Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Utilization of amino acids and dipeptides by *Lactobacillus plantarum* from orange in nutritionally stressed conditions

F.M. Saguir[1,*], I.E. Loto Campos[1] and M.C. Manca de Nadra[1,2,*]

1 Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina
2 Cerela, Tucumán, Argentina

Keywords
amino acids and peptides, *Lactobacillus plantarum*, metabolism, nutrition.

Correspondence
Dr Maria C. Manca de Nadra, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán and Cerela, 4000, Tucumán, Argentina.
E-mail: mcmanca@fbqf.unt.edu.ar

*Career Investigator of CONICET – Argentina

2007/0376: received 9 March 2007, revised 5 September 2007 and accepted 1 November 2007

doi:10.1111/j.1365-2672.2007.03708.x

Abstract

Aims: To investigate amino acid and dipeptide utilization by *Lactobacillus plantarum* N4 isolated from orange peel, in a nutritionally depleted medium based on MRS (Mann, Rogosa, Sharpe).

Methods and Results: In MRS with 0·1 g l^{-1} of meat extract and without peptone and yeast extract, growth increased when essential and stimulatory amino acids and nonessential amino acid were added to the medium. Replacement of the essential amino acid, leucine, and the nonessential amino acid, glycine, by leucyl-leucine (Leu-Leu) and/or glycyl-glycine (Gly-Gly) significantly enhanced growth. Essential amino acids were mainly consumed and the dipeptides were almost completely used at the end of growth. Leucine and glycine accumulated internally from the peptides were higher than from the free amino acids. Glucose utilization increased in the media containing dipeptides compared with the medium containing free amino acids.

Conclusions: In a N-depleted medium, Leu-Leu and/or Gly-Gly were more effective than the respective amino acids in supporting growth of the microorganism. The more efficient internal accumulation of glycine and especially leucine from dipeptides confirmed the ability of the strain to assimilate mainly complex nitrogen molecules rather than simple ones.

Significance and Impact of the Study: The ability of *Lact. plantarum* N4 to efficiently use dipeptides could contribute to spoilage development in the natural medium of the organism, orange juice.

Introduction

Lactic acid bacteria (LAB) have been implicated in the spoilage of soft drinks, fruit juices and related products. Tajchakavit *et al.* (1998) reported that *Lactobacillus* and *Leuconostoc* spp., can multiply in apple juice producing an undesirable buttermilk flavour because of diacetyl production, a fermented flavour because of organic acid production and swelling of packages because of CO_2 production. The rate of growth of these bacteria is dependent on their ability to utilize the substrates available in the medium. LAB generally have complex nutritional requirements for growth especially with regard to nitrogen sources (Amoroso *et al.* 1993; Elli *et al.* 1999; Saguir and Manca de Nadra 2002; Hébert *et al.* 2004). Saguir and Manca de Nadra (2007) demonstrated that *Lact. plantarum* N4 and N8 from oranges had absolute requirements for cysteine, glutamic acid, isoleucine, leucine, threonine and valine for growth: strain N4 also required lysine and tryptophan. These authors also described an improved synthetic medium for conducting metabolic and genetic studies in *Lact. plantarum* where cell densities of 10^9 CFU (colony forming units) ml^{-1} were achieved.

Utilization of amino acids is necessary for a number of physiological roles such as protein synthesis, intracellular pH control, generation of metabolic energy or redox potential and resistance to stress (Fernadez and Zúñiga 2006). Arena *et al.* (1996) reported that strains of *Lact. plantarum* grew in natural and centrifuged orange juice. However, orange juice is generally low in free

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology

amino acids. So, conversion of peptides to free amino acids and their subsequent utilization may have considerable nutritional value for bacterial development. Foucaud *et al.* (2001) studied the utilization of peptides as a source of essential amino acids for 12 LAB of dairy origin with multiple amino acid requirements and a large set of intracellular peptidases. They demonstrated that the nutritional value of peptides varied with the strain and peptide composition, and that *Lactococcus lactis* had an advantage over *Leuc. mesenteroides*. Aredes Fernandez *et al.* (2003) showed that dipetides can serve as sources of essential amino acids for *Pediococcus pentosaceus*. More recently, Aredes Fernandez and Manca de Nadra (2006) reported that in a mixed culture of a proteolytic *Oenococcus oeni* strain and a nonproteolytic *Ped. pentosaceus* strain from wines, the proteolytic system of the heterofermentative micro-organism increased the release of peptides and amino acids into the complex medium enhancing the growth of both bacteria. In milk fermentations, the proteolytic system of LAB plays a key role because it enables these bacteria to grow in milk, thereby ensuring successful fermentation. Proteolysis of casein is initiated by a cell-envelope proteinase that degrades the protein into oligopeptides that are subsequently taken up by the cells via specific peptide transport systems and are further degradaded into shorter peptides and amino acids by the concerted action of various intracellular peptidases. The proteolytic system of *L. lactis* has been investigated to the extent that a complete model for casein breakdown, transport and degradation of casein-derived peptides, and regulation has been established (Savijoki *et al.* 2006).

