STUDIES ON THE PRODUCTION OF 4-ETHYLPHENOL BY *DEKKERA* spp. YEASTS

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INTRODUCTION

Yeasts from the genus *Dekkera* (or its *fungi imperfecti* form, *Brettanomyces*) are often associated with wine spoilage (especially in barrel-aged red wines).

These yeasts produce large amounts of acetic acid from glucose metabolism and are known to metabolize wine phenolic acids (p-coumaric and ferulic acids), producing volatile compounds (mainly 4-ethylphenol and 4-ethylguayacol), which give a characteristic aroma to wines, often described as “horse sweat”, “animal” or "leather" Although these aromas are often regarded as negative, some winemakers consider that, at low concentrations, “Brett character” can contribute positively to the complexity of the bouquet of some wines (Fugelsang, 1997).

In this work, we studied the effect of some environmental factors (substrate concentration, pH and sulfur dioxide concentration) on the production of 4-ethylphenol (4EP) using *p*-coumaric acid as substrate, by *Dekkera anomala* and *Dekkera bruxellensis*. *p*-Coumaric was chosen for being the most abundant hydroxycinnamic acid in wine.

MATERIALS AND METHODS

In this work we used *Dekkera bruxellensis* strain PYCC 4801 and *Dekkera anomala* strain PYCC 5153 (Portuguese National Yeast Collection, Fundação Calouste Gulbenkian, Lisbon, Portugal). These species were chosen because of their exceptional abillity to convert *p*-coumaric acid to 4EP. Cellular growth was monitored spectrophotometrically and 4EP concentration was determined by GC-FID using the method described by Bertrand (1981).

Initially, the effect of p-coumaric acid concentration (up to 500 mg l^{-1}) on growth of *D. anomalla* and *D. bruxellensis* in liquid YM growth medium (DIFCO, Detroit, USA), was evaluated. Afterwards, the kinetics of 4EP production during yeast growth and the production of 4EP with different initial concentations of p-coumaric acid (up to 100 mg l^{-1}) were measured in order to study the conversion yield, at various substrate concentration levels, in YM growth medium. The effects of pH and sulfur dioxide on growth and 4EP production were also studied, using YM growth medium, for both yeasts. All experiments were repeated to confirm the results

