP rather than into ethanol [22,24].

It is well-known that these acidic end products (and hydrogen peroxide when produced) of both homo- and heterofermentations tend to inhibit the growth and metabolic activities of other microorganisms which may also be present in the culture environment. Other end products can accumulate, particularly during heterofermentations, and these products also exhibit antagonistic activity. Formic acid, acetoin, 2,3-butanediol, and diacetyl are active in this connection.

The antagonistic activities associated with other organic acids present (e.g., malic and citric acid) can vary inasmuch as these acids can be further metabolized under certain circumstances. For example, malic acid can be decarboxylated to lactate and CO_2 [33], citric acid can be utilized as an electron acceptor in similar processes [31]. Also lactic acid can be degradated anaerobically to either formic acid and acetic acid in the presence of citrate [34], or to acetic acid, CO_2, and H_2 [35].

4. ANTIMICROBIAL ACTIVITY

4.1. Fermentation end products

As mentioned above, accumulation of acid end products increases the antimicrobial activity in fermented products [36]. The acid production and the accompanying pH decrease extend the lag phase of sensitive organisms [37]. Ingram et al. [38] have defined the following three factors to be important for the preservative action of acid substances: (i) the effect of solely pH, (ii) the extent of the dissociation of the acid and (iii) a specific effect of the molecule itself. The antimicrobial activity of organic acids having more than four carbons generally increases at constant pH with the chain length [39,40]. However, due to their low solubility in water, acids with chain lengths greater than C_{10} or C_{11} are not particularly efficient in this regard, and those with chain lengths greater than C_8 are usually ineffective against Gram-negative bacteria [36]. Lipophilic acids such as acetic and lactic acid in their undissociated form can penetrate the microbial cell and interfere with essential metabolic functions such as substrate translocations and oxidative phosphorylation, and reduce the intracellular pH [36,37]. The concentration of the undissociated acid in relation to the dissociated acid is related to the pK_a value. The minimal inhibitory concentration of an undissociated acid (MIC_{undiss}) for a spoilage organism is usually constant within the pH interval existing in a fermented product, whereas the inhibitory concentration of the total acid is rapidly increasing (See Fig. 1). Acetic acid is reported to be more inhibitory than lactic acid, especially against yeasts and molds [36]. This can be explained by the extent of dissociation since acetic acid has between two and four times more of the acid in the undissociated state at a pH interval between 4.0 and 4.6 compared to lactic acid.

Information on the relationship between inhibition of microbial growth and relative concentrations of the dissociated vs. the undissociated acid has been available for at least 60 years [41]. Despite this information many view the inhibitory effect of acids in silages as related solely to pH effects [23]. A pH below 4.6 (acid foods) is regarded as safe in pasteurized foods with respect to

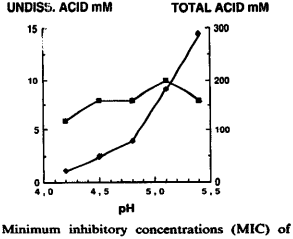

Fig. 1. Minimum inhibitory concentrations (MIC) of total lactic acid (◆); and undissociated lactic acid (■): over a pH range between 4.2 and 5.4. A silage strain of *Enterobacter* sp. was used as an indicator organism (L. Östling and S.E. Lindgren, unpublished results).

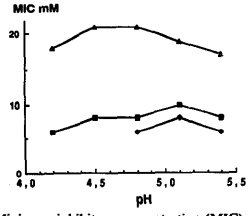

Fig. 2. Minimum inhibitory concentration (MIC) of undissociated lactic acid over a pH range against silage bacteria; ◆, *Clostridium tyrobutyricum*; ■, *Enterobacter* sp.; ▲, *Propionibacterium shermanii* (L. Östling, A. Jonsson and S.E. Lindgren, unpublished results).

growth of spore-forming food pathogens [42]. In other reports, inhibitory acid concentrations are given without reporting the pH value of the system [43]. Information concerning the inhibitory activity of acid combinations is not readily available. Some reports indicate, however, that acetic and lactic acid function synergistically in inhibiting growth of *Salmonella* [44,45] and yeasts [46]. This synergism is due most likely to an increase in undissociated acetic acid which is a consequence of the strong acidic effect of lactic acid. Yeasts, molds and acid-producing bacteria are tolerant to acids and a low pH [37]. Some strains in fact are able to utilize these acids (under aerobic conditions) in an energy-yielding metabolism [36]. Others employ an inducible proton-lactate symport system which releases acids from the cells which have entered by simple diffusion [47,48].

Sensitivity to these acids varies among spoilage and pathogenic bacteria [36]. In Fig. 2 the MIC_{undiss} are expressed for various silage microorganisms. The data indicate that this MIC value is constant in a pH range between 4.5 and 5.2. Below this range the proton concentration will interfere and reduce the MIC value. Above this range the total acid concentration has a similar effect.

4.2. Carbon dioxide

CO_2 (HCO_3^-) accumulation in fermented plant products is the result of an endogenous respiration of the plant cells combined with microbial activities [23]. The influence of CO_2 on product preservation is two-fold. First, it plays a role in creating an anaerobic environment by replacing existent molecular oxygen in the product. Secondly, CO_2 per se has antimicrobial activity [49]. The protective role of CO_2 is especially important in the fermentation of silages and vegetables to prevent growth of molds.

Low concentrations of CO_2 can stimulate growth of some organisms, while high concentrations prevent growth of others. Sensitivity can vary considerably with some organisms totally insensitive to CO_2 at any level [49]. Storage in CO_2 tends to select for various *Lactobacillus* spp. [50]. Common fruit rotting organisms such as *Botrytis, Rhizopus* and *Penicillum* are not inhibited by 10% CO_2 but concentrations between 20 and 50% have strong antifungal activity [49]. The mechanism of action in this regard is unknown, but two explanations have been offered, one implicating inhibition of enzymatic decarboxylations [51], the other pointing to an accumulation of CO_2 in the membrane lipid bilayer resulting in dysfunction in permeability [52,53].

4.3. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) can be generated by LAB in the presence of oxygen through the action of flavoprotein oxidases or NADH peroxidases [24]. The use of oxygen as an electron acceptor obviously is favoured by many microbial species inasmuch as additional energy can be generated with growth rates and cell yields increasing accordingly. End product patterns are altered, acetate is usually the major end product found under these conditions [24], and the content of hydrogen peroxide can increase to effective antimicrobial levels because the LAB generally lack catalase activity [26].

The production of H_2O_2 by LAB is dependent not only on availability of oxygen, but also on particular strains present in the system [54]. Dahiya and Speck [55] and Price and Lee [56] demonstrated the inhibitory effect of H_2O_2 produced by LAB towards both *Staphylococcus aureus* and *Pseudomonas* spp. The inhibition of food-borne

pathogens by LAB has been ascribed at least in part to the activity of H_2O_2 [57]. Wheater et al. [58] showed that the H_2O_2 MIC value for *Lactobacillus lactis* was 125 $\mu g\ ml^{-1}$ but *Staphylococcus aureus* was inhibited by as little as 5 $\mu g\ ml^{-1}$. The bactericidal effect of H_2O_2 has been attributed to its strong oxidizing effect on the bacterial cell [59] and to the destruction of basic molecular structures of cell proteins [60].

