Acta Chimica Slovaca, Vol.4, No.2, 2011, 118 - 135

Importance of lactobacilli for bread-making industry

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Review

Abstract

Lactic acid bacteria are widely used in the production of fermented foods when they are used as the starter cultures. The starter cultures are defined as a preparation consisting of active or inactive microorganisms which possess desirable metabolic activity. The design of starters used in bakery industry requires prior knowledge of the biochemical characteristics and baking potential of present microflora. Performance of starters during fermentation process is usually studied by characterization of the acidification properties, such as pH value decrease, total titratable acidity increase and organic acids production. This review describes characteristics of bacteria belonging to the genera *Lactobacillus*, their metabolism and useful properties as sourdough microorganisms.

Keywords: bread, fermentation, lactic acid bacteria, sourdough, starter cultures

Introduction

Lactic acid bacteria (LAB) have been used in food fermentations for more than 4000 years. It is important to acknowledge that the widespread term „LAB“ have no official status in taxonomy and is only a general term of convenience used to describe the group of functionally and genetically related bacteria. LAB consist of bacterial genera within the *Firmicutes* comprised of about 20 genera. The main members of the LAB are genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Carnobacterium*, *Aerococcus*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weisella*. *Lactobacillus* is the largest genus of this group, comprising around 80 recognized species (Stiles and Holzapfel, 1997; Konings et al., 2000; Hutkins, 2006; Reddy et al., 2008; Kocková and Valík, 2011).

LAB have a long history of use in a variety of cereal fermentations, especially in the manufacture of baked goods (Hansen, 2002; Gobbeti et al., 2005). It has been reported that around 50 different species of LAB have been isolated from sourdough (Corsetti et al., 2007). *Lactobacillus* strains are the most frequently observed bacteria in this matrix, but the species belonging to the genera *Leuconostoc*, *Weissella*, *Pediococcus*, *Lactococcus* or *Enterococcus* have been isolated as well (Hansen, 2002; Ehrmann et al., 2005; Hervé et al., 2006).

Sourdough fermentation is an important modern microbial method based upon the ancient spontaneous process (Decock et al., 2005; Rehman et al., 2006). Sourdough is an intermediate product for dough and bread preparation and contains metabolically active microorganisms (Vogel et al., 1996; Plessas et al., 2007). LAB and yeasts play the key role in this fermentation. Major attention has been paid to the biochemistry and physiology of sourdough LAB, because they are responsible for the rheology, flavour and nutritional properties of baked goods. Even though the carbohydrate and nitrogen metabolism deserved the major interest, other biochemical mechanisms have also been part of the research interest (Katina et al., 2006; De Vuyst et al., 2007; Vracken et al., 2007).

This review focuses on the importance of lactobacilli in bakery industry and utilization of their useful properties in starter cultures preparation.

Characteristics of the genera *Lactobacillus*

Lactobacilli are described as Gram-positive, catalase-negative, non-sporing rods, whose length varies between 1.5 μm and 5 μm. They may also have a slender, curved or bend appearance and frequently are able to form chains. Colony morphology is also variable on agar plates, with some strains producing large round colonies and others producing small or irregular colonies (Görner and Valík, 2004; Hutkins, 2006).

Most of the lactobacilli are mesophilic, but the genus contains also species that are psychrotrophic, thermoduric or thermophilic. Temperature optimum varies from 30 to 45 ºC. Some species show high tolerance to salt, osmotic pressure and low water activity. Acid-tolerance is a common feature of lactobacilli and most of the strains are able to grow at pH below 4.4. The optimum pH value for their growth is 5.5 – 6.5. Some strains are ethanol-tolerant and bile-tolerant as well. Most of the species are aero-tolerant, but some of them require strict anaerobic conditions (Hutkins, 2006; Reddy et al., 2008).

The phylogeny variability of LAB can be characterised by differences of 16S *r*RNA. A phylogram based on 16S *r*RNA gene sequences of sourdough lactobacilli is reported in Fig. 1.

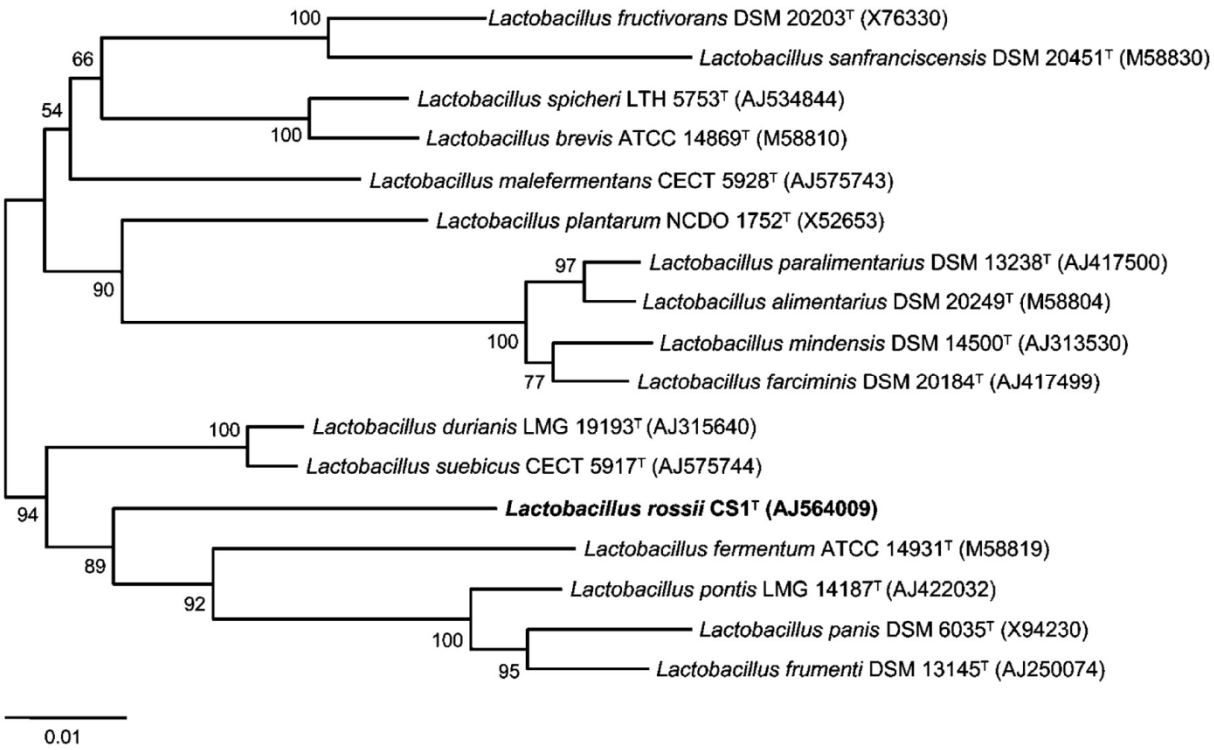

Fig. 1. Phylogenetic tree of *Lactobacillus* species commonly associated with or found in sourdough products based on 16S *r*RNA gene sequences (Corsetti et al., 2005).