MRS medium (De Man *et al.* 1960) is commonly used to study and maintain LAB. It is a rich medium with a mixture of different carbon sources (acetate, citrate, carbohydrates) and complex nitrogen sources. This complexity may interfere with the interpretation of physiological data related to the nitrogen requirements of these bacteria. So, the first aim of the present study was to characterize growth on progressively simplified MRS. In addition, utilization of free amino acids and dipeptides as sources of nonessential and essential amino acids for *Lact. plantarum* N4 in a N-depleted medium, based on MRS, was determined.

Materials and methods

Bacterial strain

Lactobacillus plantarum N4 was isolated from orange peel (Arena *et al.* 1999) and was stored at −20°C in MRS (De Man *et al.* 1960) supplemented with glycerol (30%, v/v).

Media, growth conditions and culture procedures

Different media were prepared by modifying the complex nitrogen and carbon sources of MRS. MRS without peptone and yeast extract was considered as the basal medium (BM). A modified BM containing 0·1 g l^{-1} rather than 10 g l^{-1} of meat extract, called deficient basal medium (DBM), was supplied with the following amino acids and/or dipeptides (Sigma, St Louis, MO, USA) (in mmol l^{-1}): alanine, 2·24; arginine, 1·72; cysteine, 1·65; glutamic acid, 1·02; glycine, 2·59; isoleucine, 1·52, leucine, 2·29; lysine, 2·05; phenylalanine, 1·21; threonine, 1·68; tryptophan 0·98; valine, 2·56; glycyl-glycine (Gly-Gly), 1·10 and/or leucyl-leucine (Leu-Leu) 1·10 as replacement for glycine and/or leucine, respectively, in the original amino acid mixture. All media were adjusted to pH 6·5 with 1N NaOH before sterilization at 120°C for 15 min. Amino acids and peptides sterilized by filtration through a nylon membrane (0·22 μm pore size, Millipore, Bedford, MA) were added to the sterilized media.

For the final culture in the experimental media, cells grown in MRS pH 6·5, incubated without agitation at 30°C were harvested at the end of exponential growth (8 h) by centrifugation, washed twice with sterile distilled water to avoid carry-over of essential nutrients and resuspended in sterile distilled water to an optical density $(OD)_{620\ nm}$ of 0·90. This cell suspension was used to inoculate the experimental media at a rate of 2% (v/v). All cultures were incubated statically at 30°C for 72 h.

Growth measurement

Bacterial growth was monitored at 620 nm (Spectronic Genesys 5; Milton Roy Company, Rochester, NY, USA). Cell cultures were diluted with sterile medium prior to measuring OD to maintain linearity between OD and biomass when necessary. At the same time, the CFU were determined (CFU ml^{-1}). From these data it was possible to calculate the average of growth rates. The growth rate (μ) was calculated for each replicate during exponential growth by the formula: $\mu = (1/t)\ [(\log_{10} N_t/N_0)\ 2{\cdot}303]$ where t is the time required for cells to increase from N_0 to N_t. Supernatants were collected by centrifugation at 5000 ***g*** for 10 min and then stored at −20°C for subsequent chemical analysis.

Analytical methods

Dipeptides and amino acids were analysed by reverse-phase HPLC (RP-HPLC) using an ISCO liquid chromatograph (ISCO, Lincoln, NE, USA). Samples were submitted to a precolumn derivatization with *o*-phthaldiladehyde (OPA). The reagent for derivatization consisted of

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology

200 mg OPA in 9 ml methanol, 1 ml 0·4 mol l^{-1} sodium borate pH 10 and 160 μl 2-mecaptoethanol. Solvents used for separation were: solvent A: methanol, 10 mmol l^{-1} sodium phosphate buffer, pH 7·3 and tetrahydrofuran (19 : 80 : 1) and solvent B: methanol and 10 mmol l^{-1} sodium phosphate buffer, pH 7·3 (80 : 20). Solvent gradient conditions were as follows: 6 min (0 B); 10 min (15% B); 4 min (30% B); 12 min (40% B); 16 min (80% B) and 5 min (0 B). All separations were performed on a Waters Nova-Pack C18 column (150 × 3·9 mm i.d., 60 Å, 4 μm) at a flow rate of 1·0 ml min^{-1} (Waters Corp., Milford, MA). The detection was by fluorescence using a model 121 fluorimeter (340 nm excitation filter and 425 nm emission filter). Samples were injected in triplicate onto the column, after being filtered through a 0·22 μm filter. Prior to RP-HPLC analysis, all samples were diluted with 0·4 mol l^{-1} borate buffer, pH 10. Standards of amino acids and dipeptides were used to determine the concentration of free amino acids and Gly-Gly or Leu-Leu respectively. The standard solutions were prepared by dissolving each amino acid or dipeptide in a 0·1 N HCl at a concentration of 2·5 μmol ml^{-1}. They were stored at −18°C. Aliquots of 50, 100, 200 and 500 μl of these solutions were adjusted to 25 ml with 0·4 mol l^{-1} borate buffer, pH 10. Precolumn derivatization and the column were at room temperature. D-glucose was measured by the glucose oxidase method (Wiener Laboratory, Rosario, Argentina).