In milk, H_2O_2 activates the potent antibacterial lactoperoxidase system (LPS) [61]. This activation can be caused by LAB under aerobic conditions [62]. The effect of the LPS system is quite variable. Gram-positive bacteria including LAB are minimally affected by the LPS. Gram-negative bacteria such as *Escherichia coli, Salmonella* and pseudomonads, on the other hand, are more sensitive [61]. The antibacterial mechanism of LPS is complex and has not yet been classified in detail. The major effect has, however, been referred to the oxidation of SH-groups in vital metabolic enzymes like hexokinase, aldolase and glyceraldehyde-3 phosphate dehydrogenase [61].

4.4. Diacetyl

Diacetyl (2,3-butandione) is the characteristic aroma product associated with butter. It is produced by strains within all genera of LAB [63]. Formation of diacetyl tends to be repressed during the metabolism of hexoses, but significant amounts can be produced under other conditions, e.g. in the presence of organic acids like citrate which is converted via pyruvate into diacetyl. The inhibitory activity of diacetyl against a large number of microorganisms including pathogens such as *Mycobacterium tuberculosis* has been reported from the early 1930s [64]. Spillman et al. [65] showed an inhibition of *E. coli* with as little as a few ppm diacetyl and that this bioactivity increased with decreasing pH of the system. Jay [63] also has shown the synergistic effect of pH on diacetyl's bioactivity. He also reported that yeasts and Gram-negative bacteria were more sensitive than the non-LAB, Gram-positive bacteria. LAB and clostridia seemed to be insensitive to its inhibitory activity. According to Jay [66] diacetyl interferes with arginine utilization by reacting with the arginine-binding protein of Gram-negative bacteria.

4.5. Bacteriocins and bacteriocin-like substances

Production of antagonistic substances other than metabolic end products by LAB has been known for some time. The first report along these lines was made by Rogers [67] who showed antagonistic activity for *L. lactis* against *Lb. bulgaricus*. The substance was determined to be a polypeptide and subsequently termed nisin [68]. Its antibacterial spectrum included inhibition of streptococci, staphylococci, *Bacillus* spp., clostridia and lactobacilli among others [69]. Nisin today is accepted as a food additive, especially because of its inhibitory activity towards outgrowth of spores [70]. Another inhibitory substance, diplococcin, was described for *L. lactis* ssp. *cremoris*. This substance was observed to have a narrow activity spectrum and to be effective only against other strains of *L. lactis* ssp. *cremoris* and *lactis* [71].

These two observations heralded an interest in the production of antimicrobial proteins by the LAB. These proteins are now classified as bacteriocins and are characterized by their narrow range of activity affecting primarily closely related bacteria [72]. Early investigations on bacteriocin production by lactobacilli were reported by de Klerk and Coetzee [73] for strains of *Lb. acidophilus* and *Lb. fermentum*. The bacteriocin produced by *Lb. fermentum* was later characterized by de Klerk and Smit [74]. Descriptions of bacteriocins are now available for lactobacilli, pediococci, leuconostoc, lactococci and *Streptococcus thermophilus*. Biochemical and genetic information on these bacteriocins was extensively summarized recently by Klaenhammer [75]. In this report two types of bacteriocins are distinguished; one with a narrow activity spectrum against related bacteria, the other with a broader range of activity against Gram-positive bacteria. Pathogens like *Clostridium botulinum* and *Listeria monocytogenes* are among the targets in the latter group [70]. Mehta et al. [76] reported a broad spectrum antimicrobial protein (5.4 kDa), produced by *Lb. acidophilus* and inhibitory to strains also within Gram-negative

genera such as *Salmonella, Shigella* and *Pseudomonas*.

Though extensive reports exist on bacteriocinogenic activities of LAB, only a few substances have been well characterized and the target for their activity defined.

The most well-known and best characterized bacteriocin is nisin, with a M_w of 3500 Da. The active substance contains no aromatic amico acids but L-amino acids and unusual S-amino acids like lanthionine and β-methyllanthionine. The mode of activity is not fully known, but the likely site is the bacterial membrane, and it behaves as a surface-active cationic detergent [69].

A similar activity was shown by De Klerk and co-authors [73,74,77] for the protein attached to a lipocarbohydrate component produced by *Lb. fermentum*. The inhibitory agent was bacteriocin-like and active against closely related organisms.

Barefoot and Klaenhammer [78] screened 52 strains of *Lb. acidophilus* for production of bacteriocins. A majority produced bacteriocin-like compounds inhibitory to different lactobacilli. Characterization of the bacteriocin produced by one of the strains indicated a large protein aggregate termed "lactacin B". Further purification of the substance indicated the presence of a 6000–6500-Da protein.

Honso et al. [79] have shown the presence of an inhibitory peptide having a M_w of 3500. This bacteriocin is produced by *Lb. acidophilus* and has been shown to inhibit DNA synthesis in *E. coli*. A bacteriocin produced by *Lb. helveticus* LP27, designed lactocin 27, had a narrow acting spectrum and terminated protein synthesis [80]. DNA and RNA synthesis were not affected. The active substance was found to be a glycoprotein with a M_w of 12400.

Proteins inhibitory to eukaryotic cells have also been reported. *L. lactis* ssp. *lactis* var. *diacetylactis* and *S. thermophilus*, for example, were reported to produce substances effective against molds such as *Aspergillus fumigatus, A. parasiticus* and *Rhizopus* [81].

Some reports exist about antibacterial activity of non-identified substances. These substances usually have a low molecular weight, they are non-proteinaceous and the activity is distinguishable from the acids and H_2O_2. Acidolin, a hygroscopic and thermostable substance from *Lb. acidophilus*, was reported to have a M_w of approx. 200 and to be effective against enteropathogens and spore-formers but not against closely related organisms [82]. *S. thermophilus* produces a low molecular substance ($M_w \approx 700$ Da) effective against pseudomonads, *Salmonella, Shigella, E. coli* and streptococci [83]. Silva et al. [84] have recently identified a low molecular substance ($M_w > 1000$) from a *Lactobacillus* sp. strain GG. The activity was focused against anaerobic bacteria such as clostridia, *Bacterioides* spp. and *Bifidobacteria* and members of Enterobacteriaceae, *Pseudomonas, Staphylococcus* and *Streptococcus*.

5. ANTIBIOSIS IN FOOD AND FEED PRODUCTS

5.1. Silages

Silage is produced by the controlled fermentation of crops and animal waste products [23,85]. The primary goal is to minimize loss of nutrients during extended periods of storage, thereby maintaining the food value of the product. However, a comprehensive assessment of silage quality must address potential health risks and other problems associated with the product (e.g., the spreading of clostridia spores) as well as storage stability.

Slow or incomplete fermentations favour growth of enterobacteria (i.e., members of the family *Enterobacteriaceae*) and clostridia [86]. The enterobacteria compete with LAB for available carbohydrates during the initial fermentation stages resulting in decreased production of lactic acid. If unrestrained, the enterobacteria will eventually deplete carbohydrate reserves, initiate ammonia production through varied deamination reactions, and seriously compromise the nutritional quality of the product [15]. Both saccharolytic and proteolytic groups of clostridia are also present in silages [86], not as members of the epiphytic flora but as contaminants derived from soil particles. Accumulation of saccharolytic spores like *C. tyrobutyricum* in silages is regarded as a major contamination for the spoilage of hard cheeses

[87], and proteolytic clostridia such as *C. sporogenes* are the major producers of ammonia in spoiled silages [23].

Yeasts and molds are not adversely affected by the acidic condition generated in a sucessful fermentation, and the involvement of yeasts especially in aerobic silage deterioration has been confirmed [86]. They initiate spoilage by consuming accumulated organic acids and generating heat thereby reducing the preservative potential of the product [88]. The accumulation of clostridia spores has recently been found to occur just beneath the zone for aerobic deterioration [89]. At a later stage when the temperature exceeds 45°C a shift in flora is observed and the heat is generated by the activity of thermophilic *Bacillus* spp. in the surface zone [88].

