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Full Length Research Paper

Selection of prospective infants' lactobacilli isolates for amino acids production

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A total of 9 lactic acid bacteria (LAB) isolated from Egyptian infants, belonging to lactobacilli strains, were screened and selected according to their amino acids production. The isolate strains produced different essential amino acids in fermentation of M17 medium. *Lactobacillus* strain L1 produced the highest yield of histidine 92.5 mg/l, L2 strain produced the highest amount of asparagine 59.61 mg/l, cysteine 32.96 mg/l, phenylalanine 17.49 mg/l and tyrosine 244.91 mg/l. On the other hand, *Lactobacillus* strain L3 is the most potential culture for the production of citrulline 61.12 mg/l and glutamic 68.78 mg/l. This strain also produced the highest amount of lysine, ornithine and proline, which were 57.04, 95.58 and 87.06 mg/l, respectively. While the strain L4 produced the highest amount of glycine 32.12 mg/l, strain L7 produced the highest amount of alanine 147.55 mg/l and strain L5 produced the highest amount of asparatic acid 218.09 mg/l. The present study concluded that the *Lactobacillus* strains have the potential to produce different amino acids with variance concentrations.

Key Words: Lactobacilli, *Lactobacillus*, amino acids, screening, production.

INTRODUCTION

Lactic acid bacteria are used for the fermentation of a large variety of food products. In the early days, most important feature of these organisms was the conversion of the available sugar into lactic acid in order to achieve preservation of the fermented product. In modern fermentation processes, in the dairy industry, other aspects such as flavour development and consistent product quality have become as important as preservation (Tan et al., 1993).

The concentrations of free and essential amino acids in milk are too low for the starter to obtain the maximal desired cell densities of 500 g bacteria (dry weight) /ml (Mills and Thomas, 1981). Therefore, cultures have to use their proteolytic system for releasing amino acids from the available sources (Law and Haandrikman, 1997).

Lactic acid bacteria (LAB) are the most important culture used in food fermentation (Wibowo et al., 1985). Lactobacilli are indispensable agents for the fermentation of food and feed, and they exert probiotic effects on human and animal health (Lindgren and Dobrogosz, 1990; Pereira and Gibson, 2002; Ogunbanwo et al., 2003; Pereira et al., 2003; Kabir et al., 2004, 2005, 2009a, b).

L-Amino acids are known to play a wide range of roles in nature. They are the constituents of proteins and act as precursors of other compounds such as nucleic acids, haemo group, hormones and neurotransmitters (Pereira et al., 2003; Voet and Voet, 1995). From the nutritional point of view, the enrichment of food and diets with L-amino acids improves food assimilation, intensifies the metabolism of fatty acids and avoids damage to the central nervous system. The Food and Agriculture Organization together with the World Health Organization (FAO/WHO, 1985) have established a table of essential L-amino acids (EAA). Their report estimates the amino acid re] in starter lactococci (Roudot-Algaron and Yvon, 1998; Yvon et al., 1997; Gao et al., 1997). However, relatively little is known about the ability of non-starter lactobacilli to metabolise amino acids. These are the predominant

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microorganisms in mature Cheddar (Fox et al., 1998). The addition of selected lactobacilli to cheese milk with the objective to accelerate or improve cheese quality resulted in increased levels of free amino acids in cheese, accompanied by increased flavour intensity (Muir et al., 1996; Trepanier et al., 1992; Fernandez-Espla and Fox, 1998). It is uncertain which source of energy is used by the non-starter lactobacilli during their growth in cheese after lactose depletion. A number of lactobacilli were shown to utilise some amino acids and peptides as an energy source in addition to galactose and ribose residues, N- acetyl-galactosamine and sialic acid, derived from nucleic acid and casein degradation (Williams et al., 2000). Transamination was described to be one of the possible mechanisms for amino acid breakdown by lactococci in cheese (Gao et al., 1997; Yvon et al., 1997). A-Ketoglutarate was found to be an effective amino group acceptor for lactoccocal aminotransferases and enhanced the conversion of amino acids to flavour compounds in experimental cheeses (Yvon et al., 1998).