Statistical analysis

To validate the methods, Student's *t*-test was used. Three replicate determinations were carried out.

Results

Growth of *Lactobacillus plantarum* N4 in modified MRS

Table 1 shows the maximum growth rates (μ_{max}) and cell densities of *Lact. plantarum* N4 in MRS when different carbon and nitrogen sources were progressively omitted or modified. In MRS, the micro-organism had a growth rate of 0·63 h^{-1} and reached a final biomass of $3{\cdot}5 \times 10^9$ CFU ml^{-1} at 8 h of incubation. Omission of yeast extract from MRS decreased the growth rate and final cell concentration by 17·5% and 7%, respectively, whilst the individual omission of peptone did not produce any significant change in these parameters. When yeast extract and peptone were simultaneously omitted (BM), the growth parameters decreased in a similar way as in the medium without yeast extract, indicating that the inhibitory effect on growth was mainly because of the absence of yeast extract rather than peptone. In BM, reduction of meat extract from 10 to 5 g l^{-1} decreased the growth rate, although no significant change was observed in the final population. Further reductions in the meat extract concentration (from 10 to 1 or 0·1 g l^{-1}) significantly decreased both growth parameters. In BM containing 0·1 g l^{-1} of meat extract (DBM), the micro-organism had a significantly reduced growth rate and final cell density 1 log cycle lower than in the BM. DBM was used to study the effect of amino acids and peptides under limited growth conditions.

Table 1 Growth of *Lactobacillus plantarum* N4 in modified MRS media

Culture medium	μ (h^{-1})	Log CFU ml^{-1}
MRS	0·63 ± 0·03	9·50 ± 0·45
MRS without yeast extract	0·52 ± 0·02	8·88 ± 0·31
MRS without peptone	0·60 ± 0·02	9·41 ± 0·38
MRS without yeast extract and peptone (BM)*	0·50 ± 0·02	8·83 ± 0·44
BM containing 5·0 g l^{-1} of meat extract	0·37 ± 0·02	8·48 ± 0·43
BM containing 1·0 g l^{-1} of meat extract	0·23 ± 0·01	7·94 ± 0·40
BM containing 0·1 g l^{-1} of meat extract	0·07 ± 0·02	7·71 ± 0·37

Values are the means of three independent experiments ±SD.
CFU, colony forming units.
*This medium was considered as basal medium (BM) and had an initial concentration of 10 g l^{-1} of meat extract.

Effect of amino acids and peptides

In the DBM, no significant effect was observed when the essential amino acid leucine and/or the nonessential amino acid glycine were added separately or together to DBM (data not shown). Addition of the eight essential amino acids for *Lact. plantarum* N4 (glutamate, cysteine, isoleucine, leucine, lysine, valine, threonine and tryptophan), the three stimulatory amino acids, alanine, arginine and phenylalanine, and the nonessential amino acid, glycine, (Saguir and Manca de Nadra 2007) to DBM displayed a positive impact on bacterial growth. The growth rate increased from 0·07 to 0·11 h^{-1} and final cell numbers from 7·7 to 8·1 log CFU ml^{-1} (Fig. 1). In DBM supplemented with the amino acid solution, the effect of Leu-Leu and/or Gly-Gly in place of the corresponding free amino acids on bacterial growth was investigated. As shown in Fig. 2, addition of Leu-Leu and/or Gly-Gly increased both growth parameters. The stimulatory effect was more pronounced for the dipeptide supplying leucine (an essential amino acid) than for the dipeptide supplying glycine (a nonessential amino acid). Moreover, when Leu-Leu was added in place of free leucine, the