Health hazards associated with silages can be attributed to bad conditions for silage fermentations. Kalac and Woolford [90] have reviewed the potential health problems caused by mycotoxins, listeriosis and botulism.

The major antagonistic activity in silage fermentations is attainment of a rapid decrease in pH and anaerobic conditions [23]. A reduction in water activity (a_w) has an additional synergistic effect on the antibiosis [15]. A rapid decline in pH has a strong initial inhibitory effect on the number of enterobacteria [91], and a pH below 4.2 is recommended for storage of silages under anaerobic conditions [23]. At this low pH clostridia are inhibited. However, this value (i.e., pH 4.2) seems to be based on the effect of inorganic acids. It is known that inhibition occurs at higher pH when organic acids are present [92]. Therefore, a more reliable indicator for conditions which limit growth of clostridia is the MIC_{undiss}-acid value. Jonsson [93] showed that MIC_{undiss}-acid values for *C. tyrobutyricum* strains were 4.6–9.6 mM of lactic acid. This level of acidity is reached rapidly in the initial phase of the silage process, which indicates that a complete inhibition of clostridial activity should be easy to achieve. In reality, however, clostridia are commonly encountered in farm-scale silages, indicating that conditions such as uniform distribution of organic acids and anaerobiosis are diffcult to insure during fullscale silage-making. The concentration of the undissociated acid which inhibits growth of *C. butyricum* and *C. sporogenes* is at least 2 and 4 times lower, respectively, compared to the concentration which inhibits *C. tyrobutyricum* [93].

Yeasts and molds in silages can grow at pH levels as low as 3.5 [79]. Under aerobic conditions yeasts isolated from silage are able to consume organic compounds such as lactic, acetic, citric, malic and succinic acids [94–96]. This metabolic activity initiates the deterioration and points to the importance of anaerobicity in the silo.

Homofermentative LAB starter cultures can be added to the silage in order to compensate for the low number of these bacteria present on the crops [15]. Most inoculants used in early work consisted of dairy starters which yielded variable and inconsistant results [97]. Today LAB are selected specifically for their activity in silage crops. It is important that strains selected for such use are genetically stable, able to carry-out a rapid homofermentation of hexoses at ambient temperatures, and exhibit no proteolytic activity [15]. Woolford and Sawczyc [98] reported that a starter culture must rapidly establish a low pH and subsequently dominate over the epiphytic microflora to be of value in this regard. Evaluation of different mixtures of inoculants has shown that a mixture of *Pediococcus acidilactici* and *Lb. plantarum* is most favourable for silage fermentation [15]. The pediococci rapidly initiate the fermentation and *Lb. plantarum* reduces the pH to the desired low level [99]. The composition of the inoculum and the amount applied affect the subsequent rate of acid production and ammonia release during the fermentation [15].

The antibiosis of LAB against yeasts and molds is weak and the effect of starter cultures on storage stability in terms of yeast and mold growth is inconsistent at best [15].

The fermentable sugar content of many inoculated silages is too low to initiate production of stabilizing levels of lactic acid. This can be solved by addition of carbohydrate such as molasses or by enhancing their production through use of malt enzymes [11]. The benefits of combining enzymes with inoculants have been evaluated in recent years [13]. The potential value of such a combination is based on the fact that silage crops often

contain low levels of fermentable sugars, while also containing high levels of polymers such as pectins, cellulose, hemicellulose, starch and fructans. A genetically engineered strain of *Lb. plantarum* was recently constructed for use as a silage starter culture capable of circumventing the expensive addition of an enzyme. The organism was transformed by electroporation: an α-amylase gene and an endoglucanase gene were incorporated into its chromosome [100].

Lactic acid fermentation of fish and slaughterhouse waste products is a low-cost method for preserving these materials [101]. Two major problems are associated with the production of fish silages. One involves the high level of proteolytic activity present in the material which creates a demand for additional acid generation during storage in order to keep the pH at a constant low level. The other problem involves the growth of moulds on uncovered surfaces [85]. A pH below 4.5 is regarded as safe with respect to *C. botulinum* [102]. The importance of a rapid fermentation aided by addition of a LAB inoculant and a sugar source on the inhibition of pathogens has been shown by Cooke et al. [9].

Reports on antibacterial activities of organisms isolated from silages are rare. Lindgren and Clevström [103] reported such activities in LAB preparations isolated from fermented fish and forage silages. The activities were heat-sensitive, had an apparent $M_w > 10\,000$, and were effective against *B. cereus*, *S. aureus*, *C. perfringens* and *L. lactis* var. *cremoris*.

5.2. *Vegetables*

Fermentation of vegetables such as cabbages, olives and cucumbers yield products highly appreciated owing to their aroma and palatability. Fresh vegetables contain high numbers of an epiphytic microflora consisting of numerous spoilage bacteria and only small numbers of LAB. The fermentation is controlled by the addition of sodium chloride and performed under anaerobic conditions. All four genera of LAB are generally involved in the fermentation [20]. Problems associated with fermentation of cucumbers include gas formation by yeasts, coliform bacteria and LAB [104].

Fleming and co-workers believe that the vegetable fermentation occurs in four stages involving also a secondary fermentation caused by yeasts and a postfermentation caused by aerobic surface growth of oxidative yeasts, molds and bacteria. A rapid pH decrease is important for the reduction of the activity of pectinolytic enzymes of microbial and vegetable origin [20]. Storage stability is enhanced by strong acidity and a reduction in the level of available sugar [104]. The primary inhibitor against yeasts in soy sauce, for example, was identified as acetic acid [105].

Evaluation of inoculants to be used for fermented vegetables indicates the need for culture diversity and the importance of heterofermentations. However, evaluation of pure cultures of different homo- and heterofermenters indicates that epiphytic *Lb. plantarum* always completes the fermentation [106].

The importance of bacteriocin production in fermented vegetables has been addressed by Fleming et al. [107]. They suggest that production of bacteriocins by *P. cerevisiae* might explain appearance of pediococci during the initial stages of fermentation. A bacteriocin, pediocin A, was found to be active against *C. botulinum*, *C. sporogenes*, *S. aureus*, *Lb. brevis* and *L. monocytogens* [108,109]. Also plantaricin A has been observed for a *Lb. plantarum* strain isolated from fermented cucumber [110]. The proteinaceous substance has a narrow activity and antagonizes closely related competitors in fermenting vegetables.

5.3. *Meat and fish*

Meat and fish are rich in an assortment of nutrients and are therefore an excellent environment for microbial growth. Spoilage of chilled fresh meat and fish products is caused by growth of a psychrotrophic Gram-negative flora consisting of organisms within the *Pseudomonas / Acinetobacter / Alcaligenes* group. Pathogenic micro-organisms contaminating raw meats generally include: *Staphylococcus aureus, Salmonella* spp., *Clostridium botulinum* and *Listeria monocytogenes* and for fish, *Vibrio parahaemolyticus* [111]. The possible occurrence of these organisms as con-

taminants creates a need for strict handling and processing of food products.

Temperature reduction is the major method used to retard microbial profileration in these products. Vacuum packing and controlling the atmosphere are two additional methods used to improve the shelf-life of meats. Both methods cause an ecological change in the microflora which is dominated primarily by LAB [112]. The presence of LAB is generally beneficial for storage due to their production of natural antagonistic substances. However, production of odours, off-flavours and slime can be negative factors associated with LAB growth. Some heterofermentive LAB in particular have been associated with these latter traits [113].