RESULTS

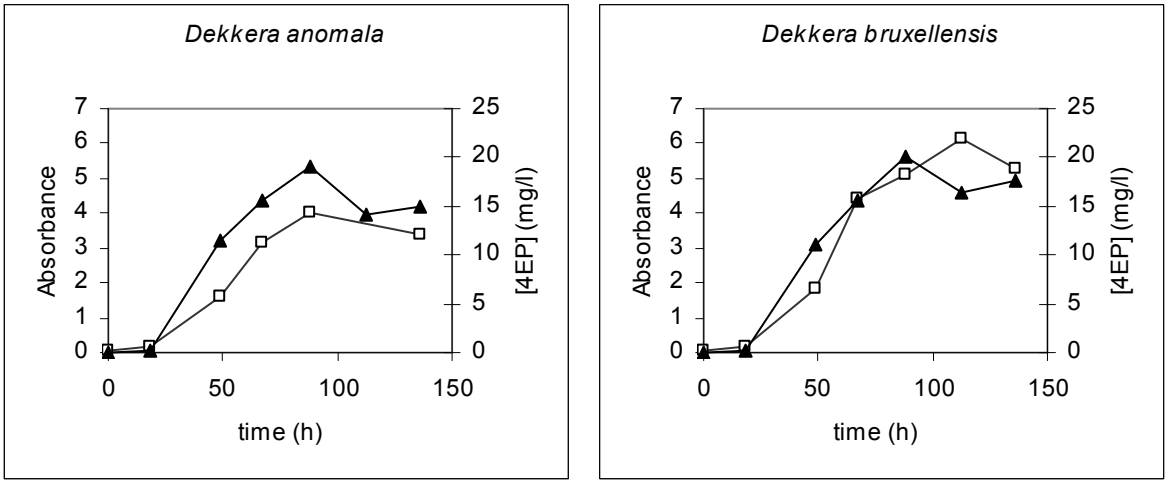

Figure 1 – Production of 4-ethylphenol (4EP) by *D. anomala* PYCC 5153 and *D. bruxellensis* PYCC 4801during growth in YM medium (pH 4.5) supplemented with 20 mg l^{-1} *p*-coumaric acid; (▲) – 4EP concentration, (‾) – corrected absorbance (at 660 nm)

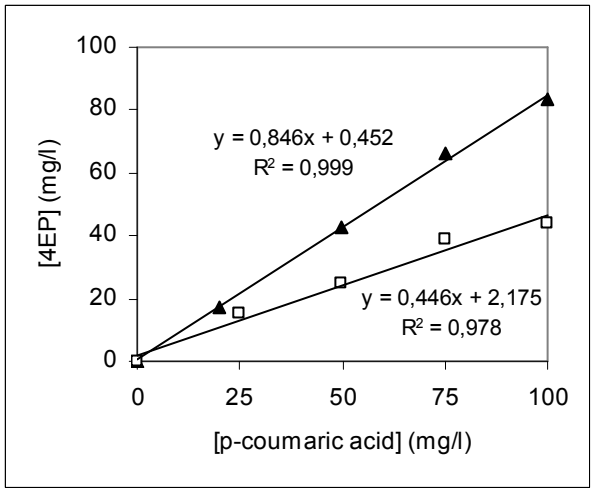

Figure 2 – Production of 4-ethylphenol (4EP) by (▲) *D. anomala* PYCC 5153 and (‾) *D. bruxellensis* PYCC 4801 in YM medium (pH 4.5) with different initial concentrations of p-coumaric acid.

Table 1 – Influence of pH on 4-ethylphenol (4EP) production of *D. anomala* PYCC 5153 and *D. bruxellensis* PYCC 4801 in YM medium supplemented with *p*-coumaric acid at two different concentration levels.

		D. anomala		*D. bruxellensis*	
pH	[p-coum.] (mg l^{-1})	Absorbance (max)	[4EP] (mg l^{-1})	Absorbance (max)	[4EP] (mg l^{-1})
3,0	25,0	3,60	22,8	4,11	20,9
3,5	25,0	4,82	19,6	4,67	21,5
4,0	25,0	4,23	22,4	5,48	21,5
4,5	25,0	4,69	22,6	5,05	22,2
3,5	50,0	4,54		5,72	42,8
4,0	50,0	4,14	48,6	6,72	45,8
4,5	50,0	5,14	45,8	6,34	48,4
5,0	50,0	5,16	44,5	6,78	46,6

DISCUSSION

Growth of both *D. anomala* and *D. bruxellensis* was only affected negativelly at *p*-coumaric acid concentration levels higher than 100 mg l^{-1} (results not shown). This negative effect of p-coumaric acid on growth was stronger in the case of *D. anomala* than in the case of *D. bruxellensis* and was previously described for other yeasts (Baranowski *et al.,* 1980). The results obtained in the kinetics experiments indicate that, apparently, 4EP production is coincident with growth, for both yeasts, indicating that it might occur simultaneously with sugar metabolism (Figure 1). The yield of conversion of p-coumaric acid to 4EP was higher with *D. anomala* (around 85%) than with *D. bruxellensis* (around 45%). At the concentration range tested, there is an apparently linear relationship between the initial p-coumaric acid level and 4EP concentration (Figure 2). At pH levels between 3.0 and 5.0 there were no apparent differences on 4EP production by both yeasts, though low pH (3.0-3.5) affected slightly cell growth (Table 1). Preliminary results suggest that, at low free sulfur dioxide concentrations, growth of both yeasts is more affected than the production rate of 4EP.

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