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Short communication

Glycerol metabolism in *Lactobacillus collinoides*: production of 3-hydroxypropionaldehyde, a precursor of acrolein

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Abstract

Lactobacillus collinoides is a lactic acid bacterium commonly found in fermenting apple juice. Although this bacterium is not particularly involved in malolactic conversion, the presence of *L. collinoides* in cider may have serious consequences on the product. *L. collinoides* is indeed considered to be responsible for the transformation of glycerol to 3-hydroxypropionaldehyde (3-HPA), a precursor of acrolein that spoils the product quality by generating bitter tastes. The purpose of our work was to evaluate the influence of environmental and culture conditions on the conversion of glycerol to 3-HPA in *L. collinoides*, and to obtain a DNA probe of the gene coding for glycerol dehydratase, the enzyme responsible for this conversion.

Keywords: *Lactobacillus collinoides*; 3-HPA; Glycerol dehydratase

1. Introduction

The cider alteration known as 'piqûre acroléique', results from the chemical transformation of 3-hydroxypropionaldehyde (3-HPA) to acrolein, a lachrymatory agent responsible of undesirable peppery flavours. The 3-HPA, precursor of acrolein, is derived from glycerol, one of the most important by-products of alcoholic glucose fermentation by yeasts.Glycerol is converted into 3-HPA by a coenzyme B12-dependent dehydratase (EC 4.2.1.30) (Smiley and Sobolov, 1962). This conversion requires anaerobic conditions and a univalent cation. Furthermore, it is inhibited by vitamin B12. The 3-HPA is subsequently reduced to 1,3-propanediol by an NADH-linked dehydrogenase (1,3-PD oxidoreductase; EC 1.1.1.202) or spontaneously transformed by thermal intramolecular dehydration to acrolein (Schütz and Radler, 1984). This transformation is enhanced by low pH and hence is favored in cider during distillation (Fig. 1).

Acrolein production during cider elaboration has been correlated with the presence of an heterofermentative bacterium: *Lactobacillus collinoides* (personal communication by ADRIA-Normandie). This lactic acid bacterium commonly found in cider (Carr and Davis, 1972) is involved in the metabolism of quinic acid, the second most abundant organic acid after malic acid in cider, to dihydroshikimic acid (Carr et al., 1954, 1957). Preliminary studies have shown that this organism exhibits a natural resistance to conditions encountered during the fermentative process (Laplace et al., 1999).

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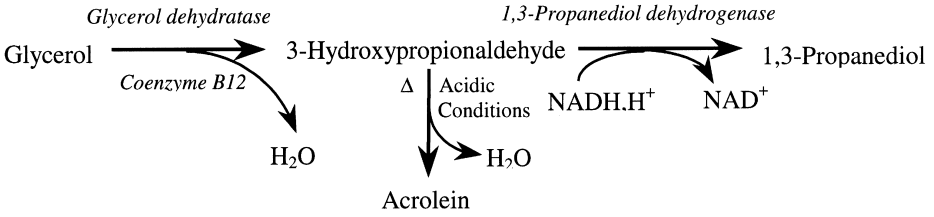

Fig. 1. Metabolic pathway from glycerol to 1,3-propionaldehyde via the intermediate 3-HPA.

The purpose of this work was to study the glycerol metabolism in *Lactobacillus collinoides,* as a function of the glucose concentration. Furthermore, in this report we describe the molecular cloning of a fragment of the gene encoding the glycerol dehydratase in *L. collinoides*. This work presents a first approach to examine the potential use of a molecular probe to detect *L. collinoides* in early stages of cider elaboration.

2. Materials and methods

2.1. Bacterial strain and culture condition

The present study was performed with a strain of *L. collinoides* isolated from a French cider and kindly provided by the ADRIA-Normandie (Villers-Bocage, France). This strain has been deposited in the publicly accessible culture collection BCCM/LMG (accession number LMG 18850). Cultures were grown at 30°C without shaking in 10-ml glass tubes containing 9 ml of MRS medium, pH 6.5 (De Man et al., 1960), supplemented with 0.1 M glucose unless specified in Section 3.

2.2. Analytical methods

Glucose and glycerol were enzymatically analysed by means of Roche reagents (Meylan, France). The assay for 3-HPA content was based on the colorimetric method of Circle et al. (1945), a method that is specific for acrolein detection.

2.3. General molecular methods

Restriction endonuclease, alkaline phosphatase and ligase were obtained from Roche (Meylan) and used according to the furnished instructions. PCR was carried out in a 25-μl volume with 1 μg of chromosomal DNA of *L. collinoides* using Ready To Go PCR beads (Pharmacia Biotech). The annealing temperature was 5°C below the melting temperature of primers; 30 cycles were performed and PCR products were purified using the QIAquick Kit (QIAGEN). DNA sequence was analysed using the Mac Vector™ (Kodak, Scientific Imaging Systems) program and databases searches were performed with the BLAST program (Altschul et al., 1990). Other standard techniques were carried out as described by Sambrook et al. (1989).

3. Results and discussion

The first results showed that in *L. collinoides*, the pathway of acrolein synthesis is independent of glucose degradation. Indeed, on medium with glucose as sole carbon source, no production of acrolein was observed. Moreover, *L. collinoides* was not able to grow in a medium containing glycerol as sole carbon source and thus to produce the precursor of acrolein (3-HPA). As both glucose and glycerol are present simultaneously in fermented apple must, we have studied the glycerol metabolism in *L. collinoides* in a media containing 54 mM glycerol and various glucose concentrations; 54 mM glycerol correspond to the concentration usually found in cider after alcoholic fermentation. Under these conditions, *L. collinoides* was able to grow and also to convert glycerol into 3-HPA. An optimal rate of glycerol conversion was observed for a molar ratio glycerol/glucose equal to 3 which is in good agreement with the value reported by Sobolov and Smiley (1960) (Fig. 2). The consumption of glycerol and glucose suggests that they are utilised concomitantly.