Carbohydrate metabolism of lactobacilli

Lactobacilli are fermentative bacteria. Depending on the metabolic pathway of sugar fermentation, they are divided into homofermentative or heterofermentative group (Table 1.). Some species have the genetic and physiological capability to ferment sugars by both ways and, therefore, they are referred to as facultative heterofermentative bacteria (Hutkins, 2006; O´Toole and Lee, 2006).

Homofermentative lactobacilli produce more than 85 % of lactic acid from glucose. Hexoses are metabolised *via* the enzymes of the Embden-Mayerhoff pathway (Patel et al., 2011). They ferment 1 mol of glucose to 2 mol of pyruvate, generating a net yield of 2 mol of ATP per molecule of glucose metabolized. Pyruvate is then reduced to L- or D-lactate by the enzyme lactate dehydrogenase. Lactic acid is the major product of this fermentation (Fig. 2.). $NADH+H^+$ formed during the glyceraldehyde-3-phosphate dehydrogenase reaction must be re-oxidized by lactate-dehydrogenase, to maintain the $[NADH+H^+]/[NAD^+]$ balance (Hutkins, 2006; O´Toole and Lee, 2006; Gänzle et al., 2007; Reddy et al., 2008; Hudecová and Šimkovič, 2009).

Table 1. Division of sourdough lactobacilli according to their fermentation pathway (Corsetti et al., 2007).

Obligate homofermentative	Facultative heterofermentative	Obligate heterofermentative
Lactobacillus acidophilus	*Lactobacillus casei*	*Lactobacillus fermentum*
Lactobacillus delbrueckii	*Lactobacillus paralimentarius*	*Lactobacillus sanfranciscensis*
Lactobacillus farciminis	*Lactobacillus plantarum*	*Lactobacillus reuteri*
Lactobacillus amylophilus	*Lactobacillus pentosus*	*Lactobacillus buchneri*
Lactobacillus amylovorus	*Lactobacillus casei*	*Lactobacillus brevis*
Lactobacillus crispatus		*Lactobacillus acidifarinae*
Lactobacillus mindensis		*Lactobacillus fructivorans*
Lactobacillus johnsoniii		*Lactobacillus frumenti*
		Lactobacillus hilgardii
		Lactobacillus panis
		Lactobacillus pontis
		Lactobacillus rossiae
		Lactobacillus siliginis
		Lactobacillus spicheri
		Lactobacillus zymae

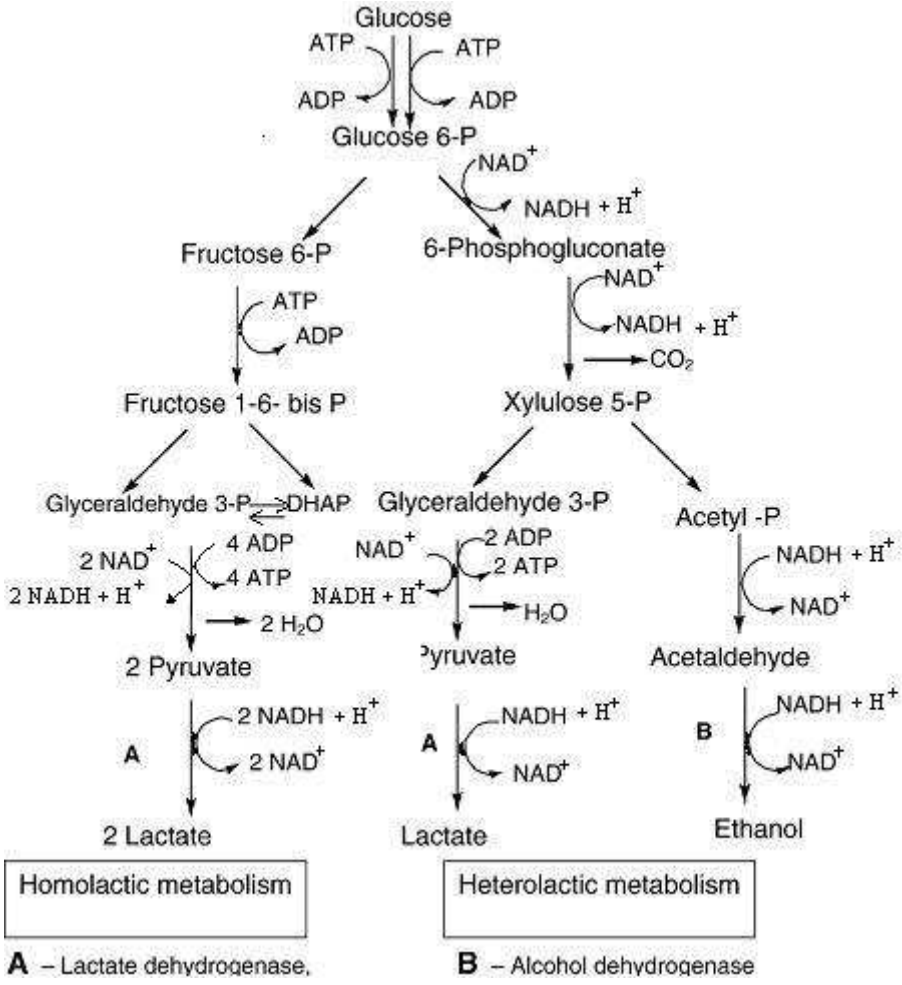

Fig. 2. Metabolism of LAB (Reddy et al., 2008).

Heterofermentative lactobacilli produce only 50 % of lactic acid from glucose. The hexoses are metabolised *via* the phosphoketolase pathway. In obligate heterofermentative bacteria, aldolase is absent, and instead of this enzyme the phosphoketolase is present. These bacteria ferment 1 mol of glucose into 1 mol of lactic acid, 1 mol of ethanol, and 1 mol of CO_2 (Patel et al., 2011; Ravyts and De Vuyst, 2011). 1 mol of ATP is generated per mol of glucose, resulting in less growth per mol of glucose metabolised (Fig. 2.). Oxidation of $NADH+H^+$ and maintenance of the $[NADH+H^+]/[NAD^+]$ balance occurs *via* the two reductive reactions catalysed by acetaldehyde dehydrogenase and alcohol dehydrogenase (Hutkins, 2006; O´Toole and Lee, 2006; Gänzle et al., 2007; Reddy et al., 2008; Hudecová and Šimkovič, 2009).

