EFFECT OF AMINO ACIDS AND PEPTIDES ON GROWTH OF PEDIOCOCCUS PENTOSACEUS FROM WINE

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***Abstract*-- Lactic acid bacteria are characterized by numerous nutritional requirements. The influence of amino acids and peptides on the growth of *Pediococcus pentosaceus* c1 isolated from argentinean wines was determined. Cells were growth in synthetic media and in the same media added with the following dipeptides: leucine-leucine; leucine-proline; methionine-proline and glycine-glycine. In the complete medium, *P. pentosaceus* c1 reached a final biomass of $1x10^8$ cfu ml^{-1} with a growth rate of 0.17 h^{-1}. When leucine, methionine and glycine were independently removed from complete medium, cell biomass was lower than 90% indicating that these amino acids are essentials to bacterial growth. In absence of proline the parameters of growth were similar to those obtained on control medium, indicating that proline was not essential for bacterial growth. The growth was partially restored in the medium without methionine (80 %), after addition of the dipeptide methionine-proline, and a growth of 90 % was reached by the addition of leucine-proline to the medium without leucine. When leucine-leucine was added to the medium without leucine, 100 % of cell growth was observed and when glycine-glycine was incorporated to the medium without glycine, growth parameters were increased. Dipeptides could supply the essential amino acid requirements of *P. pentosaceus* c1. The lower specific growth rate (20 %) and cell biomass (70 %) obtained when the four dipeptides were added to the medium without leucine, methionine, glycine and proline, suggest that a limited uptake of amino acids by *P. pentosaceus* c1 could be caused by a substantial concentrations of dipeptides.**

***Keywords*-- *Pediococcus pentosaceus*, Wine, Amino acids**

I. INTRODUCTION

In the wine industry, lactic acid bacteria including *Oenococcus oeni*, *Pediococcus pentosaceus* and *Lactobacillus hilgardii* are commonly used as starters to carry out malolactic fermentation (MLF) (Davis *et al.*, 1988). Nevertheless, despite the use of starter cultures, MLF very often remains difficult to induce due to the cumulative inhibitory effect of low pH, high alcohol and SO_2 content (Wibowo *et al.*, 1988; Britz and Tracey, 1990). These difficulties are also thought to have, as their origin, the nutrient composition of wines. The free amino acid content of wines could be of great significance. The wine lactic acid bacteria have numerous nutritional requirements for growth, especially nitrogen sources. The majority of amino acids are either stimulatory or essential for their growth (Garvie, 1967; Tracey and Britz, 1989; Fourcassie *et al.*, 1992; Amoroso *et al.*, 1993). The conversion of peptides to free amino acids and its subsequent utilization by lactic acid bacteria could satisfy their amino acid requirements. The aim of this work is to analyze the effect of free amino acids and peptides during growth of *P. pentosaceus* in a chemically defined medium to evaluate their respective roles in growth of this microorganism.

II. MATERIALS AND METHODS

A. Organism and inoculum preparation

Pediococcus pentosaceus c1 was isolated from Argentinian red wine (Manca de Nadra and Strasser de Saad, 1987; Strasser de Saad and Manca de Nadra, 1987). The cells were cultivated in MRS broth (De Man *et al*, 1960), which contained (g l^{-1}): proteose peptone, 10; beef extract, 10; yeast extract, 5; dextrose, 20; sorbitan mono-oleate complex, 1; ammonium citrate, 1; magnesium sulphate, 0.1; manganese sulphate, 0.05; di-sodium hydrogen phosphate, 2 and 150 ml tomato juice, in distilled water, pH 6.5 at 30°C. At the end of the exponential growth phase, the cells were harvested and inoculated in an adaptation medium. After 24 h incubation, the cells of the last transfer were harvested by centrifugation, washed twice with sterile distilled water to avoid carry-over nutrients and resuspended in sterile distilled water (OD_{620} = 0.90). This bacterial suspension was inoculated into the synthetic medium or modified synthetic media at the rate of 4%.

B. Media and growth conditions

Tucumán synthetic medium (Ledesma *et al.*, 1977) was used as basal medium with the following composition (in g l^{-1}): D-glucose, 10; potassium acetate, 10; potassium dihydrogen orthophosphate, 2; sodium thioglycollate, 0.5; magnesium sulphate.$7H_2O$, 0.15; manganese sulphate.$4H_2O$, 0.02; ferrous