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology

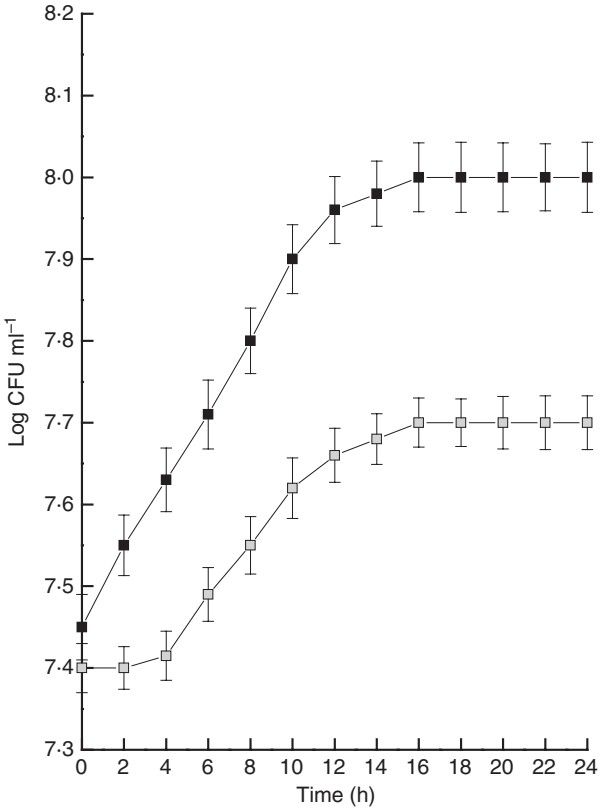

Figure 1 Effect of amino acid mixture on the growth of *Lactobacillus plantarum* N4 in deficient basal medium. (□) Without amino acid mixture; (■) with amino acid mixture.

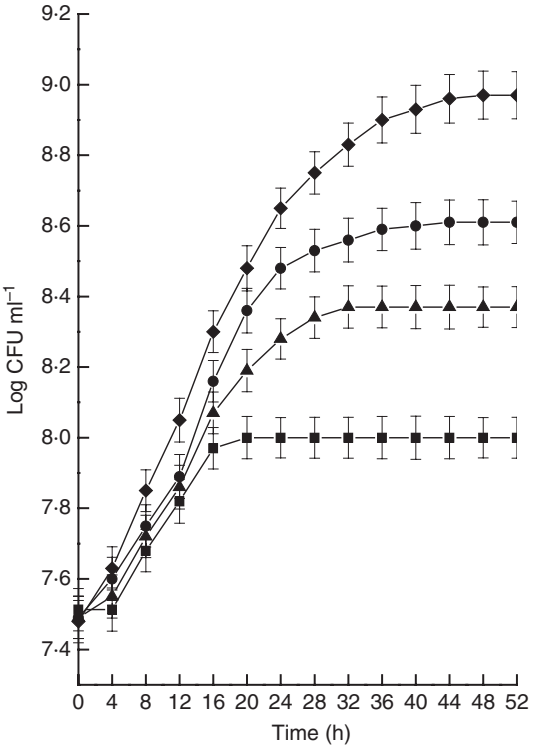

Figure 2 Effect of dipeptides as sources of the respective amino acids on the growth of *Lactobacillus plantarum* N4 in deficient basal medium supplemented with the amino acid mixture. (■) Without dipeptides; (▲) with Gly-Gly in place of glycine; (●) with Leu-Leu in place of leucine; (◆) with Leu-Leu + Gly-Gly in place of the respective amino acids.

maximum growth rate increased by 97% and the number of cells doubled. When both dipeptides were simultaneously incorporated into the medium instead of the respective free amino acids, maximum bacterial growth was raised at 48 h of incubation, after which growth ceased. In this circunstance, the extent of growth was enhanced up to 150% in relation to that obtained in the same medium supplemented with the amino acid mixture.

Utilization of amino acids

Figure 3 shows amino acids utilization by *Lact. plantarum* N4 in DBM containing the amino acid mixture (control medium) and in the same medium with Leu-Leu or Leu-Leu and Gly-Gly as sources of the respective free amino acids, measured at the end of exponential growth phase (20, 36 and 48 h of incubation for control medium, medium with Leu-Leu instead of leucine and medium with Leu-Leu and Gly-Gly instead of leucine and glycine respectively). The utilization of amino acids began immediately growth began in the different media. The overall amino acid decrease was 10 ± 0·41 mmol l^{-1}, which represented a change of 51%, 46% and 38% relative to the initial concentration in control medium, control medium with Leu-Leu or control medium with Leu-Leu + Gly-Gly respectively. In control medium the following amino acids, in order of decreasing concentration, were mainly consumed after 20 h of incubation: valine (1·47 mmol l^{-1}), lysine (1·31 mmol l^{-1}), alanine (1·14 mmol l^{-1}), threonine (1·08 mmol l^{-1}), cysteine and arginine (1·07 mmol l^{-1}); lowest consumption was found for the aromatic amino acids trytophan (0·14 mmol l^{-1}) and phenylalanine (0·44 mmol l^{-1}). When Leu-Leu replaced free leucine consumption of glycine and tryptophan increased 21% and 9%, respectively, compared with those in the control medium, at the end of exponential growth. With Leu-Leu and Gly-Gly instead of the respective amino acids, a larger decrease of residual alanine, arginine, glutamic acid, phenylalanine and tryptophan compared with the control medium occurred. This effect was more pronounced for the aromatic amino acids phenylalanine (35·8%) and tryptophan (60%). When Gly-Gly was used instead of glycine, similar results to those in the control medium were