Despite their metabolic diversity they are very fastidious about growth factors and require nutrient-rich environments. Except carbohydrates as the source of carbon and energy, lactobacilli demand nucleotides, amino acids and vitamins belonging to the group B for their rapid growth (Arpai and Bartl, 1977; Marklinder and Lönner, 1992; Görner and Valík, 2004; Valík et al., 2008). Lactobacilli ferment wide range of sugars, such as glucose, fructose, lactose and maltose. Plant-derived carbohydrates cellobiose, amygdaline and trehalose may be fermented as well. Some lactobacilli species are able to ferment starch (Fig. 3.) and therefore are called amylolytic LAB (Calderon et al., 2002). Amylolytic lactobacilli are capable of the direct lactic acid production from starchy materials using extracellular α-amylase (Zhang and Chervan, 1991; Xiaodong et al., 1997; Reddy et al., 2008). *Lactobacillus amylovorus* DCE 471 is a promising starter culture for higher competitiveness among other bacteria in sourdough preparation (Leroy et al., 2006).

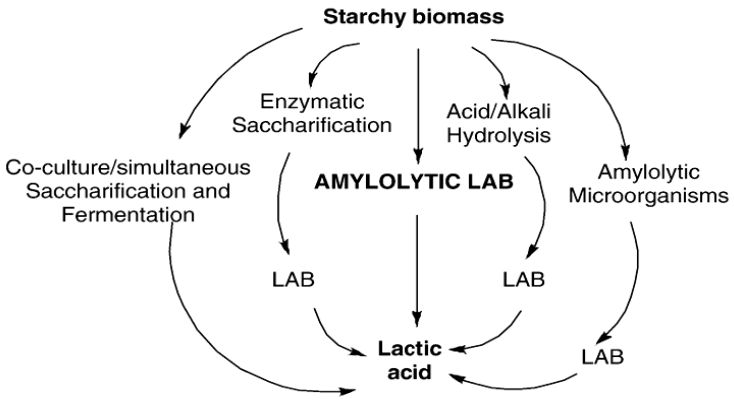

Fig. 3. Schematic representation of lactic acid production from starch as a substrate by amylolytic LAB (Reddy et al., 2008).

Important metabolites produced by lactobacilli

Lactic acid is the dominant metabolite of lactic fermentation. It possess mild aroma and therefore does not cover up weaker aroma compounds present in sourdough (Tseng and Montville, 1993). Naturally lactic acid occurs in two optical antipodes L (+) and D (-), or as a mixture of both forms. Ability to produce these isomers or their mixture relies on the genera and species of the LAB and is usually used for their classification (De Angelis et al., 2007; Plessas et al., 2008).

The acetic acid presence in the media after fermentation by the *Lactobacillus* strains can be the result of the degradation of the produced lactic acid, or of the citrate metabolism or it originates from the heterofermentative pathway (Zalán et al., 2010). Acetic acid contributes to acidification. It is well known for its strong aroma, fungicidal and antimicrobial effect and significant texture affect. The most important texture affect by acetic acid is shorter and harder gluten. On the other hand lactic is responsible for the more elastic gluten structure (Corsetti and Settanni, 2007; De Vuyst et al., 2009; Liptáková et al., 2010). The ratio between lactic and acetic acids, defined as the fermentation quotient (FQ), is an important factor that may affect the aroma profile and is also relevant for the structure of the final products (Gobbetti, 1998).

Lactobacilli are capable of production formic, caproic, propionic, butyric and valeric acids as well, which in turn exhibit antimicrobial activity. Among metabolite products of lactobacilli, the lactic and acetic acids are regarded as the main organic acids, which possess antimicrobial behaviour (Liptáková et al., 2007; Zalán et al., 2010). The efficiency of produced organic acids originates from their effect on the bacterial cytoplasmic membrane, where they affect the membrane potential and therefore inhibit the active transport through the membrane (Caplice and Fitzgerald, 1999). Lactic and acetic acid are weak, lipid-soluble acids which in undissociated form diffuse through the plasma membrane. Acetic acid diffusion is rapid. Lactic acid diffuses slowly, thanks to the lower degree of lipid solubility (Stratford, 2000). The neutral cytoplasmic pH value causes the acids dissociation. Charged anions and protons are unable to diffuse back through the plasma membrane. They accumulate in the cytoplasm, which is accompanied with the pH value decrease (Stratford, 2000; Liptáková et al., 2007). Diffusion may run till the equality in concentration of acids on the both sides of the membrane (Escalante et al., 2010). If the concentration of preservative acids is sufficient, the accumulated protons overcome cytoplasmic buffering and lower internal pH (Stratford, 2000). Moreover the decreased pH value creates unsuitable growth conditions for many pathogens and contaminating bacteria (Kazatelová, 2003; O´Toole and Lee, 2006).

Carbon dioxide (CO_2) is produced by heterofermentative LAB. It supports anaerobic environment and is toxic to some aerobic food microorganisms (Caplice and Fitzgerald, 1999), because it precludes the cell membrane to reduce and balance the internal and external pH value (Plocková et al., 1996; Caplice and Fitzgerald, 1999; Kazatelová, 2003). CO_2 produced by heterofermentative lactobacilli also improves the capacity of the dough by retaining the gas in it (Gobbetti, 1998).

Production of volatile and antimicrobial compounds

It is well known that the greatest amount of aroma substances is formed during the baking. Sourdough fermentation is essential for achieving the acceptable flavour, because chemically acidified breads are not able to ensure suitable sensory quality (Kaseleht et al., 2011). Different starter cultures produce various volatile compounds. Good knowledge of volatiles production by lactobacilli may be used for desired starter cultures selection (Gobbetti, 1998; Rehman et al., 2006).

Heterofermentative LAB produce preferentially ethyl acetate, but hexyl acetate, ethyl hexanoate and isopenthyl acetate occur as well (Rehman et al., 2006; Navrhus and Sørhaug, 2006; Kaseleht et al., 2011). During citrate metabolism of homofermentative LAB α-acetolactate is non-enzymatically converted to the flavour compound of the bread crumb – diacetyl (2,3-butanedione) (Gänzle et al., 2007). Heterofermentative LAB do not produce diacetyl. They probably lack pathway for its synthesis (Fig. 4.) or the redox potential is too high to allow oxidation of acetoin. Diacetyl formation during growth in sourdough was reported for strains of *L. plantarum, L. farciminis, L. helveticus, L. alimentarius* and *L. acidophilus.* Apart from diacetyl, acetaldehyde, 2- and 3-methylbutanol are produced, too (Gänzle et al., 2007; Kaseleht et al., 2011).

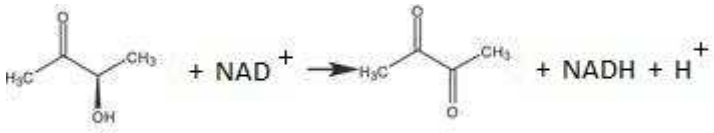

Fig. 4. Reaction pathway of diacetyl production from acetoin (Kaseleht et al., 2011).