sulphate.$7H_2O$, 0.01; tween 80, 1.1 and (in mg l^{-1}): adenine, 50, cytilyc acid, 50; deoxyguanosine, 50; guanine-HCl, 50; thymidine, 50; uracil, 50; p-aminobenzoic acid, 0.01; vitamin B_{12}, 0.01; calcium pantothenate,1; D-biotin, 0.01; folic acid, 0.1; niacin, 1; piridoxal ethyl acetal HCl, 0.5; riboflavin, 0.5; thiamine HCl, 1. Amino acid concentrations are given in Table 1. To adapt cells from complex medium to synthetic medium, the cells were precultivated in adaptation medium where the amino acids source was substituted by tryptone (4 g l^{-1}). Different synthetic media were prepared by modifying the composition of nitrogen source of the basal medium as mentioned in the text. The modified basal medium with dipeptides contained (in mmol l^{-1}): Gly-Gly, 2.2; Gly-Ala, 4.4 or 2.2; Leu-Leu, 0.2; Leu-Pro, 0.45 or 0.34; Pro-Asp, 0.34 or 1.5 and Met-Pro, 0.34 as a replacement for Gly, Ala, Leu, Pro, Asp and Met respectively in the original amino acid mixture. All media were adjusted to pH 6.5 with 1N NaOH before sterilization. The adaptation and synthetic media were sterilized in autoclave, with heating stopped immediately on reaching 121ºC. Cysteine-HCl and peptides sterilized by filtration were added to the sterilized media. All cultures were incubated at 30ºC for 5 days.

C. Growth measurement

Bacterial growth was monitored by periodic measurement of optical density at 620nm. At the same time, the colony-forming units (cfu ml^{-1}) were determined by plating 0.1 ml of inoculated media on appropriate media. Growth of *P. pentosaceus* c1 on peptides was tested in basal medium containing all amino acids except for one amino acid, which was supplied in the form of a dipeptide.

Table 1: Amino acids in the basal medium

Compound	Concentration (g l^{-1})
L-Glutamic acid	0.15
D,L-Alanine	0.20
L-Arginine	0.005
L-Asparagine	0.90
L-Cysteine-HCl	0.20
L-Phenylalanine	0.04
L-Histidine-HCl	0.05
L-Isoleucine	0.05
L-Leucine	0.06
L-Lysine	0.05
L-Methionine	0.05
L-Proline	0.04
L-Serine	0.10
L-Tyrosine	0.004
L-Threonine	0.05
L-Tryptophan	0.05
L-Valine	0.03
L-Glycine	0.3

III. RESULTS

A. Free amino acids effect on *Pediococcus pentosaceus* c1 growth.

In the complete synthetic medium *P. pentosaceus* c1 reached a cell density of $1x10^8$ cfu ml^{-1} at 48 h of incubation at 30ºC with a growth rate of 0.17 h^{-1}. An important lag phase was observed (12 h) before growth began (Fig. 1).

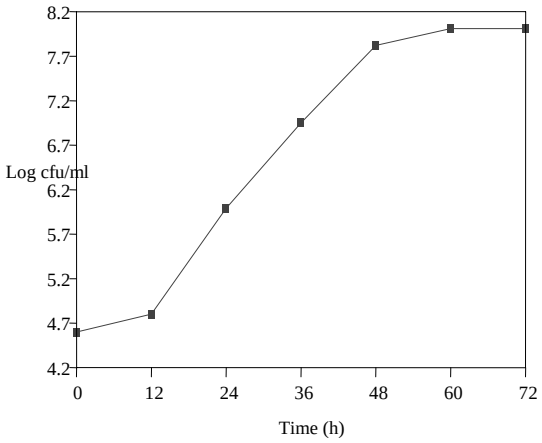

Fig. 1. Growth of *P. pentosaceus* c1 in basal medium.

The amino acids effect on growth rate and lag time of *P. pentosaceus* c1 in Tucumán synthetic medium was determined by omitting each amino acid in turn (Table 2).

In absence of arginine, cysteine, glutamate, glycine, hystidine, isoleucine, leucine, methionine, phenylalanine, threonine, triptophane, tyrosine or valine no growth was observed.

Removal of proline from the medium did not affect growth rate but lag phase was 12 h longer than that observed in basal medium. Omission of asparagine, aspartate, lysine or alanine from basal medium diminished growth rate to 0.14, 0.14, 0.12 and 0.13 h^{-1} respectively, but also resulted in lag times of about 24 h except to serine. Similar results were observed when glutamate or serine were omitted from a simplified synthetic medium for *Lactococcus lactis* subspecie *lactis* NCDO 2118 growth (Cocaing-Bousquet *et al*., 1995). *P. pentosaceus* c1 required 13 amino acids for growth and four of them had stimulatory effect. Serine and proline were not essential for its growth.

These results demonstrate that *P. pentosaceus* c1 requires for growth more amino acids than other lactic acid bacteria from wine as was determined for *Oenococcus oeni* strains by Saguir and Manca de Nadra (1997).

Table 2. The effect of omission of amino acids from basal medium on the growth rate and lag time for *P. pentosaceus* c1.

