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Nutritional requirements and amino acids utilization by lactic acid bacteria from wine -A short review

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Abstract
It is well known that lactic acid bacteria need multiple nutritional requirements for growth. They require numerous amino acids and other growth factors to develop in synthetic media. In this review the amino acid requirements, the effect of L-malic acid and citric acids metabolism on these requirements and the catabolism of amino acids by lactic acid bacteria from wine are discussed, with emphasis on arginine and its amino acid derivates: citrulline and ornithine.

Key words: Lactic acid bacteria, wine, amino acids, metabolism, nutritional requirements.

Introduction

The conversion of L-malic acid into L-lactic acid and carbon dioxide by lactic acid bacteria (LAB) reduces the acidity of wines conveniently, improves their organoleptic characteristics and makes them microbiologically more stable. This malolactic fermentation (MLF) can be achieved by *Oenococcus oeni* (formerly *Leuconostoc oenos*[1]), *Lactobacillus* spp. and *Pediococcus* spp., of which *O. oeni* is the best adapted to the low pH and high ethanol concentration that characterize wine after alcoholic fermentation[2-4]. LAB have been characterized by their numerous nutritional requirements, especially of nitrogen sources. Garvie[5] has reported that *O. oeni* has an absolute need for arginine, cysteine, glutamic acid, isoleucine, tyrosine and valine, whereas certain strains also required aspartic acid, glycine, histidine, leucine, methionine, phenylalanine, serine, threonine and tryptophan for optimum growth in synthetic media. Thus, difficulties in inducing MLF may also be caused by the nutrient composition in wine, besides the effect of a low pH, high ethanol and SO_2 contents. The free amino acid concentration in wine is limited. Arginine and proline are usually considered as the major components of grape juices and wines[6]. Colagrande et al.[7] have reported the presence of only small amounts (less than 10 mgl^{-1}) of methionine, leucine or isoleucine in Champagne-based wine. Regarding essential amino acids, only arginine and glutamic acid appear to be available at high enough concentrations at the end of alcoholic fermentation[8]. On the other hand, the metabolism of amino acids by LAB has implications for the quality and safety of fermented foods. Amino acid catabolism could play an important role in the ability to obtain energy in nutrient-limited environments. However, the catabolic pathway of many amino acids remains partially undefined in LAB. Although arginine in grape juice is almost entirely metabolized by yeast during vinification, it is still present at the end of alcoholic fermentation. Three pathways for the catabolism of arginine have been described in LAB. Morita et al.[9] have postulated that strains of *Lactobacillus fermentum* are able to produce nitric oxide from arginine. The authors assume that this reaction is catalyzed by nitric oxide synthase with citrulline as a possible co-product. Kuensch et al.[10] have reported on the stoichiometric conversion of arginine into ornithine by different strains of *O. oeni* in reactions linked to the urea cycle. Sponholz[11] has demonstrated the excretion of urea into the medium during arginine catabolism by strains of *O. oeni* and *Lactobacillus brevis* from wine via the arginase-urease pathway:

$$\text{Arginine} + H_2O \xrightarrow{\text{Arginase}} \text{Ornithine} + \text{Urea}$$

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} CO_2 + 2\,NH_3$$

Manca de Nadra et al.[12] have studied the arginine dehydrolase system (ADI) in homo and hetero fermentative LAB. This is an energy generating system and catalyses the hydrolysis of arginine through the action of three enzymes:

$$\text{Arginine} + H_2O \xrightarrow{\text{Arginine deiminase}} \text{Citrulline} + NH_3$$

$$\text{Citrulline} + P_i \xrightleftharpoons{\text{Ornithine transcarbamylase}} \text{Ornithine} + \text{Carbamyl-}P_i$$

$$\text{Carbamyl-}P_i + ADP \xrightleftharpoons{\text{Carbamate kinase}} ATP + NH_3 + CO_2$$

Studies of the presence of ADI pathway enzymes in LAB [12,22] collectively indicated that the pathway typically occurs in heterofermentative lactobacilli, whereas it is a variable trait in *Lactococcus lactis, O. oeni, Lactobacillus helveticus, Lactobacillus jensenii* and *Lactobacillus leichmanii*. Furthermore, Manca de Nadra et al.[12] and Crow and Thomas[16] have reported that numerous LAB have an incomplete ADI pathway. The objective of this review is to evaluate the current knowledge of the amino acid requirements by LAB from wine, predominantly *O. oeni*, the effect of L-malic acid and citric acid on the requirements and amino acid catabolism,

with emphasis on arginine and its derivates: citrulline and ornithine. Reference is made with respect to the amino acid metabolism by LAB isolated from vegetables.