The primary antimicrobial compounds produced by sourdough LAB are lactic and acetic acid, diacetyl, acetaldehyde, hydrogen peroxide, carbon dioxide and ethanol, but these compounds strongly affect the sensory acceptance of food when present in inhibitory concentrations. Moreover the organic acids antimicrobial effect depends on their non-dissociated molecule form. But LAB are able to produce bacteriocins – low molecular weight

compounds (Navrhus and Sørhaug, 2006; Liptáková et al., 2007; Gänzle et al. 2009; Zalán et al., 2010). They are commonly categorized into three groups: class I – lantibiotics, which are small and heat-stable peptides that contain thioether amino acid like lantionine; class II – small, heat-stable and hydrophobic peptides with an antilisteral activity composed out of one (IIa) or two (IIb) polypeptide chain; class III – consists of large, heat-labile and hydrophilic proteins (Messens and De Vuyst, 2002; Yi et al., 2010). Bacteriocins weaken the integrity of the cell membrane by the non-enzymatic mechanisms, or possess the ability inhibit the cell wall synthesis (Taylor, 2005). A few bacteriocins or bacteriocin-like compounds have been identified, isolated and characterised (Navrhus and Sørhaug, 2006; Liptáková et al., 2010). For example, plantaricin ST31 produced by *L. plantarum* ST31 inhibits several Gram-positive bacteria (Todorov et al., 1999), bavaricin A produced by *L. bavaricus* MI401 is effective against *Listeria* strains and some Gram-negative bacteria (Kaiser and Montville, 1996; Todorov et al., 1999), and bacteriocin-like inhibitory substance BLIS C57 by *L. sanfranciscensis* C57 prevents spoilage by Gram-positive bacteria including *Listeria* strains (Corsetti et al., 1996).

Reutericyclin is an antibiotic produced by *L. reuteri* which possess effectiveness in inhibition of Gram-positive bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus* and toxigenic *Clostridia*. Reutericyclin is produced in active concentrations during fermentation of sourdough. Starter cultures with ability to produce this compound were successful in ropiness prevention (Gänzle et al. 2009).

Table 2. Inhibitory substances produced by lactobacilli isolated from sourdoughs and their antimicrobial spectrum (Todorov et al., 1999; Messens and De Vuyst, 2002; Narvhus and Sørhaug, 2006; Yi et al., 2010).

Inhibitory substance	Producer	Antimicrobial spectrum
bavaricín A	*L. sakei* (formerly *bavaricus*), *L. curvatus*, *L. plantarum*, *L. sakei* MI401	*Listeria* ssp., Gram-negative bacteria
BLIS C57	*L. sanfranciscensis* C57	*Listeria* ssp., Gram-positive bacteria
plantaricin ST31	*L. plantarum* ST31	Gram-positive bacteria
reutericyclin	*L. reuteri* LTH2584	*Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridia* ssp.

LAB metabolites with antifungal properties include propionate, phenyl lactate, hydroxyphenyl lactate, several cyclic dipeptides or 3-hydroxy fatty acids (Gänzle et al. 2009). These compounds are active only when co-operate with other antimicrobial agents and were shown to extend the mould-free shelf-life of bread (Gänzle et al., 2009; Poutanen et al., 2009).

The use of a protective culture should be considered as an additional safety factor, with the ability of improving the microbiological safety of the food. Implementations of these cultures can improve the good manufacturing practices. It happens mainly thanks to limiting the growth and survival of food-borne pathogens and food spoilage organisms (Messens and De Vuyst, 2002).

Exopolysaccharides production

Sourdough lactobacilli secrete structural variety of polysaccharides termed as exopolysaccharides - EPS (Tieking and Gänzle, 2005; Gänzle et al., 2007) into the environment, and are able to replace hydrocolloids in gluten-free sourdoughs (Galle et al., 2011). All relevant EPS in sourdough are homopolysaccharides (Tieking and Gänzle, 2005), consisting of identical monosaccharides D-glucose or D-fructose and, on the basis of the constituent saccharide unit they are divided into two major groups: glucans and fructans, which significantly influence dough and baked goods properties (Patel et al., 2011). Homopolysaccharides are synthesized from sucrose by extracellular glucan- and fructosyl-transferases (glycosyl transferases), where sucrose commonly present in cereal flours (Gänzle, 2009) is used as the glycosyl donor (Tieking and Gänzle, 2005; Gänzle et al., 2007; Minervini et al., 2010). These transferases (transglycolases) have ability to use the energy of the glycosidic bond of sucrose to transfer the glucose or fructose moiety to the glycosyl acceptor molecule (Tieking and Gänzle, 2005).

In bakery industry, homopolysaccharides reuteran and levan are used. The water soluble glucan reuteran produced by *L. reuteri* strains LB 121, ATCC 55730 and 35-5 (Patel et al., 2011) comprises of 70 % α-(1,4) and few α-(1,6) glycosidic bonds (Fig. 5a.). Levan (Fig. 5b) (synthesized by *L. sanfranciscensis* strain LTH 2590 and *L. reuteri* LB 121) is fructan having fructose linked by β-(2,6) glycosidic bonds. If both are present, they positively influence the rheology of dough (Badel et al., 2011).

EPSs produced by bread associated lactobacilli favourably influence host of technological properties of dough and bread by facilitating water absorption, softening the gluten content of the dough, improving the dough rheology and machinability, increasing specific loaf volume, retarding bread staling and prolonging shelf-life (Tieking and Gänzle, 2005; Patel et al., 2011). They particularly relevant for the textural quality of gluten-free breads that do not contain wheat, rye or barley and thus lack wheat gluten or rye pentosans that are capable of water binding and gas retention at the dough stage (Gänzle, 2009). In situ

production of homopolysaccharides in sourdough was reported to be more effective than external addition (Galle et al., 2011).