Antagonisms resulting from LAB have been evaluated as a means to prolonged shelf-life and to inhibit the number of pathogens found in non-fermented meat and fish products. Selected LAB cultures have been added to ground beef [114], deboned poultry [115], vacuum packed beef steaks [116] and shrimp [117]. The active principle in lieu of acid production is usually obscure, and the inhibition is generally thought to be the result of H_2O_2 production and/or specific antibacterial substances such as bacteriocins [16].

Fermented meat products, mainly sausages, are appreciated by the consumer because of their aromas and flavours. Improvement in stability and hygiene of such products can be ascribed to the conversion of sugars to lactic acid [118].

Traditionally, production of these sausages relied on a "natural fermentation" promoted by LAB naturally occurring in the raw material. This flora was favoured by addition of salt, sugar and nitrite and processes such as drying and smoking [16]. The successful use of inoculants for dairy products raised an interest in using LAB for meat fermentations [16], and organisms within the facultative homofermentative LAB genera together with the genus *Micrococcus* have been evaluated for this purpose [119].

Improvement in shelf-life and hygiene of fermented products can be attributed to the combined effects of salt, pH, nitrite and water activity [16]. The major influence of starter cultures seems to reside in their ability to rapidly reduce the pH of the product, and addition of sugars is important in order to provide an optimal nutrition for these bacteria [120]. The content of nitrite, for example, can be lowered without a reduction in protection against *C. botulinum* when bacon was inoculated with *Lb. plantarum* and sucrose added [131].

Antimicrobial products such as H_2O_2, bacteriocins and related substances are believed to be involved in the antibiosis of fermented meat products, but their precise role in this regard has yet to be determined. Schillinger and Lücke [122] screened 221 strains of lactobacilli isolated from meat products to evaluate the antagonistic activity of these bacteria. The intention was to find antagonistic strains which could be used in low-acid foods. Six strains of *Lb. sake* exhibited an activity which was caused by a proteinaceous substance effective against various LAB and *Listeria monocytogenes*.

Bacteriocin production by inoculants can cause problems. Houle et al. [123] showed that mixtures of rapidly growing strains of inoculants for meat fermentation could retard each other's growth through antibiosis caused by bacteriocins.

Fermentation of fish-cereal vegetable mixtures is widely used in the orient [124]. The products are home-made and consumption can cause health problems [125]. Conditions for a proper production are usually lacking, and use of inoculants can have a beneficial influence on the hygiene of these products.

Involvment of LAB in aroma production and spoilage of marinated fish products has been reviewed by Blood [126], but no information was given concerning their contribution to antibiosis during storage.

Information about LAB naturally occurring on raw fish is scarce. Two reports show the existence of lactobacilli on herring [127]. One reports lactobacilli on fish caught in the artic region [128]. This *Lactobacillus* resembled *Lb. plantarum* and it produced an antibacterial substance with a M_w between 700 and 1500.

5.4. Dairy products

Starter cultures were introduced early this century in the dairy industry, mainly to guarantee a

uniform product quality in butter, cheese and cultured milk products. Traditionally milk fermentation and cheese making have been valuable processes to increase storage stability of an important nutrient easily subjected to a rapid spoilage. However, fresh milk can also transport organisms pathogenic to man (e.g., *Mycobacterium tuberculosis*) and mastitis bacteria including: *S. aureus*, *S. agalactiae*, *E. coli* and *Klebsiella pneumoniae*. And pasteurization of milk does not reduce the number of spores of *Clostridium tyrobutyricum*. This organism is transmitted from bad silages via the intestine, polluted udders and milk to the cheese curd. The spoilage is characterized by a blowing caused by hydrogen. The effect of LAB antagonism in the control of spoilage bacteria and pathogens in raw milk, fermented milk and cheese products has been extensively summarized [8,18,129], and the antagonistic properties have been attributed mainly to acids, bacteriocins and related products produced by common dairy inoculants.

Modification of dairy starter cultures by gene technology is being explored today as a means of designing strains with more pronounced antimicrobial and acid generating activities. Another major interest in this regard is the postulated beneficial role of the normal microflora in gastrointestinal health and disease. LAB are thought to confer beneficial effects in this ecosystem. *Lb. acidophilus*, *Lb. bulgaricus*, *L. lactis* and *Bifidobacterium bifidum* [14,130,131] are cited most often in this connection. Indicated therapeutic values of these and other LAB include anticholesteremic properties, tumour suppression, an control over growth and colonization of potentially pathogenic microorganisms. Metchnikoff"s concepts concerning the influence of LAB on healthy conditions in the intestine continues to provoke an interest in the probiotic concept. A probiotic is defined as "organisms and substances which contribute to intestinal microbial balance" [132]. Several mechanisms have been suggested for the protective effect by LAB and enterococci including: (a) lowering of intestinal pH, (b) adhesion to the intestinal wall and thereby preventing colonization by pathogens, (c) competition for nutrients, (d) production of antibacterial substances, and (e) production of antitoxins and enhanced immunity [6].

Hydrogen peroxide production by *L. lactis* was reported to activate the LPS system in abomasum of calves, and thereby improving its antimicrobial activity [133].

Fuller [134] and Sissons [6] report the inconsistency in results describing the beneficial effects of probiotic applications. Some of the negative results are thought to be the consequence of nonadherence of the LAB to gastric and gut epithelial tissues, their inability to grow in the gut environment, and/or lack of host specificity with regard to LAB strain used.

6. *LACTOBACILLUS REUTERI* REUTERIN SYSTEM OF ANTIMICROBIAL ACTIVITY

As discussed above, acidic and other metabolic end products such as hydrogen peroxide and diacetyl as well as a variety of bacterocins are clearly recognized as agents of antimicrobial activity produced and excreted by LAB. Reports on existence of other low molecular weight antimicrobial substances produced by these bacteria are numerous [5], but to date these substances have neither been identified nor their existence confirmed by other investigations. A notable exception in this regard is the recent discovery of reuterin production by *Lb. reuteri* first reported by Axelsson et al. [135].

Lb. reuteri was first isolated by Lerche and Reuter [136] but classified at the time as *Lb. fermentum* Type II. It is now a recognized species of heterofermentative *Lactobacillus* inhabiting the gastrointestinal (GI) tract of humans and animals; it can be isolated also from meat products [28]. It is perhaps the dominant heterofermentive species in the GI tract [137,138]. Recent studies showed the presence of *Lb. reuteri* in all regions of the proximal GI tract (i.e. stomach to ileum) in nursed piglets within 1 to 2 days after birth [139].

Lb. reuteri appears to be unique among lactobacilli, and among bacteria in general for that matter, in its ability to produce and excrete reuterin during anaerobic metabolism of glycerol [140]. Reuterin is a potent, broad spectrum anti-

microbial substance effective against Gram-negative and Gram-positive bacteria, yeast, fungi and protozoa. Reuterin has been isolated, purified and identified as an equilibrium mixture of monomeric, hydrated monomeric, and cyclic dimeric forms of 3-hydroxypropionaldehyde (3-HPA) [140–142]. It is the first low molecular weight antimicrobial substance produced by a *Lactobacillus* to be chemically identified, other than the classic end products described above. A coenzyme B_{12}-dependent glycerol dehydratase catalyses conversion of glycerol into reuterin, and an NAD^+-dependent oxidoreductase catalyses reduction of reuterin to 1,3-propanediol. Both enzymes have been purified and characterized [141,143.144]. These enzymes constitute a pathway for use of glycerol as an alternate hydrogen acceptor by *Lb. reuteri* during carbohydrate heterofermentations. Growth rates and cell yields are increased significantly when glycerol is available for this purpose [144]. A few other bacterial species, e.g., *Klebsiella,* are able to use glycerol in a similar manner. However, these species produce 3-HPA only as a transient metabolic intermediate which is immediately reduced to 1,3-propanediol [145]. *Lb. reuteri* appears to be unique in its ability (i) to produce more 3-HPA than is reduced, and (ii) to excrete the excess 3-HPA, thereby imparting antimicrobial activity to the surrounding environment.

