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Screening of representative cider yeasts and bacteria for volatile phenol-production ability

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ABSTRACT

Representative cider microorganisms (47 yeast strains and 16 bacterial strains) were studied for their ability to produce volatile phenols in a synthetic medium simulating cider conditions and supplemented with the necessary precursors. The various strains were tested for cinnamoyl esterase activity and only *Lactobacillus collinoides* were able to hydrolyse chlorogenic acid. Phenolic acid decarboxylase (PAD) activities were observed for 6 yeasts and 4 bacterial species allowing them to produce vinylphenols from hydroxycinnamic acids. On the other hand, 4 bacterial species exhibited phenolic acid reductase (PAR) activities leading to the formation of hydroxyphenylpropionic acids. *Brettanomyces/Dekkera anomala* and *L. collinoides* were able to produce 4-ethylcatechol (4-EC) and 4-ethylphenol (4-EP) from caffeic and *p*-coumaric acid, respectively, indicating that both species exhibit PAD and vinylphenol reductase (VPR) activities. In the experimental conditions used, the production of ethylphenols by *L. collinoides* was faster than the one observed for *D. anomala*.

1. Introduction

For the last 25 years, fermented beverage producers and researchers have become increasingly interested in volatile phenols. Indeed, these molecules can be considered as major organoleptic defect markers of various fermented alcoholic beverages. In these products, volatile phenols were usually associated with “animal”, “horsey”, “leather”, “phenolic” or “spicy” aromatic notes. The sensory impact of these compounds was particularly studied in beers (Halcrow et al., 1966), in malt whisky fermentations (Van Beek and Priest, 2000) and in wines (Chatonnet et al., 1992b; Etievant et al., 1989; Romano et al., 2009). In red wines, volatile phenols can confer “animal” defects and a loss of “fruity” aromatic notes, which are highly detrimental to wine quality (Gerbaux and Vincent, 2001).

Volatile phenols have been found to be mainly produced by microorganisms present in the product (Heresztyn, 1986; Rodríguez et al., 2009). The first step necessary for their biosynthesis is the production of hydroxycinnamic acids (caffeic, *p*-coumaric and ferulic acids) from hydroxycinnamic esters by cinnamoyl esterase activities (Chatonnet et al., 1992a). Endogenous cinnamoyl esterase activities have been reported in barley (Humberstone and Briggs, 2002) and barley malt (Sun et al., 2005). Hernandez et al. (2007) have shown that caftaric and coutaric acids are substrates of some lactic acid bacteria (LAB) which can exhibit cinnamoyl esterase activities during malolactic fermentation of wine, increasing the concentration of hydroxycinnamic acids. On the other hand, *Aspergillus niger* industrial pectinase preparations have shown to be the main cause of cinnamoyl esterase activities in wines (Barbe and Dubourdieu, 1998; De Vries et al., 1997; Juge et al., 2001).

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Volatile phenols biosynthesis has been mainly studied in wine. In this product, the main microorganisms associated with volatile phenol production correspond to *Brettanomyces*/*Dekkera* yeasts (Chatonnet et al., 1995; Edlin et al., 1998; Martorell et al., 2006). The phenolic acid decarboxylase (PAD) (also known as the hydroxycinnamate decarboxylase) and vinylphenol reductase (VPR) activities (Fig. 1) of these yeasts enable them to produce 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) from *p*-coumaric and ferulic acids, respectively (Chatonnet et al., 1992b; Heresztyn, 1986). This 4-EP production from *p*-coumaric acid by *Brettanomyces*/*Dekkera bruxellensis* takes place mainly at the end of exponential phase and in the beginning of stationary phase of growth (Dias et al., 2003). However, some studies have indicated that other wine yeasts, *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, were able to produce 4-vinylphenol (4-VP) and 4-vinylguaiacol (4-VG) from *p*-coumaric and ferulic acids, respectively (Chatonnet et al., 1989; Smit et al., 2003). Some bacteria belonging to the *Bacillus subtilis*, *Lactobacillus brevis*, *Pediococcus pentosaceus* and *Pediococcus* sp. species have also been shown to be able to produce vinylphenols (Cavin et al., 1998; Couto et al., 2006; De las Rivas et al., 2009). Moreover, some *Lactobacillus plantarum* strains were shown to harbour both the phenolic acid reductase (PAR) and PAD activities, allowing for the production of both phloretic acid (PA) and 4-VP from *p*-coumaric acid (Barthelmebs et al., 2000).

In beer, *Brettanomyces*/*Dekkera* yeasts and *Pediococcus* bacteria are the main microorganisms responsible for volatile phenol production (Martens et al., 1997; Spaepen et al., 1981; Thurston and Tubb, 1981). However, various beer bacteria can produce vinylphenols (4-VG from ferulic acid and 4-VP from *p*-coumaric acid): *Enterobacter*, *Hafnia*, and *Klebsiella* (Lindsay and Priest, 1975). Several *Saccharomyces* beer strains were also shown to be able to produce 4-VG (Coghe et al., 2004; Mc Murrough et al., 1996).