Most publications about amino acid metabolism by lactic acid bacteria focus on catabolism of single amino acids by cell-free extracts and demonstrate that some cheese micro-organisms have the enzymatic potential to carry out reactions leading to the formation of cheese flavour compounds (Christensen et al., 1999). However, the conditions in cheese are more complex since cheese contains proteins, peptides, amino acids and bacterial cells that are able to grow under stress conditions in an environment lacking fermentable carbohydrates. In this study, we screened the lactobacilli isolates for their amino acids production in the log phase by measuring the amino acids in the culture growth supernatants.

MATERIALS AND METHODS

Strains and culture conditions

Nine lactic acid bacteria were identified previously as lactobacilli (Mostafa et al., 2006). M17 (Merck, Germany) medium was used as a cultural medium for the production of amino acids. Each isolate strain was inoculated in 10 ml of M17 medium consisting of 2% peptone, 1% yeast extract and 2% glucose. Precultures were grown for 48 h at 37°C under constant shaking at 200 rpm. The cultures were then prepared inoculating 10^6 cfu/mL of each preculture in 100 ml of M17 medium for 8 h at 37°C with shaking at 200 rpm, after which the supernatants were harvested by centrifugation at 10000×g for 10 min at 4°C and stored at -20°C.

Amino acids analysis

For protein precipitation, 200 ml of sulphosalicylic acid (10%) was added to 500 ml of the culture in 2.0 ml tube and shacked well; thetube was stored at 4°C for 10 min. The upper clear solution was dissolved in the lithium citrate diluting buffer in the ratio 1:1. Then, all the samples were analyzed by Amino acid analyzer Sykam S 433 (Eresing, Germany) to analyze the amino acids. Ninhydrin reagent was used (750 ml dimethyl sulphoxide, 20 g ninhydrin, 0.6 hydrindantin and 250 ml of 4 M lithium acetate buffer pH 5.2) . Three buffers A, B, C and regeneration solution were used as a Amino acid analyzer buffer system; buffer A (11.3 g lithum citrate, 6.0 g citric acid, 50 ml methanol, 9.0 ml HCl (36.5%) and add water to final volume of 1l, pH 2.95), buffer B (18.8 g lithium citrate, 4.2 g lithium chloride, 6 ml HCl and add water to final volume of 1l, pH 4.2), buffer C (18.8 g lithium citrate, 50.9 lithium chloride, 10 ml HCl and add water to final volume of 1l, pH 1.4) and regeneration solution (12.6 g lithium hydroxide in 1l).

RESULTS AND DISCUSSION

Bacteria could produce amino acid via proteolytic activity of protein/peptide substrates. Proteins or large peptides in media should be degraded into small peptides by exoproteases, and after transport into the cells, the translocated small peptides are then cleaved to amino acids by intracellular peptidases (Exterkate and de Veer, 1987). It has been reported that *L. helveticus* is the most proteolytic species towards a wide range of substrates and has a very efficient proteolytic system involving general aminopeptidase (Kunji et al., 1996; Khalid et al., 1991; Masahiro et al., 1995).

Nine strains were studied for the production of free amino acids in cultivation medium. These strains were isolated from infants' faeces and identified previously (Mostafa et al., 2006). They were aerobically incubated at 37ºC with shacking at 200 rpm in the medium M17 broth. The tested strains produced some amino acids with different concentration. Figures 1 and 2 reveal that all of the lactobacilli strains showed produceability of citrulline and glycine with amounts of the production varied from 4.05 to 61.12 mg/l; citrulline and 1.19 to 32.12 mg/l for glycine. *Lactobacillus* strain L3 is the most potential strain for the production of citrulline, which produced 61.12 mg/l, while the strain L4 produced the highest amount of glycine which was 32.12 mg/l. On the other hand, five *Lactobacillus* strains produced different concentration of alanine with the level of production ranged from 32.40 to 147.55 mg/l, and the higher producer strains were L7 and L3 (Figure 1). Five of tested strains production, L2, L3, L5, L6 and L, produced asparagine, the highest amount of asparagine 59.61 mg/l was produced by L2 strain. Only the L5 strain produced 218.09mg/l aspartic acid and none of the other tested *Lactobacillus* strains could produce aspartic acid (Figure 1).

Figure 2 shows that glutamic acid was produced by all tested lactobacilli strains except strain L1; the highest yield 68.78 mg/l was found in the medium inoculated by the strain L3. Most of the tested strains produced cysteine and the strain L2 produced the highest amount of cysteine 32.96 mg/l. *Lactobacillus* strains L1 and L3 produced histidine, and L1 strain produced the highest yield 92.5 mg/l.

