Available online at www.sciencedirect.com
ScienceDirect
International Journal of Food Microbiology 121 (2008) 253–261
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Glycerol metabolism and bitterness producing lactic acid bacteria in cidermaking

G. Garai-Ibabe [a,*], I. Ibarburu [a], I. Berregi [a], O. Claisse [b], A. Lonvaud-Funel [b], A. Irastorza [a], M.T. Dueñas [a]

[a] Facultad de Ciencias Químicas, Dpto de Química Aplicada, Paseo Manuel de Lardizabal 3, 20018, San Sebastián, Spain
[b] UMR Oenologie-Ampélologie; INRA; Université Victor Segalen Bordeaux 2, 351, Cours de la Libération, 33405, Talence Cedex, France

Received 21 November 2006; received in revised form 27 September 2007; accepted 2 November 2007

Abstract

Several lactic acid bacteria were isolated from bitter tasting ciders in which glycerol was partially removed. The degradation of glycerol via glycerol dehydratase pathway was found in 22 out of 67 isolates. The confirmation of glycerol degradation by this pathway was twofold: showing their glycerol dehydratase activity and detecting the presence of the corresponding gene by a PCR method. 1,3-propanediol (1,3-PDL) and 3-hydroxypropionic acid (3-HP) were the metabolic end-products of glycerol utilization, and the accumulation of the acrolein precursor 3-hydroxypropionaldehyde (3-HPA) was also detected in most of them. The strain identification by PCR-DGGE *rpoB* showed that *Lactobacillus collinoides* was the predominant species and only 2 belonged to *Lactobacillus diolivorans*. Environmental conditions conducting to 3-HPA accumulation in cidermaking were studied by varying the fructose concentration, pH and incubation temperature in *L. collinoides* 17. This strain failed to grow with glycerol as sole carbon source and the addition of fructose enhanced both growth and glycerol degradation. Regarding end-products of glycerol metabolism, 1,3-PDL was always the main end-product in all environmental conditions assayed, the only exception being the culture with 5.55 mM fructose, where equimolar amounts of 1,3-PDL and 3-HP were found. The 3-HPA was transitorily accumulated in the culture medium under almost all culture conditions, the degradation rate being notably slower at 15 °C. However, no disappearance of 3-HPA was found at pH 3.6, a usual value in cider making. After sugar exhaustion, *L. collinoides* 17 oxidated lactic acid and/or mannitol to obtain energy and these oxidations were accompanied by the removal of the toxic 3-HPA increasing the 1,3-PDL, 3-HP and acetic acid contents.

Keywords: Lactic acid bacteria; Glycerol; Bitterness; 3-Hydroxypropionaldehyde; Cider

1. Introduction

In the Basque Country (Northern Spain), natural ciders are produced in small cider factories using exclusively fresh cider apples and without sugar and CO_2 addition. Cidermaking process starts in October–November and apple juices are obtained from the pressing of numerous varieties of cider apples. Juices are fermented in small tanks (5000 to 10,000 l) to dryness and usual oenological procedures (sulphur dioxide treatment, clarification or correction of the acidity) are not applied. The alcoholic and malolactic fermentation occur spontaneously with indigenous yeasts and lactic acid bacteria of the musts (Del Campo et al., 2003). The content of the main components of apple juices and ciders are showed in Table 1.

After alcoholic and malolactic fermentation, high levels of a fundamentally heterofermentative lactic microbiota is found, with *Lactobacillus* species and *Oenococcus oeni* being the most abundant (Dueñas et al., 1994). Since natural ciders are not microbiologically stabilized, the metabolisation of residual carbon sources (fructose, glycerol, lactic acid) by LAB could lead to undesirable alterations such as acetification (Dueñas et al., 1994), ropiness (Dueñas et al., 1995) and bitterness.

In wine and cider, bitterness is an alteration characterized for an unpleasant bitter taste and is associated with the presence of acrolein in these beverages. The combination of acrolein with

* Corresponding author. Tel.: +34 943 01 5318; fax: +34 943 01 5270.
E-mail address: gaizka_garai@ikasle.ehu.es (G. Garai-Ibabe).

0168-1605/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2007.11.004

Table 1
Characteristic values for some chemical parameters of apple musts and ciders

		g l^{-1}					
	pH	Density	Fructose	Glycerol	Malic acid	Lactic acid	Acetic acid
Apple must	3.4–3.9	1055–1045	70–95	0	3–8	0	0
Cider	3.5–4.0	1000–997	0–5	3–6	0	3–5	0.5–3

polyphenols leads to the formation of bitter compounds. Lactic acid bacteria belonging to the genus *Lactobacillus* have been described as responsible for this alteration by a particular metabolic pathway of glycerol. This compound is dehydrated to 3-hydroxypropionaldehyde (3-HPA), which can be transformed into acrolein by chemical dehydratation under acidic and/or heat conditions (Lonvaud-Funel, 2002). A mixture of monomeric, hydrated monomeric and cyclic dimeric forms of 3-HPA is commonly know as reuterin (Talarico and Dobrogosz, 1989).