In cider, it was previously shown using *Lactobacillus paracollinoides* (described as *Lactobacillus pastorianus* var. *quinicus*) cider strain cell extracts that the first stage in chlorogenic acid metabolism was the hydrolysis to caffeic and quinic acids (Whiting and Carr, 1957). Both products were further metabolised and hydrocaffeic acid (HCA) and 4-ethylcatechol (4-EC) were produced from caffeic acid. Later, it was shown that *p*-coumaric acid is metabolised by *L. paracollinoides* with the formation of PA and 4-EP in basal medium (Whiting and Carr, 1959). Thus, this *L. paracollinoides* strain isolated from cider presented cinnamoyl esterase, PAR, PAD and VPR activities. Moreover, a *Lactobacillus collinoides* strain isolated from apple juice and a *Lactobacillus mali* strain isolated from cider, were able to produce 4-VP from *p*-coumaric acid via PAD activity (Couto et al., 2006). However, these activities were observed in MRS/tomato juice (50:50) with 500 mg/l of *p*-coumaric acid and these culture conditions are very different from cider in terms of medium composition and precursor concentrations. In French ciders, the maximum level of *p*-coumaric acid was found to be 19 mg/l whereas the quantity of caffeic acid can reach 142 mg/l (Alonso-Salces et al., 2004). Moreover, volatile phenol precursor contents have been shown to be very different in wines and ciders. In wine, the main precursors correspond to tartaryl esters of hydroxycinnamic acids (Burns et al., 2000) reported to lead mainly to 4-EP and 4-EG. In cider, chlorogenic (or caffeoylquinic) acid, which is the quinyl ester of caffeic acid, is the main ester of hydroxycinnamic acids and therefore a potential source for caffeic acid via cinnamoyl esterase activity and thus for 4-vinylcatechol (4-VC) and 4-EC via PAD and VPR activities. Recently, we showed that 4-EC is the main volatile phenol (reaching up to 162 mg/l) in French ciders with phenolic off-flavour defects (Buron et al., 2011).

The fermentation of French ciders is performed by indigenous microorganisms as no starters are used. First, an oxidative phase characterized by the predominance of *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Hanseniaspora valbyensis* and *Candida* sp. yeasts is observed. It is then followed by the alcoholic fermentation during which *Saccharomyces uvarum* and *S. cerevisiae* species are dominant (Le Quéré and Drilleau, 1996). *Zygosaccharomyces*, *Pichia*, *Candida*, *Torulaspora*, *Rhodotorula*, *Cryptococcus* and *Brettanomyces*/*Dekkera* yeasts, originating from apples or the environment, have been previously isolated from ciders (Beech, 1993; Michel et al., 1988; Morrissey et al., 2004). Finally, spontaneous malolactic fermentation may occur and is performed by LAB belonging to various genera and species: *Oenococcus oeni*, *L. brevis*, *L. mali*, *L. collinoides*, *Leuconostoc mesenteroides*, *Pediococcus* sp. (Carr and Whiting, 1971; Coton et al., 2006).

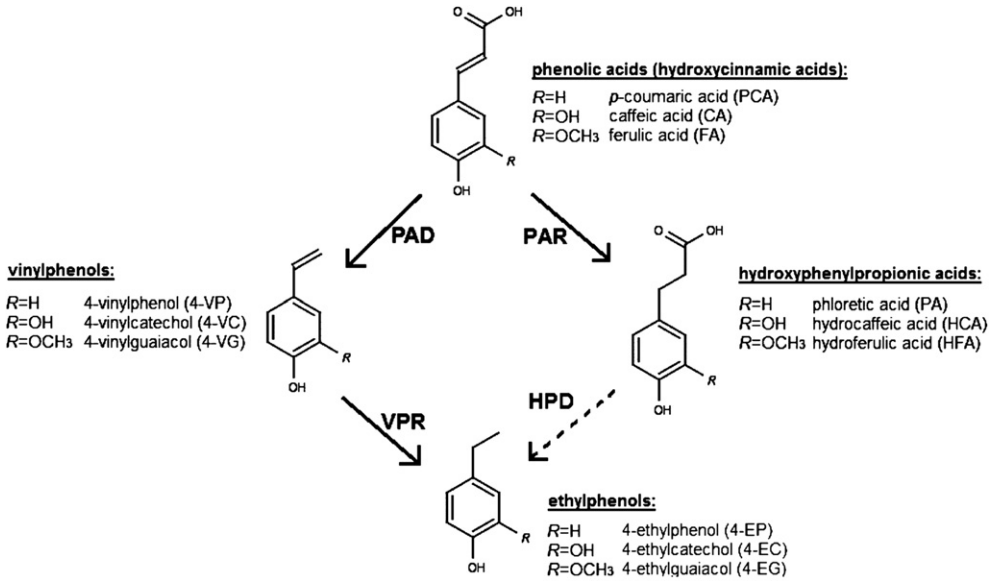

Fig. 1. Biosynthesis pathways of volatile phenols from hydroxycinnamic acids in cider. PAD, phenolic acid decarboxylase; VPR, vinylphenol reductase; PAR, phenolic acid reductase; HPD, putative hydroxyphenylpropionic decarboxylase.