The characteristics of production of amino acids by *L. delbrueckii* subsp. *lactis* (ATCC 12315), *L. casei* (NRRL-B1445, *L. delbrueckii* (NRRL-B445), and *L. heveticus* (NRRL- B1937) were investigated by Kibeom et al., (2001) . They estimated a variation in concentration of amino acids (classified into alanine, aspartate, glutamate,

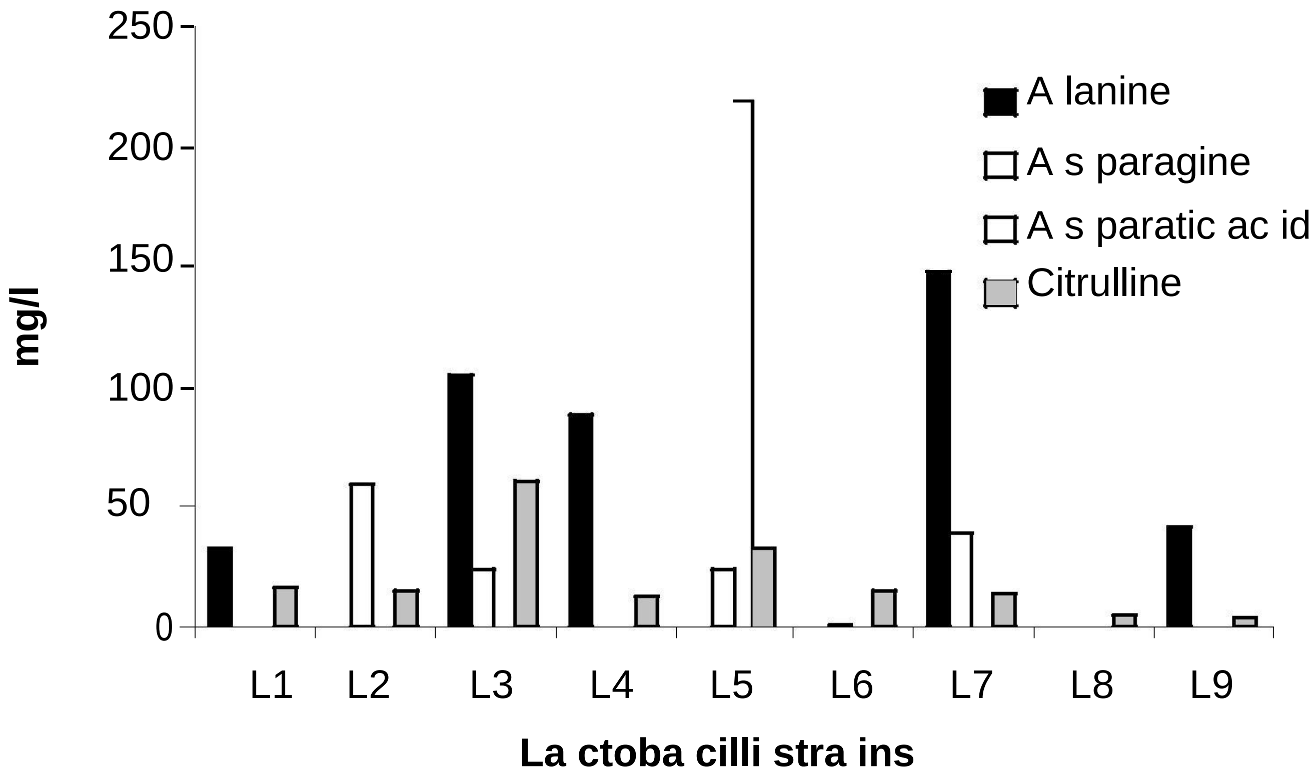

Figure 1. Production of alanine, asparagine, aspartic acid and citrulline by *Lactobacillus* strains.