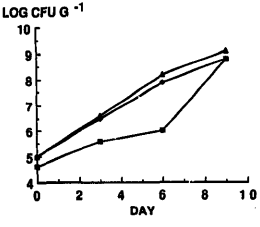

Fig. 3. Levels of Gram-negative bacteria in herring fillets stored in 100% N_2 at 5°C: ▲, Non-treated control; ◆, Treatment with *Lb. reuteri* 1068 (non-reuterin producer) and glycerol; ■, Treatment with *Lb. reuteri* 1063 (reuterin producer) and glycerol (5 replicates). Treated samples were dipped in a solution containing 10^9 bacteria ml^{-1} and 250 mM glycerol (M. Berglund and S.E. Lindgren, unpublished results).

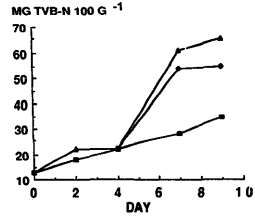

Fig. 4. Amount of total volatile basic nitrogen (TVB-N) in herring fillets stored in 100% N at 5°C (M. Berglund and S.E. Lindgren, unpublished results). For legends see Fig. 3.

It can be seen from data shown in Figs. 3 and 4 that surface treatment of herring with a mixture of *Lb. reuteri* and glycerol can significantly improve the shelf-life of the product by retarding growth of spoilage bacteria and by reducing the accumulation of total volatile basic nitrogen (TVB-N) during storage in a controlled atmosphere at 2°C. The effect is compared to treatment with a reuterin-negative strain of *Lb. reuteri* and a non-treated control. Reuterin added to ground beef inhibits growth of *E. coli* and other microorganisms contaminating this product [70].

REFERENCES

[1] Frazier, W.C. and Westhoff, D.C. (1978) In: Food Microbiology, 3rd edn., pp. 366–391. McGraw-Hill, London.

[2] Beck, T. (1978) The microbiology of silage fermentation. In: Fermentation of silage—a review (McCullough, M.E., Ed.) pp. 61–115 National Feed Ingredients Association. West Des Moines, IA.

[3] Pederson, C.S. (1979) Microbiology of food fermentation, 2nd edn., pp. 153–209. Avi, Westport, CI.

[4] Steinkraus, K.H. (1982) Fermented foods and beverages: the role of mixed cultures. In: Microbial interactions and communities (Bull, A.T. and Sluter, J.H., Eds.), pp. 407–442. Academic Press, London.

[5] Fernandes, C.F., Shahani, K.M. and Amer, M.A. (1987) Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products. FEMS Microbiol. Rev. 46, 343–356.

[6] Sissons, J.W. (1989) Potential of probiotic organisms to prevent diarrhoea and promote digestion in farm animals —A review. J. Sci. Food Agric. 49, 1–13.

[7] Hurst, A. and Collin-Thomsson, D.L. (1979) Food as a bacterial habit. In: Advances in Microbial Ecology (Alexander, M., Ed.), pp. 79–134. Plenum Press, New York and London.

[8] Gibbs, P.A. (1987) Novel uses for lactic acid fermentation in food preservation. J. Appl. Bact. Symp. Suppl. 51S–58S.

[9] Cooke, R.D., Twiddy, D.R. and Alan Reilly, P.J.A. (1987) Lactic-acid fermentation as a low-cost means of food preservation in tropical countries. FEMS Microbiol. Rev. 46, 369–379.

[10] Sharpe, M.E. (1981) The genus *Lactobacillus*. In: The procaryotes (Starr, M.P., Stolp, H, Truper, H.G., Balows, A. and Schlegel, H.G., Eds.), pp. 1635–1639. Springer-Verlag KG, Berlin.

[11] Nilsson, R. and Rydin, C. (1965) A new method of ensiling foodstuffs and feedstuffs of vegetable and animal origin. Enzymologia 11, 126–142.

[12] Setälä, J. (1988-89) Enzymes in grass silage production. Food biotechnology 2, 211–225.

[13] Seale, D.R. (1987) Bacteria and enzymes as products to improve silage preservation. In: Developments in silage (Wilkinson, J.M. and Stark, B.A., Eds.), Chalcombe Publications, Marlow.

[14] Kilara, A. and Trcki, N. (1984) Use of lactobacilli in foods—Unique benefits. Dev. Industr. Microbiol. 25, 125–138.

[15] Seale, D.R. (1986) Bacterial inoculants as silage additives. J. Appl. Bacteriol. Symp. Suppl. 9S–26S.

[16] Bacus, J.N. and Brown, W.L. (1981) Use of microbial cultures: Meat products. Food Technol. 35, 74–83.

[17] Bacus, J.N. (1984) Update: Meat fermentation 1984. Food Technol. 38, 59–63.

[18] Speck, M.L. (1981) Use of microbial cultures: Dairy products. Food Technol. 35, 71–73.

[19] Prentice, G.A. and Neaves, P. (1986) The role of microorganisms in the dairy industry. J. Appl. Bact. Symp. Suppl. 43S–57S.

[20] Fleming, H.P. and McFeeters, R.F. (1981) Use of microbial cultures: Vegetable products. Food Technol. 35, 84–88.

[21] Chassy, B.M. (1985) Prospects for improving economically significant *Lactobacillus* strains by genetic technology. Trends Biotechnol. 3, 273–275.

[22] Kandler, O. (1983) Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek 49, 209–224.

[23] McDonald, P. (1981) The Biochemistry of Silage. Wiley and Sons, Chichester.

[24] Condon, S. (1987) Responses of lactic acid bacteria to oxygen. FEMS Microbiol. Rev. 46, 269–280.

[25] Schleifer, K.H. (1986) Gram-Positive Cocci. In: Bergey's Manual of Systematic Bacteriology. Vol. 2. (Sneath, P.H.A., Ed.), pp. 999–1103. Williams and Wilkins Co., Baltimore, MD.

[26] Kandler, O. and Weiss, N. (1986) Genus *Lactobacillus*. In: Bergey's Manual of Systematic Bacteriology. Vol. 2. (Sneath, P.H.A., Ed.), pp. 1208–1234. Williams and Wilkins Co., Baltimore, MD.

[27] Schleifer, K.H., Kraus, J., Dvorak, C., Kilpper-Bälz, R., Collins, M.D and Fischer, W. (1985) Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen nov. Syst. Appl. Microbiol. 6, 183–195.

[28] Gottschalk, G. (1986) Lactate fermentation. In: Bacterial Metabolism. 2nd edn., pp. 214–224. Springer-Verlag, Berlin.

[29] Thomas, T.D., Ellwood, D.C. and Longyear, V.M.C. (1979) Changes from homo- to heterofermentation by *Streptococcus lactis* resulting from glucose limitation in anaerobic chemostat culture. J. Bacteriol. 138, 109–117.