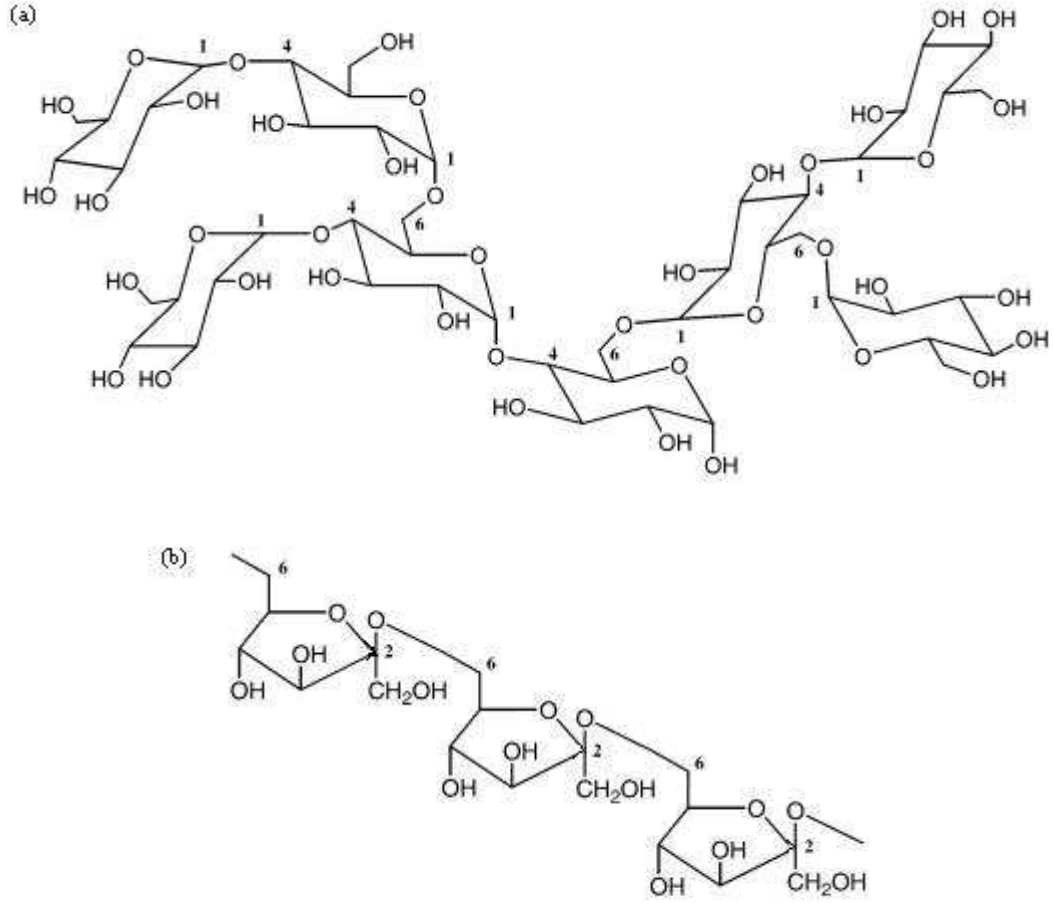

Fig. 5. Structures of exopolysaccharides reuteran (a) and levan (b) produced by sourdough lactobacilli (Badel et al., 2011).

Lactobacilli proteolytic metabolism

While the majority of sourdough lactobacilli does not exhibit cell-wall associated proteinase activity (Gänzle et al., 2007), acidification induced by saccharides degradation activates endogenous cereal proteases which release peptides and amino acids (De Vuyst et al., 2009). Lactobacilli use peptides predominantly in order to fulfil their demand of complex nitrogen. They are capable of significant support of the growth of sourdough lactobacilli (Gänzle et al., 2007).

Amino acids are of crucial importance for the final bread flavour, either as precursors for certain flavour compounds or as free amino acids that contribute to the flavour on their own (Ravyts and De Vuyst, 2011). Amino acids are used by lactobacilli for protein synthesis

as well as the source of energy via arginine deiminase (ADI) pathway (Fig. 6.) The widespread metabolic trait involves conversion of the arginine into ornithine with the concomitant generation of ATP and NH_3, giving the strain energetic advantage and protection against acid stress (Weckx et al., 2010). The key degradation reaction of amino acids during fermentation is the Ehrlich pathway leading to the formation of aldehydes or to the corresponding alcohols, while during the baking process the Strecker reaction takes place. This reaction leads to creation of aldehydes, too (Hoffmann and Schieberle, 2000; Corsetti and Settanni, 2007; De Vuyst et al., 2009).

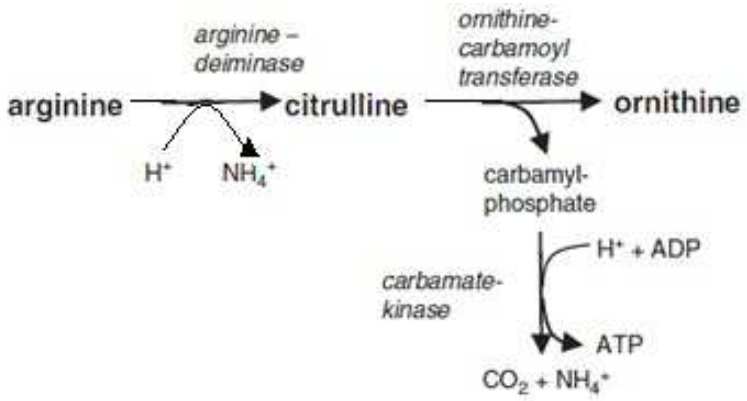

Fig. 6. Arginine-deaminase pathway in LAB (Gänzle et al. 2009).

Proteins in the dough determine its rheological properties (viscosity, elasticity), gas retaining ability and, therefore, the final loaf volume and shape (Vermeulen et al., 2006; Gänzle et al., 2008). Proteolysis of cereal proteins during fermentation and subsequent metabolism of peptides and amino acids affects flavour, volume and texture of bread and enables to develop new products for people with gluten intolerance (Gänzle et al., 2007; 2008). Catabolic reactions such as deamination, decarboxylation, transamination and side-chain modification may provide keto-acids, NH_3, amines, aldehydes, acids and alcohols, which are essential for taste and aroma of baked goods (Gobbeti et al., 2005; Kocková and Valík, 2011).

Overview of biochemical changes during sourdough fermentation is summarised in the Fig. 7.