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology

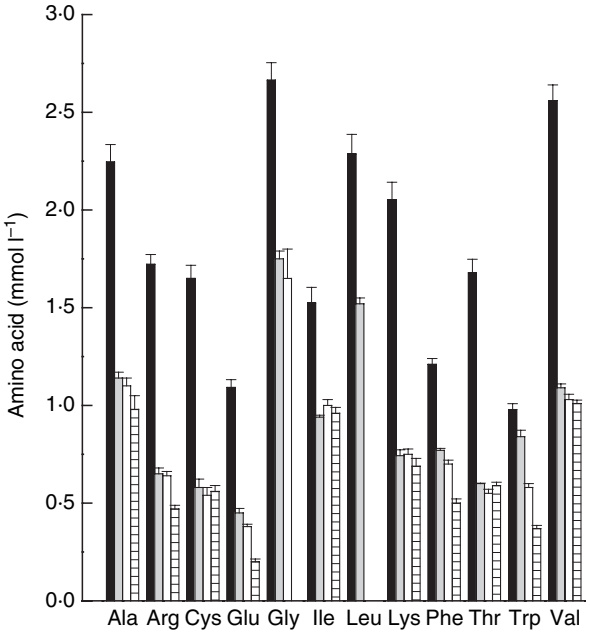

Figure 3 Concentrations of different amino acids in cultures of *Lactobacillus plantarum* N4 at the end of exponential growth phase. (■) Uninoculated medium; (▨) control medium; (□) control medium + Leu-Leu instead of leucine; (▤) control medium + Leu-Leu and Gly-Gly instead of leucine and glycine respectively.

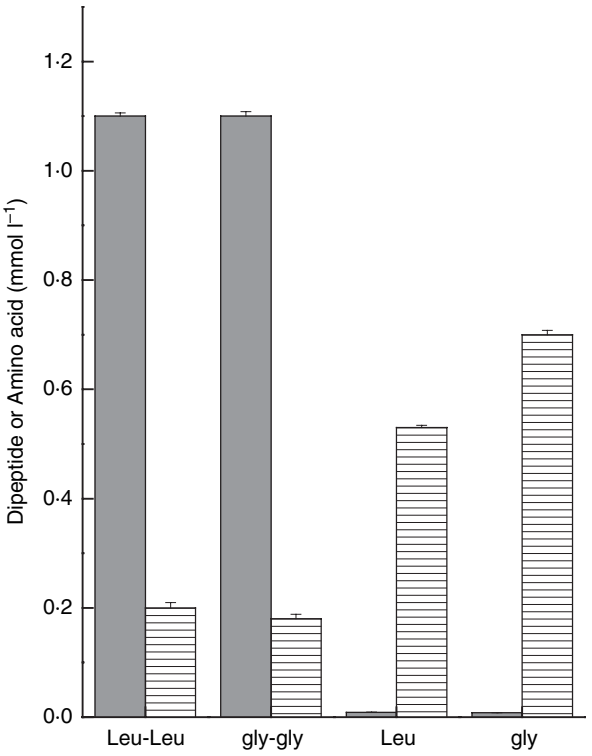

Figure 4 Changes in concentrations of residual dipeptides and the parallel liberation of amino acids in *Lactobacillus plantarum* N4 grown in deficient basal medium with the amino acids mixture and Leu-Leu + Gly-Gly instead of leucine and glycine respectively, at the end of exponential growth. ▨, 0 h; ▤, 48 h.

obtained, except for increased alanine consumption (data not shown).

Utilization of dipeptides

Figure 4 shows the dipeptide consumption at the end of growth of *Lact. plantarum* N4 in DBM containing the amino acid mixture and Leu-Leu and Gly-Gly instead of leucine and glycine respectively. The dipeptide concentration decreased significantly and, after 48 h of incubation represented approx. 91% of the initial levels of Leu-Leu and Gly-Gly. While the dipeptides decreased, the concentrations of the hydrolysis products in the cell-free medium increased by 0·53 mmol l^{-1} for leucine and 0·70 mmol l^{-1} for glycine. The fraction of leucine and glycine accumulated internally from dipeptides corresponded to 1·27 and 1·14 mmol l^{-1} respectively. In the control medium, leucine and glycine consumption were 65% and 25% lower, respectively, than the fraction accumulated internally from the corresponding dipeptides. Moreover, considering the leucine and glycine internalized from the dipetides, overall consumption of amino acids increased from 10·5 mmol l^{-1} in control medium to 13 mmol l^{-1} in the same medium supplemented with the dipeptides. Similar results were obtained when Leu-Leu or Gly-Gly utilization were individually analysed (data not shown).