[30] Eltz, R.W. and Vandemark, P.J. (1960) Fructose dissimilation by *Lactobacillus brevis*. J. Bacteriol. 79, 763–766.

[31] McFeeters, R.F. and Chen, K.-H. (1986) Utilization of electron acceptors for anaerobic mannitol metabolism by *Lactobacillus plantarum*. Compounds which serve as electron acceptors. Food Microbiol. 3, 73–81.

[32] Schütz, H. and Radler, F. (1984) Anaerobic reduction of glycerol to propanediol-1.3 by *Lactobacillus brevis* and *Lactobacillus buchneri*. Syst. Appl. Microbiol. 5, 169–178.

[33] Daeschel, M.A. (1988) A pH control system based on malate decarboxylation for the cultivation of lactic acid bacteria. Appl. Environ. Microbiol. 54, 1627–1629.

[34] Lindgren, S.E., Axelsson, L.T. and McFeeters, R.F. (1990) Anaerobic L-lactate degradation by *Lactobacillus plantarum*. FEMS Microbiol. Lett. 66, 209–214.

[35] Kandler, O., Schillinger, U. and Weiss, N. (1983) *Lactobacillus bifermentans* sp. nov., nom. rev., an organism forming CO_2 and H_2 from lactic acid. Syst. Appl. Microbiol. 4, 408–412.

[36] Baird-Parker, A.C. (1980) Organic acids. In: Microbial Ecology of Foods (Silliker, J.H., Ed.), pp. 126–135. Academic Press, New York.

[37] Smulders, F.J.M., Barendsen, P., van Logtestijn, J.G., Mossel, D.A.A. and van Der Marel, G.M. (1986). Review: Lactic acid: considerations in favour of its acceptance as a meat decontaminant. J. Food Technol. 21, 419–436.

[38] Ingram, M, Ottoway, F.J.H. and Coppock, J.B.M. (1956) The preservative action of acid substances in food. Chemistry and Industry 42, 1154–1165.

[39] Woolford, M.K. (1975) Microbial screening of the straight chain fatty acids (C_1-C_{12}) as potential silage additives. J. Sci. Food Agric. 26, 219–228.

[40] Woolford, M.K. (1975) Microbial screening of food preservatives, cold sterilants and specific antimicrobial agents as potential silage additives. J. Sci. Food Agric. 26, 229–237.

[41] Rogers, L.A. and Wittier, E.O. (1928) Limiting factors in the lactic fermentation. J. Bacteriol. XVI, 211–229.

[43] Hobbs, G. (1986) Ecology of food microorganisms. Microb. Ecol. 12, 15–30.

[43] Ahamed, N. and Marth, E.H. (1989) Behavior of *Listeria monocytogenes* at 7, 13, 21 and 35°C in tryptose broth acidified with acetic, citric or lactic acid. J. Food Protect. 52, 688–695.

[44] Rubin, H.E. (1978) Toxicological Model for a two-acid system. Appl. Environ. Microbiol. 36, 623–624.

[45] Adams, M.R. and Hall, C.J. (1988) Growth inhibition of food-borne pathogens by lactic and acetic acids and their mixtures. Int. J. Food Sci. Technol. 23, 287–292.
[46] Moon, N.J. (1983) Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. J. Appl. Bacteriol. 55, 453–460.
[47] Warth, A.P. (1977) Mechanism of resistance of *Saccharomyces bailii* to benzoic, sorbic and other weak acids used as food preservatives. J. Appl. Bacteriol. 43, 215–230.
[48] Cassio, F., Leao, C. and Van Uden, N. (1987) Transport of lactate and other short-chain monocarboxylates in the yeast *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 53, 507–513.
[49] Clark, D.S. and Takács, J. (1980) Gases as presesrvatives. In: Microbial Ecology of Foods (Silliker, J.H., Ed.), pp. 170–180. Academic Press, London.
[50] Blickstad, E., Enfors, S.-O. and Molin, G. (1981) Effect of hyperbaric carbon dioxide pressure on the microbial flora of pork stored at 4 or 14°C. J. Appl. Bacteriol. 50, 493–504.
[51] King, A.D. Jr. and Nagel, C.W. (1975) Influence of carbon dioxide upon the metabolism of *Pseudomonas aeraginosa*. J. Food Sci. 40, 362–366.
[52] Sears, D.F. and Eisenberg, R.M. (1961) A model representing a physiological rate of CO_2 at the cell membrane. J. General Physiol. 44, 869–887.
[53] Eklund, T. (1984) The effect of carbon dioxide on bacterial growth and on uptake processes in the bacterial membrane vesicles. Int. J. Food. Microbiol. 1, 179–185.
[54] Collins, E.B. and Aramaki, K. (1980) Production of hydrogen peroxide by *Lactobacillus acidophilus*. J. Dairy Sci. 63, 353–357.
[55] Dahya, R.S. and Speck, M.L. (1968) Hydrogen peroxide formation by lactobacilli and its effects on *Staphylococcus aureus*. J. Dairy Sci. 51, 1568–1572.
[56] Price, R.J. and Lee, J.S. (1970) Inhibition of *Pseudomonas* species by hydrogen peroxide producing lactobacilli. J. Milk Food Technol. 33, 13–18.
[57] Gilliland, S.E. and Speck, M.L. (1977) Antagonistic action of *Lactobacillus acidophilus* towards intestinal and food-borne pathogens in associative culture. J. Food Protect. 40, 820–823.
[58] Wheater, D.M., Hisch, A. and Mattic, A.T.R. (1952) Possible identity of "lactobacillin" with the hydrogen peroxide produced by lactobacilli. Nature 70, 623–626.
[59] Fooster, E.M., Nelson, F.E., Speck, M.L., Doltsch, R.N. and Olson, J.-C. (1957) Dairy Microbiology, pp. 106–107. Prentice-Hall, Englewood Cliffs, NJ.
[60] Sykes, G. (1965) Disinfection and sterilization, 2nd edn., p. 27. J.B. Lippincott, Philadelphia.
[61] Björck, L. (1985) The lactoperoxidase system. In: Natural Antimicrobial Systems, pp. 18–30. IDF., 41 Square Vergote, 1040, Brussels.
[62] Carlsson, J., Iwami, Y. and Yamada, T. (1983) Hydrogen peroxide excretion by oral streptococci and effecs on lactoperoxidase-thiocyanate-hydrogen peroxide. Infect. Immun. 40, 70–80.
[63] Jay, J.M. (1982) Antimicrobial properties of diacetyl. Appl. Environ. Microbiol. 44, 525–532.
[64] Jalander, Y.W. (1936) Diacetyl als Tuberkelbazillen tötender Bestandteil des finnischen Hoizteeres. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 180, 628–630.
[65] Spillmann, H., Puhan, Z. and Banhegyi, M. (1978) Antimikrobielle Aktivität thermophiles Lactobacillen 33, 148–153.
[66] Jay, J.M. (1986) In: Modern food Microbiology, 3rd edn., p. 275. Van Nostrand Reinhold, New York.
[67] Rogers, L.A. (1928) The inhibiting effect of *Streptococcus lactis* on *Lactobaillus bulgaricus*. J. Bacteriol. 16, 321.
[68] Mattick, A.T.R. and Hirsch, A. (1947) Further observations on an inhibitory substance (nisin) from lactic streptococci. Lancet ii, 5–7.
[69] Hurst, A. (1983) Nisin and other inhibitory substances from lactic acid bacteria. In: Antimicrobials in Foods (Branen, A.L. and Davidson, P.M., Eds.), pp. 327. Marcel Dekker Inc., New York.
[70] Daeschel, M.A. (1989) Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol. 43, 164–167.
[71] Babel, F.J. (1977) Antibiosis by lactic cultures bacteria. J. Dairy Sci. 60, 815–821.
[72] Tag, J.R., Dajam, A.S. and Wannamaker, L.W. (1976) Bacteriocins of Gram-positive bacteria. Bacteriol. Rev. 40, 722–756.
[73] De Klerk, H.C. and Coetzee, J.N. (1961) Antibiosis among lactobacilli. Nature 192, 340–341.
[74] De Klerk, H.C. and Smit, J.A. (1967) Properties of a *Lactobacillus fermenti* bacteriocin. J. Gen. Microbiol. 48, 309–316.
[75] Klaenhammer, T.R. (1988) Bacteriocins of lactic acid bacteria. Biochemie 70, 337–349.
[76] Mehta, A.M., Patel, K.A. and Dave, P.J. (1983) Isolation and purification of an inhibitory protein from *Lactobacillus acidophilus*. AC. Microbios 37, 37–43.
[77] De Klerk, H.C. (1967) Bacteriocinogeny in *Lactobacillus fermenti*. Nature 214, 609.
[78] Barefoot, S.F. and Klaenhammer, T.R. (1983) Detection and activity of Lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 45, 1808–1815.
[79] Hosono, A., Yastuki, K. and Tokita, F. (1977) Isolation and characterization of an inhibitory substance against *Escherichia coli* produced by *Lactobacillus acidophilus*. Milchwissenschaft 32, 727–730.
[80] Upreti, G.C. and Hindsdill, R.D. (1975) Production and mode of action of Lactocin 27. Bacteriocin from a homofermentative *Lactobacillus*. Antimicrobiol. Agents Chemother. 7, 139–145.
[81] Batish, U.K., Grover, S. and Lal, R. (1989) Screening lactic starter cultures for antifungal activity. Cultured Dairy Products Journal 24, 23–25.
[82] Hamdan, L.Y. and Mikolajcik, E.M. (1974) Acidolin: an antibiotic produced by *Lactobacillus acidophilus*. J. Antibiot. 27, 631–636.