Glycerol is, besides ethanol, the main product of fermentation by yeasts during wine and cider production (Lafon-Lafourcade, 1983; Del Campo et al., 2003). It contributes to smoothness and roundness on the palate and hence, its degradation has a negative influence on the sensorial quality of ciders (Piccinelli, et al., 2000). Glycerol degradation by lactic acid bacteria may occur through two different pathways. It can be assimilated by a glycerol dehydrogenase and a dihydroxy-acetone kinase to dihydroxy-acetone phosphate (DHAP), which finally reaches the glycolytic pathway ("oxidative branch") (Lonvaud-Funel, 2002). In the reductive branch, however, glycerol is converted into 3-hydroxypropionaldehyde (3-HPA) by coenzyme B_{12}-dependent glycerol and diol dehydratases (Talarico and Dobrogosz, 1990; Sauvageot et al., 2002). The 3-HPA can be subsequently reduced to 1,3-propanediol (1,3-PDL)

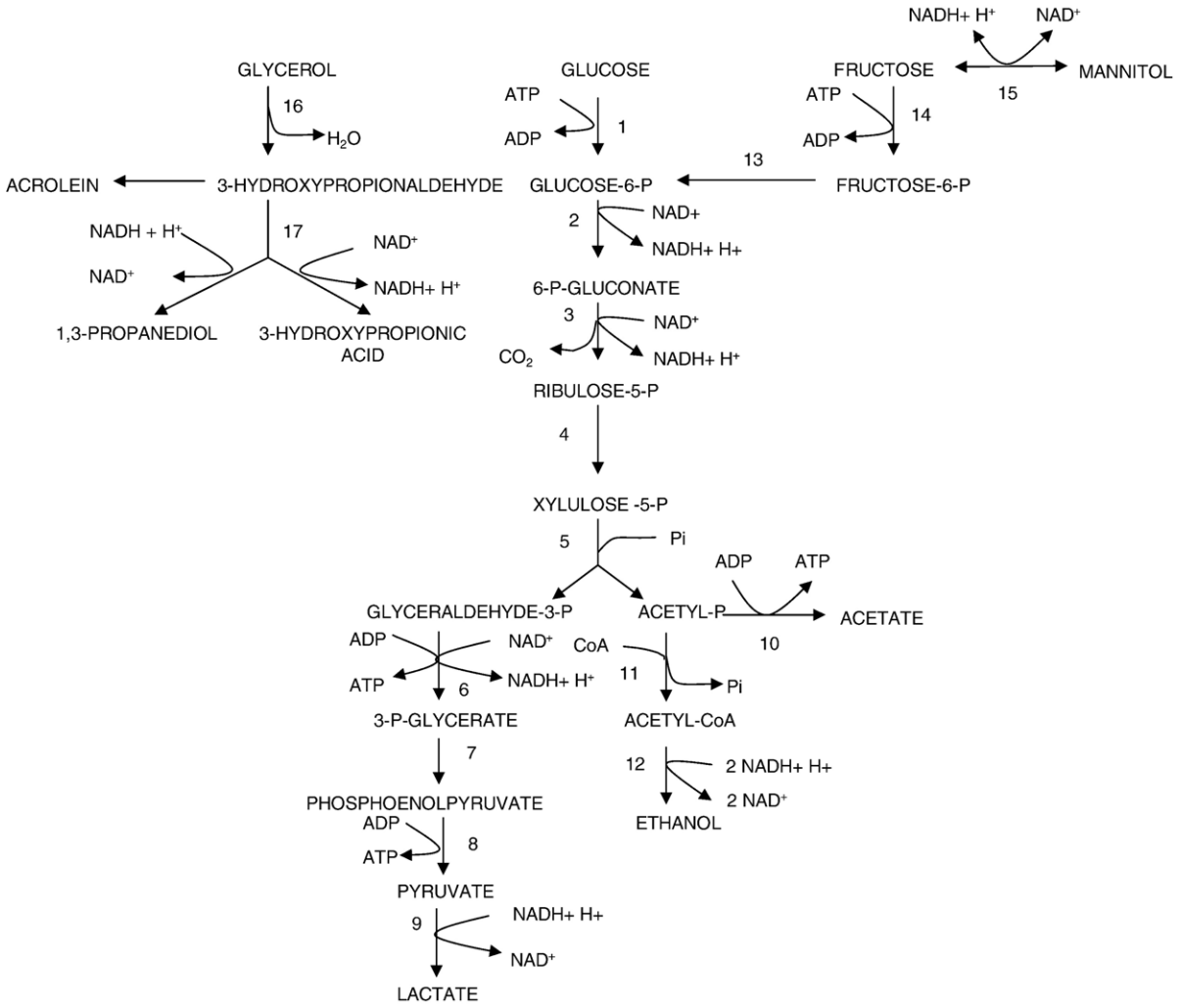

Fig. 1. Proposed pathway of heterolactic fermentation of sugars and glycerol metabolism in *L. collinoides* 17. (1) glucokinase, (2) glucose 6-phosphate dehydrogenase, (3) 6-phosphogluconate dehydrogenase, (4) epimerase, (5) phosphoketolase, (6) glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase, (7) phosphoglyceromutase and enolase, (8) pyruvate kinase, (9) lactate dehydrogenase, (10) acetate kinase, (11) phosphate acetyltransferase, (12) acetaldehyde dehydrogenase and alcohol dehydrogenase, (13) glucose phosphate isomerase, (14) fructokinase, (15) mannitol dehydrogenase, (16) glycerol dehydratase and (17) 1,3-propanediol dehydrogenase.