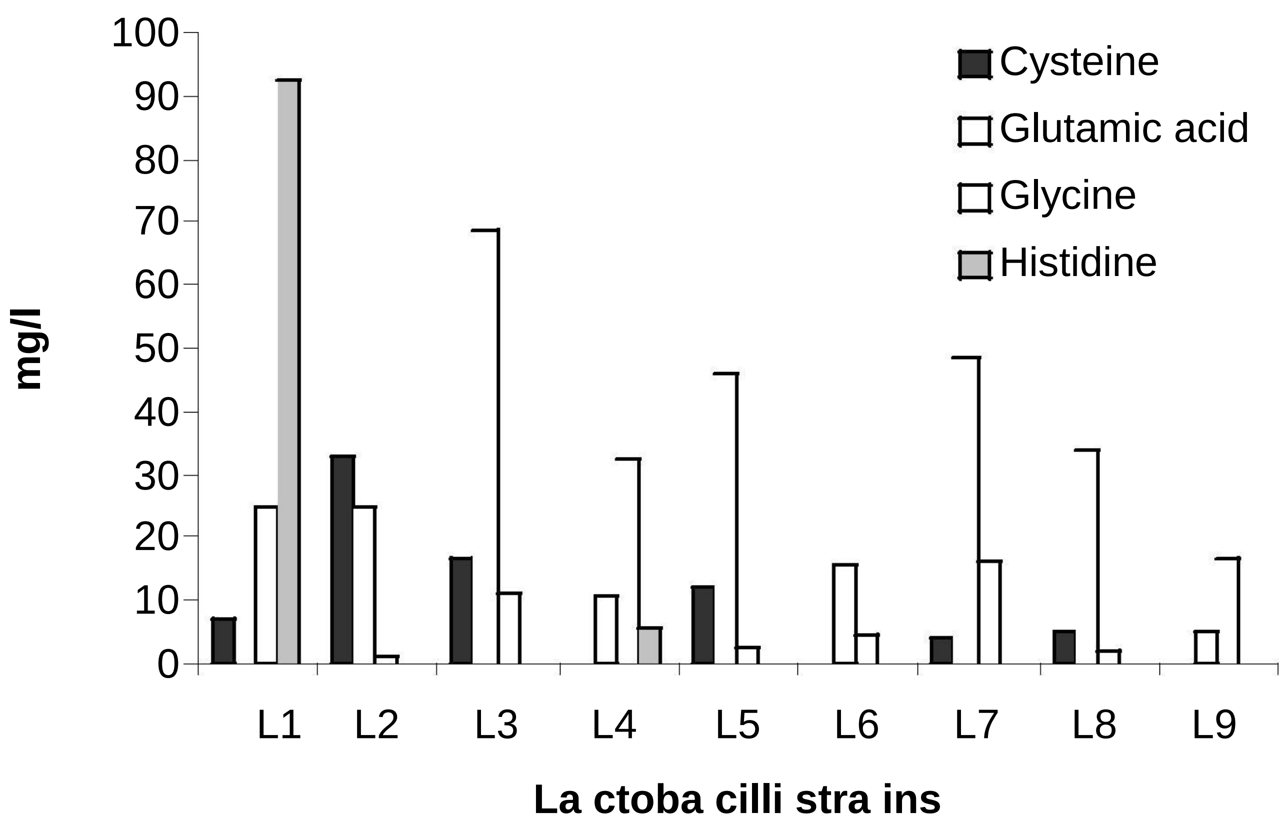

Figure 2. Production of cysteine, glutamic acid, glycine and histidine by *Lactobacillus* strains.