Effect of peptides on glucose utilization

Lactobacillus plantarum N4 consumed 20% of the initial glucose concentration at the end of exponential growth in DBM containing the amino acid mixture. When Gly-Gly, Leu-Leu or both dipeptides replaced the respective free amino acids in this medium, sugar utilization increased from 4 to 6·2, 7·2 and 8·5 g l^{-1} respectively, coinciding with the increased final biomass obtained in the presence of the one or both dipeptides (Fig. 5).

Discussion

Complex nitrogen sources such as yeast extract, casein peptone, casitone and tryptone have been shown to stimulate growth of LAB (Mikhlin and Radina 1981; Gaudreau *et al.* 1999; Hébert *et al.* 2000). However, these sources differ greatly in their proportion of free amino acids and peptides. In our study, growth of *Lact. plantarum* N4 decreased in the medium in which yeast extract rather than peptone was omitted (Table 1). This could be attributed to the limitation or exhaustion of some

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology

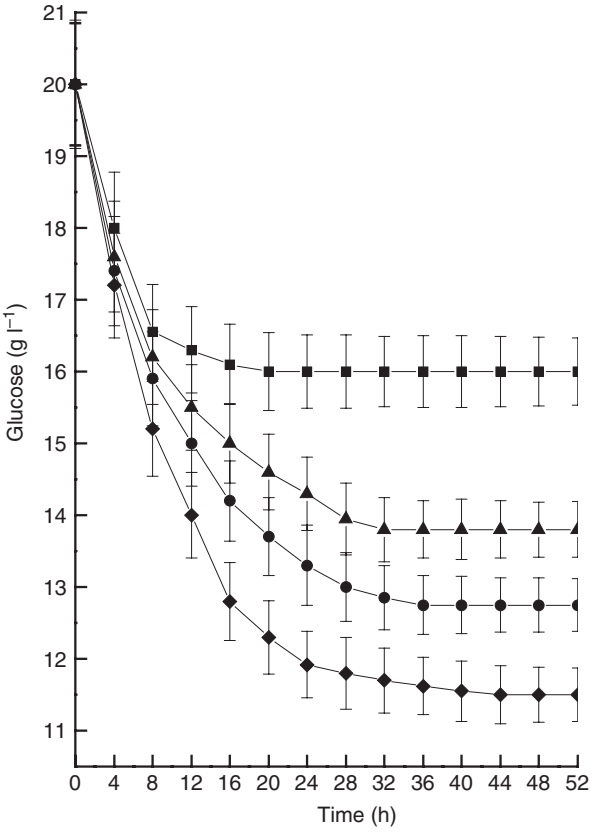

Figure 5 Changes in concentrations of residual glucose in cultures of *Lactobacillus plantarum* N4. (■) Deficient basal medium with the amino acid mixture, control medium; (▲) control medium with Gly-Gly in place of glycine; (●) control medium with Leu-Leu in place of leucine; (◆) control medium with Gly-Gly and Leu-Leu in place of the respective amino acids.

peptides or micronutrients such as vitamins and trace elements supplied by the yeast extract. Remize *et al.* (2006) demonstrated that yeast extract stimulated growth of *O. oeni* approx. 4·5-fold. Calderon *et al.* (2001) reported that among the different complex nitrogen sources tested, yeast extract was the best and was used to define a simplified medium containing only minerals and yeast extract for *Lact. fermentum*. The addition of the amino acid mixture to DBM significantly enhanced growth of *Lact. plantarum* N4 confirming that growth was restricted by the limited nitrogen source. In agreement with our results, Loubiere *et al.* (1997) reported that less growth of *L. lactis* occurred in a medium which contained only six amino acids than in a medium containing 18 amino acids. In *Lact. plantarum* N4, replacement of free leucine and/or glycine by Leu-Leu and/or Gly-Gly respectively, in the DBM containing the amino acids mixture, improved growth significantly (Fig. 2). This stimulatory effect affected not only the growth yield but also the growth rate. Thus, dipeptides were more efficient than free amino acids in supporting development of the micro-organism. The beneficial effect of the dipeptides on growth was more pronounced for the essential amino acid containing-dipeptide (leucine) than for that supplying the nonessential amino acid (glycine). Aredes Fernandez *et al.* (2004) demonstrated a more favourable effect of dipeptides containing two essential amino acids compared with the respective free amino acids on the growth rate but not on the final population of *O. oeni*. Remize *et al.* (2006) reported a preference for peptides in four strains of *O. oeni*, as growth yield was higher in the presence of nitrogen from peptides than from free amino acids. *Lactobacillus plantarum* N4 reached maximum cell numbers in the medium with both Leu-Leu and Gly-Gly instead of the respective amino acids (Fig. 2). In contrast, Aredes Fernandez *et al.* (2003) reported that addition of dipeptides in place of the corresponding amino acids decreased the growth of *Ped. pentosaceus* with respect to that obtained with free amino acids.