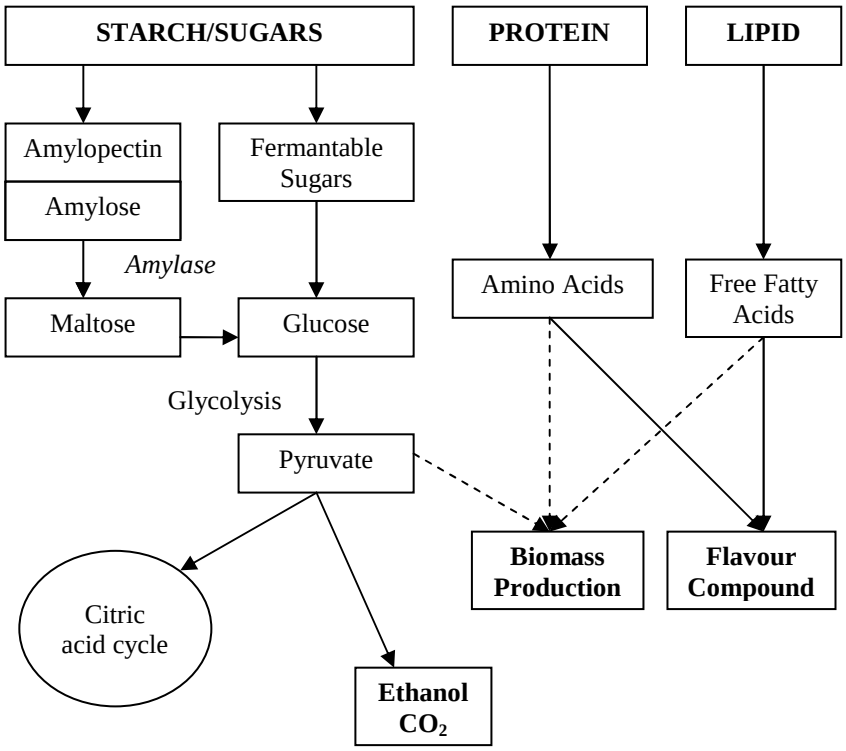

Fig. 7. Biochemical changes during sourdough fermentation (Narvhus and Sørhaug, 2006).

Useful properties of sourdough lactobacilli

The sourdough lactobacilli have earned special interest of researchers due to the benefits achieved by their use in breadmaking industry. It has been reported that the lactobacilli contribute to the improvement of the volume, texture and sensory quality of the bread, as well as to the increase of desirable bread acidity and to the improvement of bread physical and microbiological shelf-life (Paramithiotis et al., 2005; Petruláková et al., 2009a, 2010).

Rye flour is very low in gluten proteins and instead of it, starch and pentosans make an important contribution to the bread structure. The swelling and solubility of pentosans increase when LAB fermentation lowers pH. Gelatinization of starch occurs at 55 – 58 ºC. Considering that the flour amylase has temperature optimum between 50 – 52 ºC, it is crucial that the amylase is actually inactivated at the pH range that is obtained during sourdough fermentation (Narvhus and Sørhaug, 2006).

Lactobacilli fermentation may increase nutritive value of baked goods by many ways, e.g. by bioactive compounds production, retardation of starch retrogradation or phytases production (Dal Bello et al., 2005; Poutanen et al., 2009).

Phytases are enzymes playing key role in enhancing food quality rich in phytate (inositolhexaphosphate – IP_6). Phytases occur naturally in wheat and rye flours, but their

levels are not high enough to lower the phytate content. They are produced by present microflora during fermentation process. Optimum pH value for wheat flour phytases is 4.5 (Corsetti and Settanni, 2007; Poutanen et al., 2009), so the pH decrease during fermentaion raise the function of produced phytases (Corsetti and Settanni, 2007). Phytate has chelating effect and binds K, Mg, Ca, Mn, Fe and Zn to the complexes. The order of stability of mineral-phytate complexes is as follows: $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$ (Nielsen et al., 2007). These minerals then become nutritionally unavailable. Therefore the phytate is referred as antinutritive compound (Chaoui et al., 2006; Kocková and Valík, 2011).

Phytases hydrolyses IP_6 to inositolpentaphosphate (IP_5) and further to lower inositol esters ($IP_4 – IP_1$), which lose the ability to bind the minerals (Fig.8.) and these phytate complexes are less stabile (Corsetti and Settanni, 2007). Iron release from phytate is very important for people suffering from anaemia (Chaoui et al., 2006). Enzyme degradation of phytate depends on the phytases activity as well as on the flour parts size, total acidity, water content, temperature and fermentation duration (Bhatta and Goldys, 2009).

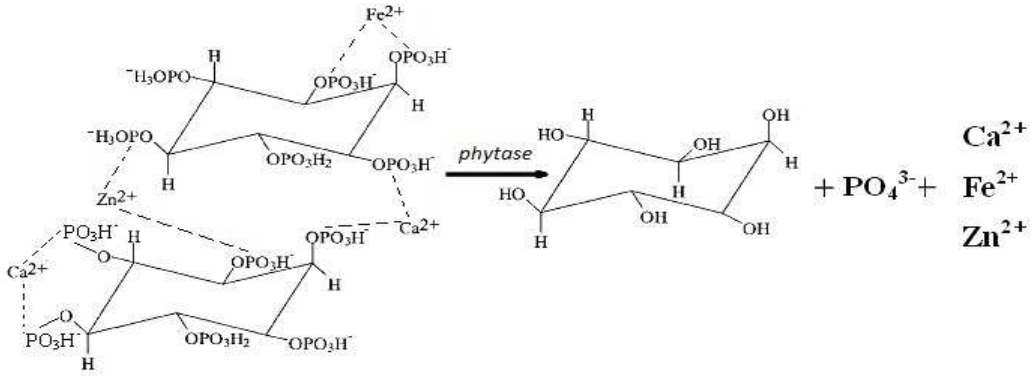

Fig. 8. Phytate degradation by sourdough lactobacilli (Kocková and Valík, 2011).

Lactobacilli have been shown to possess an outstanding potential in decreasing the celiac disease-inducing effects of gluten that has been mentioned above.

Lactobacilli as starter cultures

Nowadays, sourdough fermentation has to be predictable and consistent to ensure the maintenance of product quality and this underpins the importance of commercial starter cultures in baked goods production (Wigley, 2000). Starter cultures are defined as “the preparation containing live or inactive microorganisms with ability to create desirable metabolic activity” (Katina et al., 2006). The application of starter cultures has become the state of art. Suitable strains combination allows process standardization, mitigation of

hygienic risks and product diversification (Ammor et al., 2005; Gül et al., 2005). Starter culture development in baking industry was initially based on isolation of native microflora from sourdough, biochemical and taxonomic characterisation of the microorganisms (predominantly lactobacilli) and the selection of strains appropriate as starter cultures through time-consuming application trials (Gänzle, 2009). The superiority of commercial starters is in the improvement of the sourdough production control while ensuring reliable quality in bread production and allows the production of full sourdough in a one-stage process (Gereková et al., 2010a). Starter cultures performance within fermentation process has been referred to pH value decrease, total titratable acidity increase and organic acids production (Robert et al., 2006; Gereková et al., 2010a).

The safe character of lactobacilli attracts baking industry by simplifying the regulation and application in sourdough preparation. The genus *Lactobacillus* contains GRAS (generally recognised as safe) bacteria which means that their addition in food causes no risk for health (Badel et al., 2011). The most common lactobacilli species used as single-strain or mixed-strain starter cultures in bakery industry are: *L. sanfranciscensis, L. plantarum, L. paralimentarius, L. brevis, L. pontis, L. amylovorus, L. alimentarius, L. delbrueckii, L. crispatus, L. reuteri, L. casei,* and *L. fermentum* (Paramithiotis et al., 2005; Gänzle et al., 2007; Weckx et al., 2010).