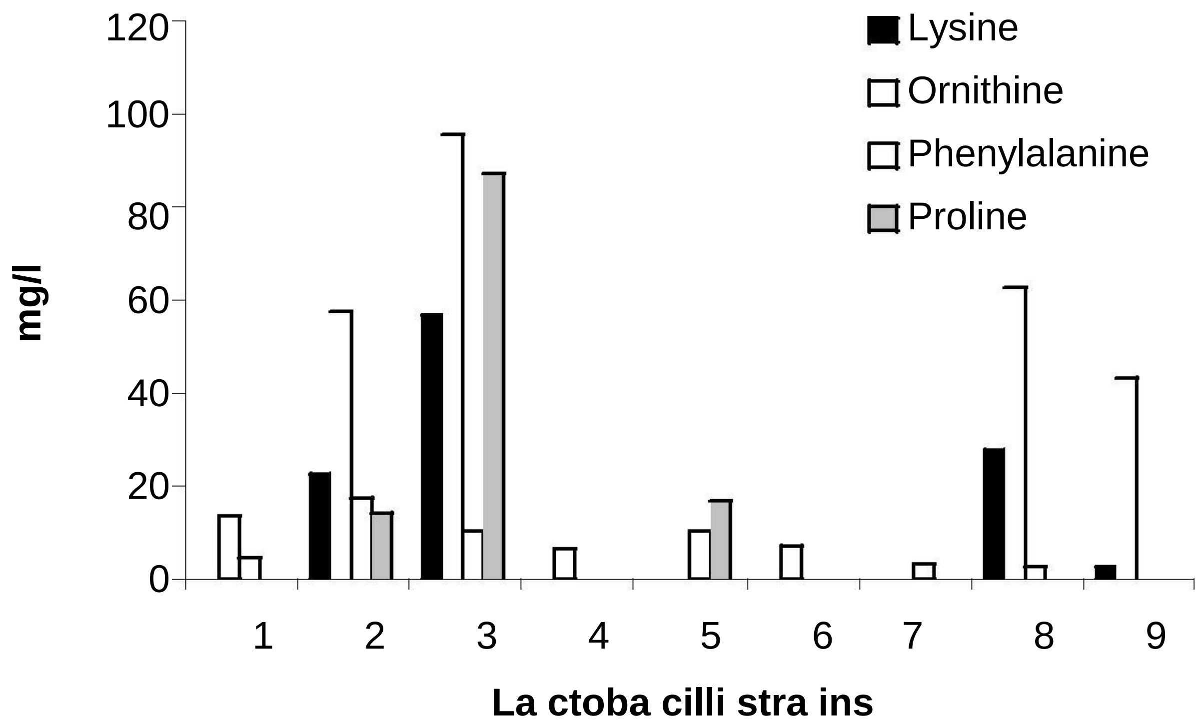

Figure 3. Production of lysine, ornithine, phenylalanine and proline by *Lactobacillus* strains.

aromatic amino acid, and histidine families). The *L. delbrueckii* (NRRL-B445) and *L. helveticus* (NRRL-B1937) had quite different characteristics in amino acids production and *L. helveticus* (NRRL-B1937) was superior in the production of amino acids as well as in cell growth (Kibeom et al., 2001).

Amino acids such as lysine, ornthine, phenylalanine and proline were produced by some of the lactobacilli strains as shown in Figure 3. Strains L2 and L3 produced these four amino acids, L2 produced the highest concentration of phenylalanine17.49 mg/l, however L3 produced a high amount of lysine, ornithine and proline, which were 57.04, 95.58 and 87.06 mg/l, respectively. Lysine is essential amino acids (Adeyemi, 1993), 42.5% of the tested *Lactobacillus* isolates were capable of lysine production (Odunfa et al., 2001). Most of the *Lactobacillus* strains produced, more of the lysine produced are excreted out of the cell than retained within. Although the differences in the *Lactobacillus* intracellular and extracellular yields were not significant over 74% of the examined *Lactobacillus* isolates produced more lysine extracellularly than within the cell (Odunfa et al., 2001). Two *Lactobacillus* strains, showed no levels of lysine retained intracellularly. Majority of the *Lactobacillus* isolates studied (70%) produced lysine at between 50 to 149 mg/ l (Odunfa et al., 2001).

Tyrosine was produced by three strains L1, L2 and L3, these strains produced 132.53, 244.91, 212.56 mg/l, respectively (Figure 4). Tyrosine and phenylalanine catabolism were investigated by using cheese flavor adjuncts of *L. casei* and *L. helveticus* under simulated Cheddar cheese-ripening (pH 5.2, 4% NaCl, 15°C, no sugar) conditions (Gummalla and Broadbent, 2001) . Tyrosine and phenylalanine catabolism was initiated by these strains by an aminotransferase is consistent with previous data for tryptophane catabolism and with other reports of aromatic amino acid catabolism in dairy lactic acid bacteria (Gao et al., 1997; Groot et al., 1998; Gummalla and Broadbent, 1999; Hemme et al., 1982).