Lactobacillus plantarum N4 had consumed between 49% and 62% of the initial amino acid level at the end of growth in the different media used, indicating that they were nutritionally required and that the cell was able to transport and use them. However, none of the amino acids were completely utilized, when bacterial growth stopped, indicating that some other compound(s) were lacking in the medium. Similar results were reported by Juillard *et al.* (1995) for *L. lactis* growing in milk. As the greatest amount of amino acid utilization was observed in the presence of both dipeptides (Fig. 3), it can be inferred that there was a relationship between the level of free amino acids utilized and the extent of growth. In addition, exponential growth of *Lact. plantarum* N4 ended at very different times in the three media. This fact could be due to greater buffering capacity of the medium by the dipeptides, which in turn lead to greater cell numbers. The essential amino acids such as valine, lysine, threonine and cysteine were mainly utilized in the different conditions. The stimulatory amino acids, alanine and arginine, were also significantly used. Arena *et al.* (1999) demonstrated that growth of *Lact. plantarum* N4 was improved by arginine, which was degraded to citrulline, ornithine and ammonium producing additional energy. In our study, isoleucine, phenyalanine and tryptophan which accounted for minor initial concentrations in the medium were poorly used by *Lact. plantarum* N4 suggesting that free amino acid consumption appeared to some extent to be linked to its availability in the medium. Similar result was reported by Remize *et al.* (2006). The presence of Leu-Leu and especially both dipeptides (Leu-Leu and Gly-Gly) as sources of the respective amino acids, strongly increased consumption of tryptophan (up to 100%) and phenylalanine (61·4%) by *Lact. plantarum* N4. A similar

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology

effect was observed for arginine, glutamate and alanine utilization, although to a lesser extent (Fig. 3). This may be linked to better incorporation of these amino acids into cell material and biomass formation than the other amino acids.

The high dipeptide utilization by *Lact. plantarum* N4 suggested that a peptide hydrolase system with high activity was present in the cells. Macedo *et al.* (2000) demonstrated that dipeptides consisting of hydrophobic substrates residues (leucine, glycine, methionine and phenylalanine) were more rapidly attacked than those with hydrophilic amino acids residues in *Lact. paracasei*. Dipeptide utilization by *Lact. plantarum* N4 was accompanied by an increase in the leucine and/or glycine efflux to the extracellular environmental (Fig. 4). Such efflux could be used by the N4 strain to exchange for other amino acids outside the cell, favouring growth of the micro-organism under poor nutritional conditions. This may explain the increase in the alanine consumption by *Lact. plantarum* N4 in the presence of Gly-Gly but not when only Leu-Leu replaced leucine in the medium. Aredes Fernandez *et al.* (2004) demonstrated that in *O. oeni*, alanine utilization was 74% higher when Gly-Gly replaced glycine in the medium. Rice *et al.* (1978) reported that in *L. lactis*, alanine, threonine and glycine were capable of exchange with 'pool' glycine.

An interesting finding was that leucine and/or to a lesser extent glycine accumulated internally from the dipeptides was higher than from the free amino acids confirming the main role of dipeptides as nitrogen sources for micro-organism growth. In *Lact. plantarum* N4 growing in presence of dipeptides significant difference in glucose consumption was determined in concordance with the higher biomass production.

In conclusion, dipeptides containing essential or nonessential amino acids were more effective than free amino acids as nitrogen sources in sustaining growth of *Lact. plantarum* N4 under nutritional stress conditions. The more efficient internal accumulation of glycine and especially leucine from the corresponding dipeptides demonstrated the preference of strain N4 for complex nitrogen molecules rather than simple ones. Finally, the ability of *Lact. plantarum* N4 to use dipeptides efficiently could satisfy its nitrogen requirements in spoilage of orange juice, which is naturally low in amino acids.

Acknowledgements

This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), Argentina. The authors thank Mr Martín Lara for his assistance during this research.

References

Amoroso, M.J., Saguir, F.M. and Manca de Nadra, M.C. (1993) Variation of nutritional requirements of *Leuconostoc oenos* by organic acids. *J Int Sci Vigne Vin* **27**, 135–144.

Aredes Fernandez, P.A. and Manca de Nadra, M.C. (2006) Growth response and modifications of organic nitrogen compounds in pure and mixed cultures of lactic acid bacteria from wines. *Curr Microbiol* **52**, 86–91.

Aredes Fernandez, P.A., Saguir, F.M. and Manca de Nadra, M.C. (2003) Effect of amino acids and peptides on growth of *Pediococcus pentosaceus* from wine. *Lat Am Appl Res* **33**, 225–229.

Aredes Fernandez, P.A., Saguir, F.M. and Manca de Nadra, M.C. (2004) Effect of dipeptides on the growth of *Oenococcus oeni* in synthetic medium deprived of amino acids. *Curr Microbiol* **49**, 361–365.