Amino acid	μ (h^{-1})	Lag time (h)
Ala	0.13	24
Arg	0	-
Asn	0.14	24
Asp	0.14	24
Cys	0	-
Glu	0	-
Gly	0	-
His	0	-
Ile	0	-
Leu	0	
Lys	0.12	24
Met	0	-
Phe	0	-
Pro	0.16	24
Ser	0.15	12
Thr	0	-
Trp	0	-
Tyr	0	-
Val	0	-

Maximum specific growth rate was 0.17 h^{-1} on basal medium
Values are the averages of three experiments

B. Effect of peptides on *Pediococcus pentosaceus* c1 growth

Figure 2 shows that *P. pentosaceus* c1 could satisfy their requirements for essential amino acids by addition of different dipeptides to the media with individual amino acid omission. The final biomass was recovered (100%) when Gly-Ala or Leu-Leu were added to media lacking glycine or leucine respectively. It was higher (50%) than that observed in basal medium when Gly-Gly was incorporated into medium without glycine. In presence of Met-Pro or Leu-Pro as source of methionine or leucine respectively, the final cell concentration was 20% lower as regards to basal medium (Fig. 2A). The growth rates (Fig. 2B) were identical to that obtained in basal medium, except when Gly was added as part of the dipeptide Gly-Gly. In this case, the growth rate increased from 0.17 to 0.23 h^{-1}.

These results suggest the presence of the peptide transport system in *P. pentosaceus* c1, which would facilitate dipeptides transport inside the cell and their hydrolysis to free amino acids by intracellular peptidases. Thus, the microorganism can supply the required essential amino acids for its growth, especially when Gly-Ala, Gly-Gly or Leu-Leu are used as Gly or Leu source. Foucaud *et al.* (1995) reported that *Lactococcus lactis* MG1363 could satisfy its requirements for essential amino acids by utilization of di and tripeptides except for Ile-Arg and Gly-His-Gly.

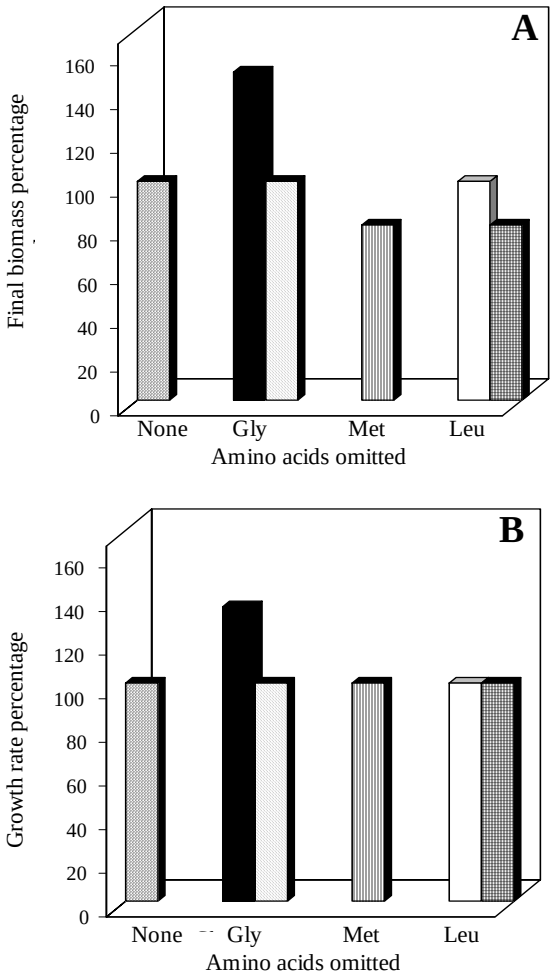

Fig. 2. Final biomass (A) and growth rates (B) of *P. pentosaceus* c1 determined in each medium deficient in one essential amino acid (indicated at the bottom) and added with the following dipeptides: Gly-Gly (■), Gly-Ala (▨), Met-Pro (▯), Leu-Leu (□) and Leu-Pro (▦). Results are expressed as percentages of final biomass and growth rate obtained on complete medium.

Figure 3 shows the results obtained on final biomass and growth rates in the media without a non-essential (proline) or stimulatory amino acids (aspartate or alanine) when supplemented with Leu-Pro, Met-Pro, Pro-Asp or Gly-Ala. No significant differences in the final cell concentrations were observed by dipeptides addition to the media deficient in one non-essential amino acid with respect to basal medium. On the other hand, when Pro-Asp was incorporated as Asp source, the growth rate increased 60% (Fig. 3B).

Figure 4 shows the final cell concentration when the medium without glycine, methionine, leucine and proline was added with Met-Pro, Leu-Pro, Leu-Leu and Gly-Gly.