Figures 5 and 6 show the different amounts of arginine, isoleucine, leucine, methionine, serine, threonine, tryptophane and valine produced by some of the lactobacilli strains. *Lactobacillus* strains L2 and L3 produced the most of these amino acids with varied amounts. The higher yield of isoleucine, leucine and serine were 2.2, 8.67 mg/l, which were produced by L2, respectively, however 7.59 mg/l serine was produced by L8. Methionine is essential amino acids (Adeyemi, 1993). Odunfa et al. (2001) reported that 25.0% of the *Lactobacillus* isolates produced methionine. They found that the highest yield was 16.1 mg/ l methionine produced by tested strains. They also found that the intracellular methionine yields of the *Lactobacillus* isolates are significantly ($P < 0.01$) higher than the extracellular yields. Most of the *Lactobacillus* cultures retain the majority of their methionine yield within the cell (Odunfa et al., 2001). Agrinine, threonine, tryptophane and valine were produced by two strains, the amounts were ranged between

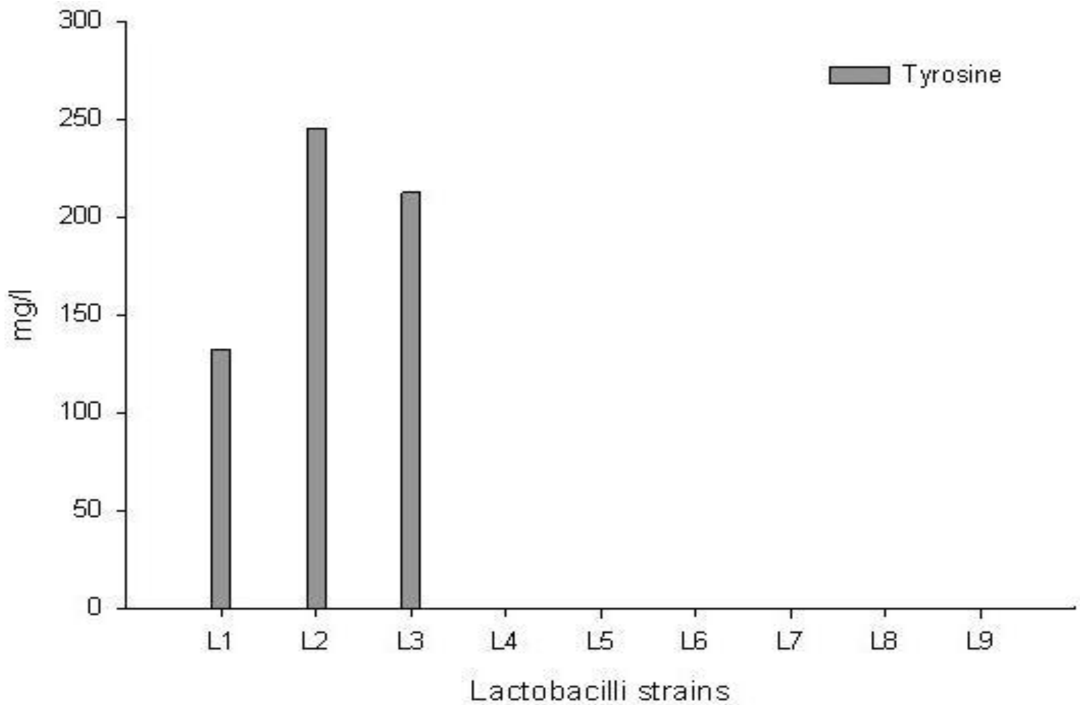

Figure 4. Production of tyrosine by *Lactobacillus* strains.