Arena, M.E., Saguir, F.M. and Manca de Nadra, M.C. (1996) Inhibition of growth of *Lactobacillus plantarum* from citrus fruits in the presence of organic acids. *Microbiol–Aliment–Nutr* **14**, 219–226.

Arena, M.E., Saguir, F.M. and Manca de Nadra, M.C. (1999) Arginine dihydrolase pathway in *Lactobacillus plantarum* from orange. *Int J Food Microbiol* **4**, 203–209.

Calderon, M., Loiseau, G. and Guyot, J.P. (2001) Nutritional requirements and simplified cultivation medium to study growth and energetics of a sourdough lactic acid bacterium *Lactobacillus fermentum* Ogi E1 during heterolactic fermentation of starch. *J Appl Microbiol* **90**, 508–516.

De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) A medium for the cultivation of Lactobacilli. *J Appl Bacteriol* **23**, 130–135.

Elli, M., Zink, R., Reniero, R. and Morelli, L. (1999) Growth requirements of *Lactobacillus johnsonii* in skim and UHT milk. *Int Dairy J* **9**, 507–513.

Fernadez, M. and Zúñiga, M. (2006) Amino acid catabolic pathways of lactic acid bacteria. *Crit Rev Microbiol* **32**, 155–183.

Foucaud, C., Hemme, D. and Desmazeaud, M. (2001) Peptide utilization by *Lactococcus lactis* and *Leuconostoc mesenteroides*. *Lett Appl Microbiol* **32**, 20–25.

Gaudreau, H., Champagne, C., Conway, J. and Degré, R. (1999) Effect of ultrafiltration of yeast extracts on their ability to promote lactic acid bacteria growth. *Can J Microbiol* **45**, 891–897.

Hébert, E.M., Raya, R.R. and De Giori, G.S. (2000) Nutritional requirements and nitrogen-dependent regulation of proteinase activity of *Lactobacillus helveticus* CRL 1062. *Appl Environ Microbiol* **66**, 5316–5321.

Hébert, E.M., Raya, R.R. and Savoy de Gioril, G.S. (2004) Nutritional requirements of *Lactobacillus delbrueckii* subsp. *lactis* in a chemically defined medium. *Curr Microbiol* **49**, 341–345.

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology

Juillard, V., Le Bars, D., Kunji, E.R.S., Konings, W.N., Gripon, J.C. and Richard, J. (1995) Oligopeptides are the main source of nitrogen for *Lactococcus lactis* during growth in milk. *Appl Environ Microbiol* **61**, 3024–3030.

Loubiere, P., Cocaign-Bousquet, M., Matos, J., Goma, G. and Lindley, N.D. (1997) Influence of end-products and nutrient limitations on the growth of *Lactococcus lactis* subsp. *lactis*. *J Appl Bacteriol* **82**, 95–100.

Macedo, A.C., Vieira, M., Poças, R. and Malcata, F.X. (2000) Peptide hydrolase system of lactic acid bacteria isolated from Serra da Estrella cheese. *Int Dairy J* **10**, 769–774.

Mikhlin, E.D. and Radina, V.P. (1981) Activation of lactic acid bacteria. *Appl Biochem Microbiol* **17**, 253–267.

Remize, F., Gaudin, A., Kong, Y., Guzzo, J., Alexandre, H., Krieger, S. and Guilloux-Benatier, M. (2006) *Oenococcus oeni* preference for peptides: qualitative and quantitative analysis of nitrogen assimilation. *Arch Microbiol* **185**, 459–469.

Rice, G.H., Stewart, F.H.C., Hillier, A.J. and Jago, J.R. (1978) The uptake of amino acids and peptides by *Streptococcus lactis*. *J Dairy Res* **45**, 93–107.

Saguir, F.M. and Manca de Nadra, M.C. (2002) Effect of L-malic and citric acids metabolism on the essential amino acid requirements for *Oenococcus oeni* growth. *J Appl Microbiol* **93**, 295–301.

Saguir, F.M. and Manca de Nadra, M.C. (2007) Improvement of a chemically defined medium for the sustained growth of *Lactobacillus plantarum*: nutritional requirements. *Curr Microbiol* **54**, 414–418.

Savijoki, K., Ingmer, H. and Varmanen, P. (2006) Proteolytic systems of lactic acid bacteria. Mini-review. *Appl Microbiol Biotechnol* **71**, 394–406.

Tajchakavit, S., Ramaswamy, H.S. and Fustier, P. (1998) Enhanced destruction of spoilage microorganisms in apple juice during continuous flow microwave heating. *Food Res Int* **31**, 713–722.

© 2008 The Authors
Journal compilation © 2008 The Society for Applied Microbiology, Journal of Applied Microbiology