Amino Acid Requirements

The amino acid requirement of four *O. oeni* strains, m, L_2, ST and X_2L, isolated from different Argentine red wines[23, 24], has been examined[25], comparing bacterial growth in a synthetic medium[26] with growth in the same medium deficient in one amino acid. *O. oeni* m required L-asparagine, L-isoleucine, L-cysteine and L-tyrosine for growth; *O. oeni* L_2 needed L-asparagine, L-phenylalanine, L-histidine, L-isoleucine, L-leucine, L-cysteine, L-threonine and L-tryptophan; *O. oeni* ST required L-glutamic acid, DL-alanine, L-asparagine, L-phenylalanine, L-histidine, L-isoleucine, L-leucine, L-cysteine, L-lysine, L-methionine, L-proline, L-serine, L-tyrosine, L-threonine, L-tryptophan, L-valine, glycine, L-aspartic acid and L-arginine and *O. oeni* X_2L L-asparagine, L-phenylalanine, L-histidine and L-methionine. Only L-asparagine was essential for growth of all strains. *O. oeni* ST was the most demanding microorganism since it needed all nineteen amino acids analyzed for growth, whereas the other strains needed only four or eight essential amino acids. Fourcassie et al.[27] studying the effect of eighteen amino acids on growth, D-glucose fermentation and malolactic activity of six strains of *O. oeni*, found that all strains had an absolute requirement for four amino acids and needed six others for optimum growth. Vasserot et al.[28] have reported for *O. oeni* grown in synthetic medium with low L-aspartic acid concentration, that with increasing L-aspartic acid concentration the maximum bacterial growth rate and maximum bacterial biomass production also increased. Saguir and Manca de Nadra[29] have reported that for *O. oeni* m growing in the absence of amino acids belonging to the aspartate family, no growth was observed when the essential amino acids L-asparagine or L-isoleucine had been removed from the basal medium and that in media deficient in methionine or threonine, the growth rate and bacterial growth were both reduced. Aredes Fernández et al.[30] have demonstrated that *Pediococcus pentosaceus* c1, isolated from Argentine wine, required more amino acids for growth than *O. oeni* strains. It required arginine, cysteine, glutamate, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine for growth in synthetic medium. Similarly, Feuillat et al.[31] have stated that *O. oeni* has the least demand for specific nitrogen compounds. Research on amino acid requirements of lactococci from vegetal origins has shown that these microorganisms were phototrophic for all amino acids when using the single omission technique[32]. Saguir and Manca de Nadra[33] have demonstrated that *O. oeni* m was unable to use essential amino acids as sole nitrogen source. The single omission technique is useful to specify nutritional characteristics of microorganisms but inadequate for the formulation of a minimal medium able to support growth.