There are bakeries in Slovakia, where the established three-stage process of sourdough preparation is still used. The most occurred lactobacilli isolated from traditional Slovak sourdoughs are: *L. brevis, L. paralimentarius, L. helveticus, L. plantarum, L. crispatus, L. delbrueckii* and *L. acidophilus* (Gereková et al., 2009; 2010a,b; Petruláková et al., 2009a,b,c; 2010). The variabilty of present lactobacilli is wide, but no commercial starters are manufactured in Slovakia (Tab.3.). Therefore the researchers at the Department of Nutrition and Food Assessment are working on the development of starter cultures containing species isolated from native sourdoughs, which could be commercially used (Petruláková et al., 2009a,b; Gereková et al., 2009; 2010a).

Table 3. Commercial starter cultures available on the market.

Commercial starter culture	Producer
Lalvain du Jour, Florapan™	LALLEMAND, Canada
Sapore®	PURATOS, USA
Böcker F, Böcker 350	BÖCKER, Germany

Conclusion

In order to prepare an ideal culture for any particular food application, it is necessary to know which properties we expect from the present microflora and to have the tools for improvement of the cultures` function. *Lactobacillus* strains are traditionally used as starter cultures in breadmaking industry. Their metabolic activities enable not only prolongation of the shelf-life by acidification, but also contribute to the sensory and the technological properties of final baked products. Nowadays, is a great demand of bread-makers to accelerate the production but maintain the quality and consumers` acceptance of baked goods. The development of starter cultures is a hot issue all-over the world but not in Slovakia. Therefore at the Department of Nutrition and Food Assessment there is an effort to fill a research niche and develop starters rivalrous to those which are already available on the market.

Acknowledgement

This work was supported by courtesy of the Slovak University of Technology Programme for the young researchers support (ZAK/04/6413), as well as of the Slovak Research and Development Agency (APVV VMSP-II-0024-09, APVV-0310-06) and of the Ministry of Education of the Slovak Republic (VEGA 1/0094/10).

References

Ammor S, Dufour E, Zagorec M, Chaillou S, Chevallier I (2005) Food Microbiology 22 (6): 529-538;

Arpai J, Bartl V (1977) In: Food Microbiology, 1st ed. (p.280). ALFA, Bratislava. ISBN: 63-138-76 (in Slovak);

Badel S, Bernardi T, Michaud P (2011) Biotechnology Advances 29 (1): 54-64;

Calderon M, Loiseau G, Guyot JP (2002) International Journal of Food Microbiology 80 (2): 161-169;

Caplice E, Fitzgerald GF (1999) International Journal of Food Microbiology 50 (1-2): 131-149;

Corsetti A, Gobbetti M, Smacchi E (1996) Food Microbiology 13 (6): 447-456;

Corsetti A, Settanni L, van Sinderen D, Felis GE, Dellaglio F, Gobbetti M (2005) International Journal of Systematic and Evolutionary Microbiology 55 (1): 35-40;

Corsetti A, Settanni L (2007) Food Research international 40 (5): 539-558;

De Angelis M, Gallo G, Corbo MR, McSweeney PL, Faccia M, Giovine M, Gobbetti M (2003) International Journal of Food Microbiology 87 (3): 259-270;

De Angelis M, Di Cagno R, Gallo G, Curci M, Siragusa S, Crecchio C, Parente E, Gobbetti M (2007) International Journal of Food Microbiology 114 (1): 69-82;

De Vuyst L, Vancanneyt M (2007) Food Microbiology 24 (2): 120-129;

De Vuyst L, Vrancken G, Ravyts F, Rimaux T, Weckx S (2009) Food Microbiology 26 (7): 666-675;

Decock P, Capelle S (2005) Trends in Food Science and Technology 16 (10): 113-120;

Ehrmann MA, Vogel RF (2005) Trends in Food Science and Technology 16 (1-3): 31-42;

Escalante A, Martínez A, Rivera M, Gosset G (2010) In Smolke ChD (Ed.), The Metabolic Pathway Engineering Handbook: Fundamentals, 1.ed., (pp.**1**-1-**1**-20). CRC Press, Boca Raton. ISBN: 978-1-4398-0296-0;

Gänzle MG, Vermeulen N, Vogel RF (2007) Food Microbiology 24 (2): 128-138;

Gänzle MG, Loponen J, Gobbeti M (2008) Trends in Food Science and Technology 19 (10): 513-521;

Gänzle MG (2009) International Journal of Food Microbiology 134 (1-2): 29-36;

Gereková P, Hybenová E, Petruláková Z, Simonidesová D, Kocková M, Valík Ľ (2009) Nakladateľstvo STU, Bratislava – Proceeding of Laboralim Conference: Recent progress in analytical methods of food: 166-169. ISBN: 978-80-227-3071-6;

Gereková P, Hybenová E, Petruláková Z, Kocková M, Valík Ľ (2010a) Opatija – Proceedings of the 5th International Congress FLOUR – BREAD `09: 354-360. ISBN: 978-953-7005-21-4;

Gereková P, Juríková N, Kocková M, Hybenová E, Valík Ľ (2010b) Nakladateľstvo STU, Bratislava – Nové trendy v potravinárstve: 106-109. ISBN: 978-80-227-3398-4;

Gobbetti M (1998) Trends in Food Science and Technology 9 (7): 267-274;

Gobbeti M, De Angelis M, Corsetti A, Di Cagno R (2005) Trends in Food Science and Technology 16 (1-3): 57-69;

Görner F, Valík Ľ (2004) In: Aplikovaná mikrobiológia požívatín, 1.ed. (p.528). Malé Centrum, Bratislava. ISBN: 80-967064-9-7.

Gül H, Özçelik S, Sağdıç O, Certel M (2005) Process Biochemistry 40 (2): 691-697;

Hansen EB (2002) International Journal of Food Microbiology 78 (1-2): 119-131;

Hervé R, Gabriel V, Lefebvre D, Rabier P, Vayssier Y, Fontagné-Faucher C (2006) LWT - Food Science and Technology 39 (3): 256-265;

Hoffmann T, Schieberle P (2000) Journal of Agricultural and Food Chemistry 48 (9): 4301-4305;

Hudecová D, Šimkovič M (2009) In: Mikrobiológia, 1.ed. (p.293). Nakladateľstvo STU, Bratislava. ISBN: 978-80-227-3194-2;

Hutkins RW (2006) In: Microbiology and Technology of Fermented Foods, 1.ed. (p.475). IFT Press, Blackwell Publishing, Oxford. ISBN: 978-0-8138-0018-9;

Chaoui A, Faid M, Belahsen R (2006) Journal of Trace Elements in Medicine and Biology 26 (4): 217-220;

Kaiser AL, Montville TJ (1996) Applied and Environmental Microbiology 62 (12): 4529-4535;