[83] Pulusani, S.R., Rao, D.R. and Sunki, G.R. (1979) Antimicrobial activity of lactic cultures: Partial purification and characterization of antimicrobial compounds produced by *Streptococcus thermophilus*. J. Food Sci. 44, 575–578.

[84] Silva, M., Jacobus, N.V., Deneke, C. and Gorbach, S.L. (1987) Antimicrobial substance from a human *Lactobacillus* strain. Antimicrob. Agents Chemother. 31, 1231–1233.

[85] Lindgren, S.E. and Pleje, M. (1983) Silage fermentation of fish or fish waste products with lactic acid bacteria. J. Sci. Food Agric. 34, 1057–1067.

[86] Woolford, M.K. (1984) The silage fermentation. Marcel Dekker Inc., New York.

[87] Bergère, J.-L. and Accolas, J.-P. (1985) Non-sporing and sporing anaerobes in dairy products. In: Anaerobic Bacteria in Habit other than Man. (Barnes, E.M. and Mead, G.C., Eds.), Microbiology symposia series No 13. Blackwell Scientific Publication, London.

[88] Lindgren, S., Pettersson, K., Kaspersson, A., Jonsson, A. and Lingvall, P. (1985) Microbial dynamics during aerobic deterioration of silages. J. Sci. Food Agric. 36, 765–774.

[89] Jonsson, A. (1990) Growth of *C. tyrobutyricum* during fermentation and aerobic deterioration of grass silage. J. Food Agric. Sci., in press.

[90] Kalac P. and Woolford, M.K. (1982) A review of some aspects of possible association between the feeding of silage and animal health. Br. Vet. J. 138, 305–320.

[91] Lindgren, S., Bromander, A. and Petterson, K. (1988) Evaluation of silage additives using scale-model silos. Swed. J. Agric. Res. 18, 41–49.

[92] Kleter, G., Lammers, W.L. and Vos, E.A. (1982) The influence of pH and concentration of lactic acid and NaCl on the growth of *Clostridium tyrobutyricum* in whey and cheese. 1. Experiments in whey. Neth. Milk Dairy J. 36, 79–87.

[93] Jonsson, A. (1989) The role of yeasts and clostridia in silage deterioration. Dissertation. Report 42. Dept. Microbiology, Swedish University of Agricultural Sciences, Uppsala.

[94] Moon, N.J. and Ely, L.O. (1979) Identification and properties of yeasts associated with aerobic deterioration of wheat and alfalfa silages. Mycopathologia 69, 153–156.

[95] Kreuger-van Rij, N.J.W. (1984) The yeasts—A taxonomic study. Elsevier, Amsterdam.

[96] Middelhouen, W.J. and Franzen, M.M. (1986) The yeast flora of ensiled whole-crop maize. J. Sci. Food Agric. 37, 855–861.

[97] Watson, S.J. and Nash, M.J. (1960) The conservation of grass and forage crops. Oliver and Boyd, Edinburgh.

[98] Woolford, M.K. and Sawczyc, M.K. (1984) An investigation into the effect of cultures of lactic acid bacteria on fermentation in silage. Strain selection. Grass and Forage Science 39, 139–148.

[99] Lindgren, S.E., Pettersson, K., Jonsson, A., Lingvall, P. and Kaspersson, A. (1985) Silage inoculation. Swed. J. Agric. Res. 15, 9–18.

[100] Scheirlinck, T., Mahillon, J., Joos, H., Dhaese, P. and Michiels, F. (1989) Integration and expression of α-amylase and endoglucanase genes in the *Lactobacillus plantarum* chromosome. Appl. Environ. Microbiol. 55, 2130–2137.

[101] Disney, G., Tatterson, I.N. and Olley, J. (1977) Recent developments in fish silage. In: Proceedings of the Conference on the Handling, Processing and Marketing of Tropical Fish, pp. 231–240. Tropical Products Institute, London.

[102] Blocher, J.C. and Busta, F.F. (1983) Bacterial spore resistance to acid. Food Technol. 37, 87–89.

[103] Lindgren, S.E. and Clevström, G. (1978) Antibacterial activity of lactic acid bacteria 1 and 2. Swedish J. Agric. Res. 8, 61–73.

[104] Daeschel, M.A., Andersson, R.E. and Fleming, H.P. (1987) Microbial ecology of fermenting plant materials. FEMS Microbiol. Rev. 46, 357–363.

[105] Noda, F., Hayashi, K. and Mizunuma, T. (1980) Antagonism between osmophilic lactic acid bacteria and yeasts in brine fermentation of soy sauce. Appl. Environ. Microbiol. 40, 452–457.

[106] Pederson, C.S. and Albury, M.N. (1961) The effect of pure culture inoculation on fermentation of cucumbers. Food Technol. 15, 351–354.

[107] Fleming, H.P., Etchells, J.L. and Costilow, R.N. (1975) Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. Appl. Microbiol. 30, 1040–1042.

[108] Daeschel, M.A. and Klaenhammer, T.R. (1985) Association of a 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl. Environ. Microbiol. 50, 1538–1541.

[109] Harris, L.J., Daeschel, M.A., Stiles, M.E. and Klaenhammer, T.R. (1989) Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. J. Food Protect. 52, 384–387.

[110] Daeschel, M.A., McKenney, M.C., McDonald, L.C. (1986) Characterization of a bacteriocin from *Lactobacillus plantarum*. Abstr. 86th Ann. Meeting, Am. Soc. Microbiol., Abstr. P13, p. 277.