Amino Acid Metabolism

The general assumption is that biomass synthesis in LAB occurs predominately from building blocks present in the culture medium[34]. However, the dissociation between catabolism of energy substrates (glucose or organic acids) and carbon assimilation from organic nitrogen sources (amino acids) may be less complete under deficient nutritional conditions, with a change in carbon flux between the different types of carbon substrates. L-malic and citric acids are undoubtedly the organic acids that LAB from wine degrade most frequently in their natural habitat. Saguir and Manca de Nadra[35] have reported that these organic acids could avoid both carbon flux and energy limitations. The growth of *O. oeni* m, L_2, ST and X_2L strains was higher in synthetic medium with L-malic acid and/or citric acid[25, 35]. In the presence of L-malic acid plus citric acid, three of the four strains assayed (m, L_2 and ST) reduced their amino acid requirements, suggesting that in terms of nutritional requirements these acids played a beneficial role. *O. oeni* X_2L, however, increased its requirements from four to nine amino acids (DL-alanine, L-asparagine, L-isoleucine, L-leucine, L-lysine, L-tyrosine, L-threonine, L-valine and L-glycine). Moreover, Saguir and Manca de Nadra[33] have demonstrated that both L-malic acid and citric acid allowed growth of *O. oeni* m, when different combinations of essential amino acids such as L-asparagine and L-isoleucine, L-asparagine and L-cysteine or L-isoleucine and L-cysteine were successively omitted from the synthetic medium. Tracey and Britz[36] have found that certain strains of *O. oeni* yielded similar growth in a synthetic medium and in the same medium with L-malic acid of which six amino acids had been omitted. Saguir and Manca de Nadra[29] have demonstrated on *O. oeni* m, that essential amino acids could be synthesized from intermediaries metabolically derived from citric acid under poor nutritional conditions. From the fermentation balance from glucose and citrate catabolism in the media assayed deprived of L-cysteine it could be inferred that glucose metabolism could be involved in the synthesis of L-cysteine. In the absence of L-asparagine or L-isoleucine, the lower production of D-lactic acid from citric acid indicates that part of the citrate could be involved in the biosynthesis of L-asparagine or L-isoleucine, via aspartate. *O. oeni* m was unable to use the dicarboxylic acid as a biosynthetic precursor for the biosynthesis of essential amino acids. L-malic acid was completely recovered as L-lactic acid in all deficient media. However, the biochemical energy gain associated with MLF should be taken into account as an additional advantage for these anabolic reactions. Ramos et al.[37] have demonstrated in *O. oeni* isolated from wine, that part of the citric acid supplied was converted into aspartate, that was not excreted into the extracellular medium. Marty-Teysset et al.[38] have reported that the citrate pathway in *Leuconostoc mesenteroides* subsp. *mesenteroides*, under certain culture conditions, was directed towards the formation of aspartate. Saguir and Manca de Nadra[39] have demonstrated that *O. oeni* m was able to produce L-asparagine in synthetic medium deprived of asparagine but supplemented with a tenfold higher concentration of L-aspartic acid. L-asparagine production increased due to the presence of L-malic acid, citric acid and ammonium sulfate. The stimulatory effect of the ammonium ion suggests that the number of amino acids retained in the medium could not supply the entire nitrogen requirement of the cell to carry out an anabolic reaction. By contrast, Cocaign-Bousquet et al.[40] have observed that growth of two *Lactococcus* strains from vegetable and dairy origin in minimal synthetic medium was not perturbed by the suppression of ammonium. Saguir[41] has demonstrated in *O. oeni* m that the addition of a high L-threonine concentration to a synthetic medium without L-asparagine but with the tenfold higher concentration of L-aspartic acid, stimulated the L-asparagine biosynthesis. This

could be attributed to the control of L-threonine biosynthesis by retro-inhibition processes. In *Lactobacillus plantarum* N4 and N8, isolated from orange[42], Arena et al.[43] have demonstrated that L-arginine was degraded to citrulline, ornithine and ammonia. The 16S rDNA sequence for *L. plantarum* N4 and N8 has been deposited by Arena and Manca de Nadra [44] in GenBank and assigned accession number AY082883 and AY082884, respectively. Growth of L. plantarum N4 was improved by the presence of arginine, citrulline or ornithine while growth of *L. plantarum* N8 was only improved by addition of L-citrulline to the medium. Arena and Manca de Nadra[44] have reported that the stimulatory effect of arginine and/or citrulline on *L. plantarum* N4 and N8 growth was lower in media enriched with tomato juice and that arginine or citrulline utilization was inversely proportional to the initial glucose concentration in tomato juice. Similarly, Arena et al.[45] have reported that glucose and arginine or citrulline were co-metabolized by *Lactobacillus hilgardii* X_1B. In this case the sugar consumption was lower in the presence of these amino acids, whereas consumption of citrulline by *O. oeni* m did not affect the amount of carbohydrate utilized. Mira de Orduña et al.[46] have found that high initial arginine concentrations led to faster degradation of fructose and glucose by *Lactobacillus buchneri* CUC-3 but such stimulatory effect was not observed for *O. oeni* MCW. There are several studies on arginine catabolism in different genera of LAB from wine, mainly in *O. oeni* [11, 47-50]. Arena et al.[45] have reported that growth of *L. hilgardii* X_1B was stimulated in the presence of arginine, citrulline or ornithine. Arginine was converted into citrulline, which was excreted into the medium and its accumulation continued throughout the period of arginine degradation. Citrulline was partially recovered as ornithine. In medium supplemented with ornithine, this amino acid was consumed to some extent and partially recovered as citrulline, suggesting the presence of both a catabolic and an anabolic ornithine transcarbamoylase. Two strains of *O. oeni*, m and X_2L, did not degrade arginine, and only the m strain degraded citrulline, which was completely recovered as ornithine, ammonia and CO_2. The citrulline utilization by this strain may be important for two reasons: the strain can gain extra growth energy from citrulline metabolism and the amino-acid diminution could avoid the possibility of ethyl carbamate (a known animal carcinogen) formation from the citrulline naturally present in wine.

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