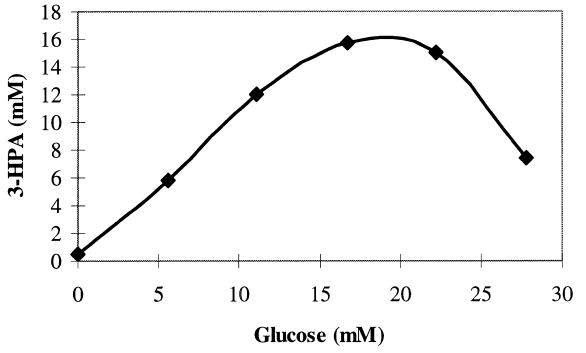

Fig. 2. 3-HPA production by *L. collinoides* with 54 mM of glycerol and varying glucose concentrations after 48 h of culture.

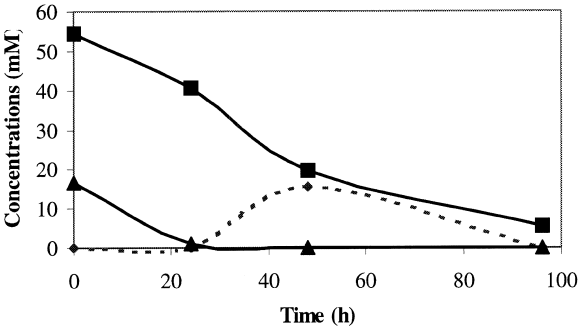

Fig. 3. Time-course of 3-HPA (♦) production from *L. collinoides* LMG 18850 with initial concentration of 16 mM glucose (▲) and 54 mM glycerol (■).

During the first hours of culture, glycerol is transformed into 1,3-propanediol by the auxiliary pathway. When glucose is exhausted, the cofactor $NADH,H^+$ production ceases, the reaction catalysed by 1,3-propanediol dehydrogenase is stopped and 3-HPA is accumulated (Fig. 3).

As the glycerol dehydratase plays a key role in glycerol metabolism and hence in potential cider alteration by acrolein, we have undertaken the cloning of the corresponding gene in order to study its regulation. A 150-bp fragment, which was obtained by PCR (using primers 5′-ATGTTT-GCTGGTTCTAACTT-3′ and 5′-CAGCTTGAAT-AGCACGAGCAGCC-3′), has been cloned and sequenced. These primers were determined from the main conserved region of enterobacter glycerol dehydratase amino acid sequence. Homology searches revealed 64, 62 and 60% identities with the gene (*dhaB*) of the large subunit of glycerol dehydratase of *Citrobacter freundii*, *Klebsiella pneumoniae* and *Clostridium pasteurianum*, respectively (Fig. 4) (Seyfried et al., 1996; Tobimatsu et al., 1996; Macis et al., 1998).

A 25-bp DNA probe has been deduced from the sequence of the amplified product. This oligonucleotide can be utilized to screen *dhaB* gene distribution in the bacterial cider microflora, allowing detection and quantification of contaminants in the first steps of the elaboration process.

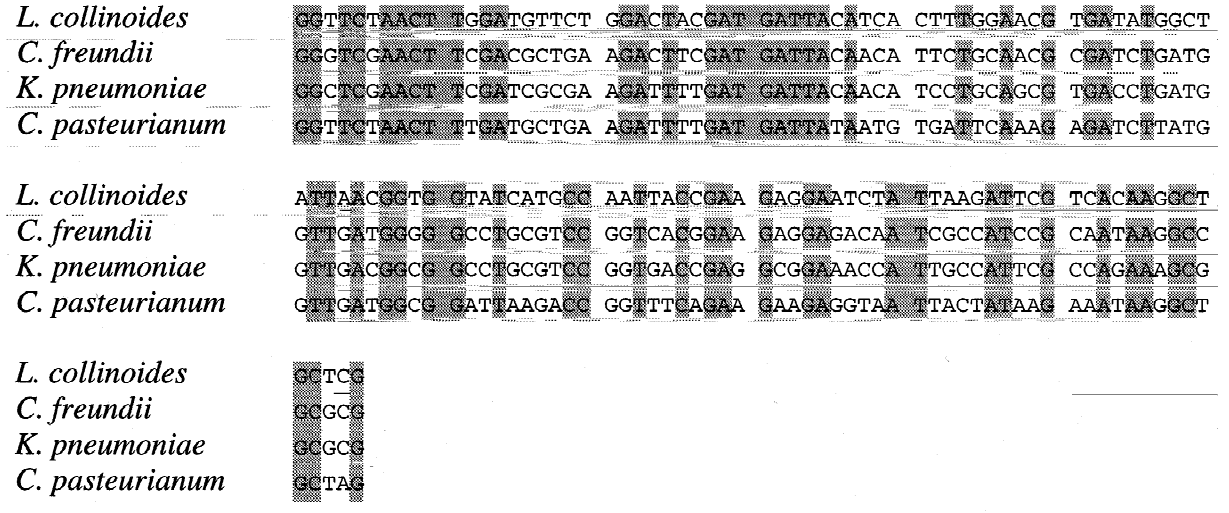

Fig. 4. Nucleotide sequence alignment of the 150-bp fragment with a region of the glycerol dehydratase genes of *C. freundii*, *K. pneumoniae* and *C. pasteurianum*. Preserved bases are indicate as dark shaded.

Work is in progress in our laboratory to obtain the entire sequence of the glycerol deshydratase gene of *L. collinoides*.

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