[111] ICMSF. Microbial ecology of foods. Vol 2. (Silliker, J.W., Ed.), Academic Press, London.

[112] Blickstad, E. and Molin, G. (1983) The microbial flora of smoked pork loin and frankfurter sausage stored in different gas atmospheres at 4°C. J. Appl. Bacteriol. 54, 45–56.

[113] Hanna, M.O., Savell, J.W., Smith, G.C., Purser, D.E., Gardner, F.A. and Vanderzant, C. (1983) Effect of growth of individual meat bacteria, on pH, color and odor of a septically prepared vacuum-packaged round steaks. J. Food Protect. 46, 216–221.

[114] Reddy, S.G., Henrickson, R.L. and Olson, H.C. (1970) The influence of lactic cultures on ground beef quality. J. Food Sci. 35, 787–791.

[115] Raccach, M. and Baker, R.C. (1978) Lactic acid bacteria as an antispoilage and safety factor in cooked, mechanically deboned poultry meat. J. Food Protect. 43, 837–841.

[116] Hanna, M.O., Hall, L.C., Smith, G.C. and Vanderzant,

C. (1980) Inoculation of beef steaks with *Lactobacillus* species before vacuum packaging. 1. Microbial consideration. J. Food Protect. 43, 837–841.

[117] Moon, N.J., Beuchat, L.R. and Hays, E.R. 1980. Evaluation of lactic acid bacteria for extending the shelf-life of shrimp. 40th Ann. Meet. Inst. Food Technol. New Orleans, LA.

[118] Deibel, R.H., Niven, C.F. and Wilson, D.D. (1961) Microbiology of meat curing. III. Some microbiological and related technological aspects in the manufacture of fermented sausages. Appl. Microbiol. 9, 156–161.

[119] Bacus,J. (1984) Update: Meat fermentation 1984. Food Technol. 38, 59–63.

[120] Bartholomew, D.R. and Blumer, T.N. (1977) The use of a commercial *Pediococcus cerevisiae* starter culture in the production of country-style hams. J. Food. Sci. 42, 494.

[121] Tanaka, N., Traisman, E., Lee, M.H., Cassens, R.G. and Foster, E.M. (1980) Inhibition of botulinum toxin formation in bacon by acid development. J. Food Protect. 43, 450–457.

[122] Schillinger, U. and Lücke, F.-K. (1989) Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 55, 1901–1906.

[123] Houle, J.-F., LaFrance, M., Julien, J.-P., Boochu, E. and Champagne, C.P. (1989) Selection of mixed cultures for meat fermentation. J. Food Sci. 54, 839–842.

[124] Adams, M.R., Cooke, R.D. and Rattagool, P. (1985) Fermented fish products of South East Asia. Trop. Sci. 25, 61–73.

[125] Graikoski, J.T. (1973) Microbiology of cured and fermented fish. In: Microbial safety of fishery products. (Chichester, C.O. and Graham, H.D., Eds.), pp. 97–112. Academic Press, London.

[126] Blood, M.R. (1975) Lactic acid bacteria in marinated herring. In: Lactic Acid Bacteria in Beverages and Food. (Carr, J.G., Cutting, C.V. and Whiting, G.C., Eds.), pp. 195–220. Academic Press, London.

[127] Kraus, H. (1961) Kurze Mitteilung über das Vorkommen von *Lactobacillus* auf frischen Herringen. Arch. Lebensm. Hyg. 12, 101–102.

[128] Schröder, K., Clausen, E., Sandberg, A.M. and Raa, J. (1979) Psychrotrophic *Lactobacillus plantarum* from fish and its ability to produce antibiotic substances. In: Advances in fish science and technology. (Conell, J.J., Ed.), pp. 480–483. Fishing, News Books, London.

[129] Hurst, A. (1972) Interactions of food starter cultures and food-borne pathogens: The antagonism between *Streptococcus lactis* and spore-forming microbes. J. Milk Food Technol. 35, 418–423.

[130] Klaenhammer, T.R. (1982) Microbiological considerations in selection and preparation of *Lactobacillus* strains for use as dietary adjuncts. J. Dairy Sci. 65, 1339–1349.

[131] Reddy, N.R., Roth, S.M, Eigel, W.N. and Pierson, M.D. (1988) Food and food ingredients for prevention of diarrhoeal disease in children in developing countries. J. Food Protect. 51, 66–75.

[132] Parker, R.B. (1974) Probiotics, the other half of the antibiotic story. Anim. Nutr. Health 29, 4–8.

[133] Reiter, B., Marshall, V.M. and Philips, S.M. (1980) The antibiotic activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system in the calf abomasum. Res. Vet. Sci. 28, 116–122.

[134] Fuller, R. (1986) Probiotics. J. Appl. Bacteriol. Symp. Suppl. 1S–7S.

[135] Axelsson, L.T., Chung, T.C., Dobrogosz, W.J. and Lindgren, S. (1989) Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. Microb. Ecol. Health Dis. 2, 131–136.

[136] Lerche, M. and Reuter, G. (1962) Das Vorkommen aerobwachsender Gram-positiver Stäbchen des Genus *Lactobacillus* Beijerinck im Darminhalt erwachsener Menschen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. 1. Abt. Orig. 185, 446–481.

[137] Kandler, O., Stetter, K.O. and Köhl, R. (1980) *Lactobacillus reuteri* sp. nov. a new species of heterofermentative lactobacilli. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1. Abt. Orig. C 1, 264–269.

[138] Sarra, P.G., Dellaglio, F. and Bottazzi, V. (1985) Taxonomy of *Lactobacillus* from the alimentary tract of chickens. Syst. Appl. Microbiol. 6, 86–89.

[139] Dobrogosz, W.J., Casas, I.A., Fagano, G.A., Talarico, T.L., Sjöberg, B.-M. and Karlsson, M. (1989) *Lactobacillus reuteri* and the enteric microbiota. In: The Regulatory and Protective Role of the Normal Microflora (Gruff, R. Midtvedt, T. and Norin, E., Eds.), pp. 283–292. Wenner-Gren International Symp., vol. 52. Macmillan Press, London.

[140] Chung, T.C., Axelsson, L., Lindgren, S.E. and Dobrogosz, W.J. (1989) *In vitro* studies on reuterin synthesis by *Lactobacillus reuteri*. Microb. Ecol. Health Dis. 2, 137–144.

[141] Talarico, T.L., Casas, I.A., Chung, T.C. and Dobrogosz, W.J. (1988) Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. Antimicrob. Agents Chemother. 32. 1854–1858.

[142] Talarico, T.L. and Dobrogosz, W.J. (1989) Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. Antimicrob. Agents Chemother. 33. 674–679.

[143] Talarico, T.L. and Dobrogosz, W.J. (1990) Purification and characterization of glycerol dehydratase from *Lactobacillus reuteri*. Appl. Environ. Microbiol. 56, 1195–1197.

[144] Talarico, T.L., Axelsson, L.T., Novotny, J., Fuizat, M. and Dobrogosz, W.J. (1990) Utilization of glycerol as a hydrogen acceptor by *Lactobacillus reuteri*: Purification of 1,3-propanediol: NAD+ oxidoreductase. Appl. Environ. Microbiol. 56, 943–948.

[145] Forage, R.G. and Lin, E.C.C. (1982) dha system mediating aerobic and anaerobic dissimilation of glycerol in *Klebsiella pneumoniae* NCIB 418. J. Bacteriol. 151, 591–599.