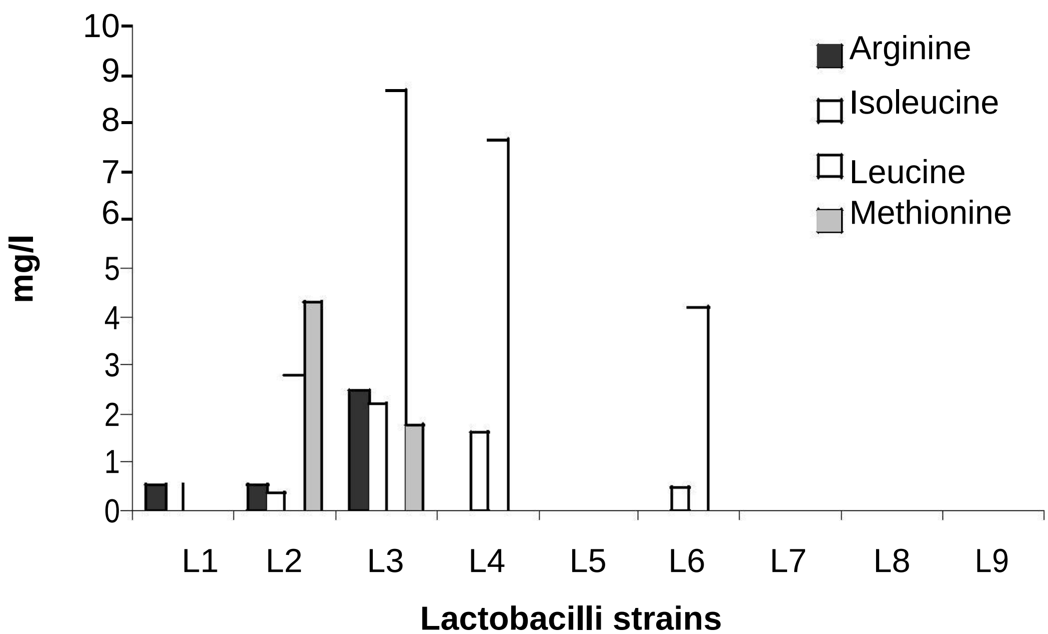

Figure 5. Production of arginine, isoleucine, leucine and methionine by *Lactobacillus* strains.

0.47 to 7.89 mg/l of threonine, and however the other amino acids were produced with amounts of 0.54 to 2.45 mg/l. Bacteria synthesize all of the 20 amino acids necessary for protein biosynthesis, in general, biosynthesis of the 20 amino acids is carried out through six independent routes, with different initial precursors.

There are six families consisting of different amino acids, as follows: 1) alanine family (alanine, valine and leucine); 2) serine family (serine and cysteine); 3) aspartate family (aspartic acid, asparagine, methionine, threonine, isoleucine and lysine); 4) glutamate family (glutamine, arginine and proline); 5) aromatic amino acid family

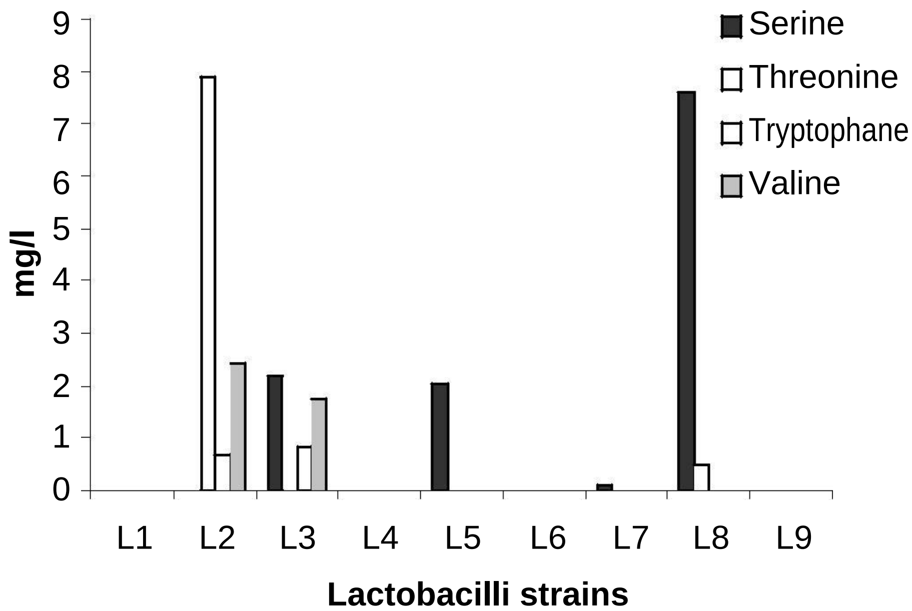

Figure 6. Production of serine, threonine, tryptophane and valine by *Lactobacillus* strains.

(tryptophan, phenylalanine and tyrosine); and 6) histidine. If all amino acid members belonging to a family above are produced (or utilized) simultaneously in the course of cultivation, it is reasonable to assume that the synthesis of the amino acids is regulated by the family-specific biosynthetic route (Kibeom et al., 2001).

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