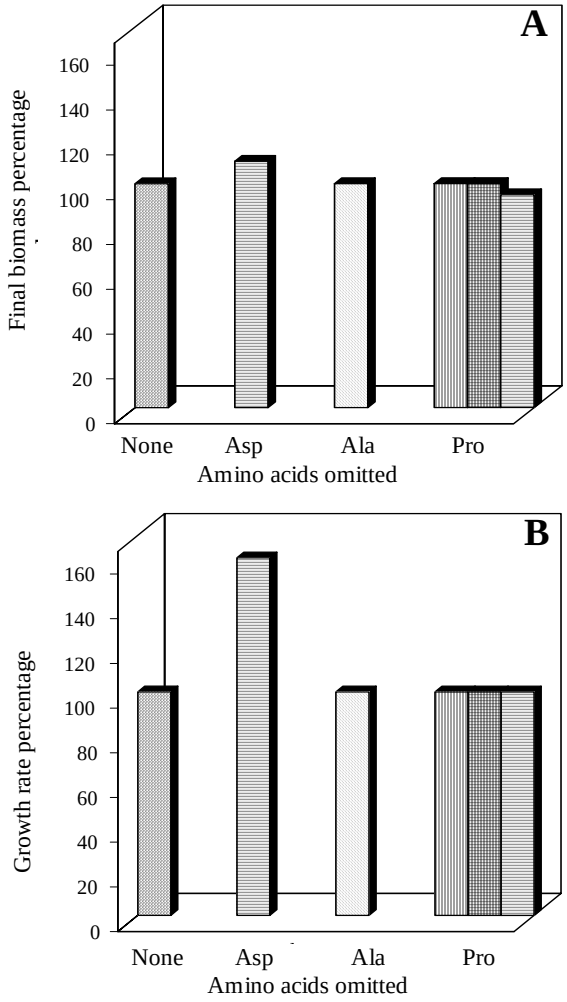

Fig. 3. Final biomass (A) and growth rates (B) of *P. pentosaceus* c1 determined in each medium deficient in one non essential amino acid (indicated at the bottom) and added with the following dipeptides: Pro-Asp ( ), Gly-Ala ( ), Met-Pro ( ) and Leu-Pro ( ). Results are expressed as percentages of final biomass and growth rate obtained on complete medium.

The final cell concentration in the medium with the four dipeptides achieved 70% respect to the cell concentration in basal medium, with a diminution in the growth rate (0.135 h^{-1}). Perhaps a limited uptake of amino acids by *P. pentosaceus* c1 could be caused by a substantial concentration of peptides.

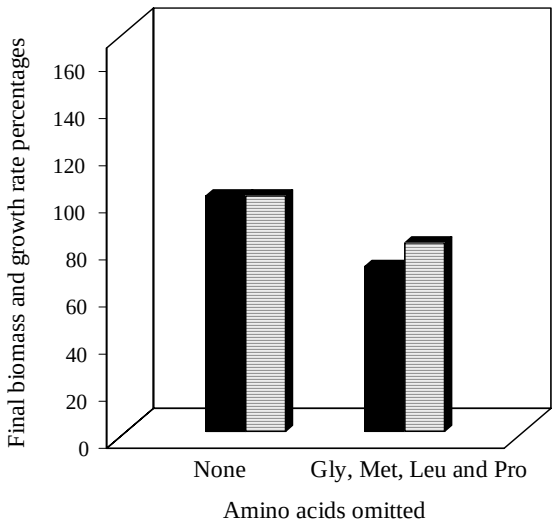

Fig. 4. Final cell concentrations ( ) and growth rates ( ) of *P. pentosaceus* c1 determined in medium deficient in four amino acid (indicated at the bottom) and added with the following dipeptides: Gly-Gly, Met-Pro, Leu-Leu, and Leu-Pro. Results are expressed as percentages of final biomass and growth rate obtained on complete medium.

IV. CONCLUSIONS

A chemically defined medium was used to study the amino acid requirements and the contribution of the dipeptides as source of amino acids for *P. pentosaceus* c1 growth. Arginine, cysteine, glutamate, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, triptophane, tyrosine and valine were essential; alanine, aspartate, asparagine and lysine were stimulatory and serine and proline were non-essential for microorganism growth.

*P. pentosaceus c*1 could satisfy the essential amino acid requirements in presence of dipeptides constituted by the required amino acids. Furthermore, in the presence of Gly-Gly as source of glycine the growth occurs with a final biomass and growth rate higher than in media containing the free amino acid. When the dipeptide Pro-Asp was added as Asp source, a stimulatory effect was also observed on *P. pentosaceus* c1 growth rate. In these cases, if the energy requirement for the uptake of one dipeptide is the same as that for the uptake of one amino acid, less energy has to be spent by the microorganism to supply its amino acid requirement.

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