Kaseleht K, Paalme T, Mihhalevski A, Sarand I (2011) International Journal of Food Science and Technology 46 (9): 1940-1946;

Katina K, Heiniö RL, Autio K, Poutanen K (2006) LWT - Food Science and Technology 39 (10): 1189-1202;

Kazatelová M (2003). Bulletin potravinárskeho výskumu 42 (1-2): 67-73;

Kocková M, Valík Ľ (2011) Potravinárstvo 5 (2): 27-40;

Konings WN, Kok J, Kuipers OP, Poolman B (2000) Current Opinion in Microbiology 3 (3): 276-282;

Leroy F, De Winter T, Adriany T, Neysens P, De Vuyst L (2006) International Journal of Food Microbiology 112 (2): 102-111;

Liptáková D, Valík Ľ, Lauková A, Strompfová V (2007) Czech Journal of Food Science 25 (5): 272-282;

Liptáková D, Hudecová A, Valík Ľ, Medveďová A (2010) Journal of Agricultural Science and Technology 4 (4): 88-98;

Lönner C, Welander T, Molin N, Dostálek M, Blickstad E (1986) Food Microbiology 3 (1): 3-12;

Marklinder I, Lönner C (1992) Food Microbiology 9 (3): 197-205;

Messens W, De Vuyst L (2002) International Journal of Food Microbiology 72 (1-2): 31-43;

Minervini F, De Angelis M, Surico RF, Di Cagno R, gänzle M, Gobbetti M (2010) International Journal of Food Microbiology 141 (1-2): 130-135;

Narvhus JA, Sørhaug T (2006) In: Hui Y (Ed), Food Biochemistry and Food Processing, 1.ed. (pp 615-637). Blackwell Publishing, Iowa. ISBN: 978-0-8138-0378-4;

Nielsen MM, Damstrup ML, Thomsen AD, Rasmussen SK, Hansen Å (2007) European Food Research and Technology 225 (2): 173-181;

O´Toole DK, Lee YK (2006) In: Lee. YK (Ed), Microbial Biotechnology: Principles and Applications (pp 227-292). World Scientific Publishing, Singapore. ISBN: 981-238-323-9;

Paramithiotis S, Chouliaras Y, Tsakalidou E, Kalantzopoulos G (2005) Process Biochemistry 40 (8): 2813-2819;

Patel S, Majumder A, Goyal A (2011). Indian Journal of Microbiology *(in press)*;

Petruláková Z, Hybenová E, Mikušová L, Gereková P, Kocková M, Šturdík E (2009a) Acta Chimica Slovaca 2 (2): 120-128;

Petruláková Z, Hybenová E, Gereková P, Kocková M, Šturdík E (2009b) Bratislava – Zbroník príspevkov konferencie Drobnicov memoriál: 66-67. ISBN: 978-80-970164-1-8;

Petruláková Z, Hybenová E, Gereková P, Simonidesová D, Kocková M, Šturdík E (2009c) Nakladateľstvo STU, Bratislava – Zbroník príspevkov konferencie Laboralim: Recent progress in analytical methods of food: 247-250. ISBN: 978-80-227-3071-6;

Petruláková Z, Hybenová E, Gereková P, Kocková M, Šturdík E (2010) Opatija – Proceedings of the 5th International Congress FLOUR – BREAD `09: 409-415. ISBN: 978-953-7005-21-4;

Plessas S, Trantallidi M, Bekatorou A, Kanellaki M, Nigam P, Koutinas AA (2007) Food Chemistry 105 (1): 187-194;

Plessas S, Fisher A, Koureta K, Psarianos C, Nigam P, Koutinas AA (2008) Food Chemistry 106 (3): 985-990;

Plocková M, Chumchalová J, Pluharová B (1996) Potravinářske vědy 14 (3): 165-174;

Poutanen K, Flander L, Katina K (2009) Food Microbiology 26 (7): 693-699;

Ravyts F, De Vuyst L (2011) Food Microbiology 28 (6): 1129-1139;

Reddy G, Altaf M, Naveena BJ, Venkateshwar M, Vijay Kumar E (2008) Biotechnology Advances 26 (1): 22-34;

Rehman SU, Paterson A, Piggott JR (2006) Trends in Food Science and Technology 17 (10): 557-566;

Robert H, Gabriel V, Lefebvre D, Rabier P, Vayssier Y, Fontagné-Faucher C (2006) LWT – Food Science and Technology 39 (3): 256-265;

Stiles ME, Holzapfel WH (1997) International Journal of Food Microbiology 36 (1): 1-29;

Taylor S (2005) In: Advances in Food and Nutrition Research, vol. 50 (p.336). Academic Press, Oxford. ISBN: 0-12-016450-7;

Stratford M (2000) In Robinson RK (Ed.), Encyclopedia of Food Microbiology, 1.ed., vol. 3, (pp.1729-1737). Elsevier, Oxford. ISBN: 978-0-08-052359-0;

Thiele C, Gänzle MG, Vogel RF (2002) Cereal Chemistry 79 (1): 45-51;

Tieking M, Gänzle MG (2005) Trends in Food Science and Technology 16 (1-3): 79-84;

Todorov S, Onno B, Sokorine O, Chobert JM, Ivanova I, Douseet X (1999) International Journal of Food Microbiology 48 (3): 167-177;

Tseng ChP, Montville TJ (1993) Biotechnology Progress 9 (2): 113-121;

Valík Ľ, Medveďová A, Liptáková D (2008) Journal of Food and Nutrition Research 47 (2): 60-67;

Vermeulen N, Kretzer J, Machalitza H, Vogel RF, Gänzle MG (2006) Journal of Cereal Science 43 (2): 137-143;

Vogel RF, Müller M, Stolz P, Ehrmann M (1996) Advances in Food Science 18 (5-6): 152-159;

Vracken G, Rimaux T, De Vuyst L, Leroy F (2007) Athens - 5th International conference predictive modeling in foods: 227-230;

Weckx S, Van der Meulen R, Maes D, Scheirlinck I, Huys G, Vandamme P, De Vuyst L (2010) Food Microbiology 27 (8): 1000-1008;

Wigley RC (2000) In Robinson RK (Ed.), Encyclopedia of Food Microbiology, 1.ed., vol. 3, (pp.2084-2094). Elsevier, Oxford. ISBN: 978-0-08-052359-0;

Xiaodong W, Xuan G, Rakshit SK (1997) Biotechnology Letters 19 (9): 841-843;

Yi H, Zhang L, Tuo Y, Han X, Du M (2010) Food Control 21 (4): 426-430;

Zalán Z, Hudáček J, Štětina J, Chumchalová J, Halász A (2010) European Food Research and technology 230 (3): 395-404;

Zhang DX, Cheryan M (1991) Biotechnology Letters 13 (10): 733-738.