Studies on the Production of 4-Ethylphenol by *Dekkera spp.* Yeasts

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ABSTRACT

The effect of some environmental factors (pH, sulfur dioxide concentration) on the production of 4-ethylphenol (4EP) using *p*-coumaric acid as substrate, by *Dekkera anomala* and *Dekkera bruxellensis*, was studied. Growth of both *D. anomala* and *D. bruxellensis* was affected negatively at *p*-coumaric acid concentration levels higher than 100 mg l^{-1}. The yield of conversion of *p*-coumaric acid to 4EP was constant, at the concentration range tested, for both yeasts, suggesting a possible linear relationship between these two factors. 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 Low molecular sulfur dioxide concentrations (>0.15 mg l^{-1}), affected dramatically growth and 4EP production by both yeasts.

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, 4-ethylphenol and 4-ethylguaiacol, respectively, 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).

Several environmental factors (substrate concentration, pH and sulfur dioxide concentration) were tested for their effect 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.

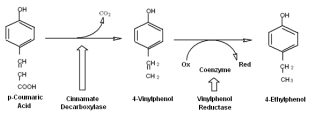

Figure 1 – Biosynthesis of 4-ethylphenol from *p*-coumaric acid by *Dekkera* yeasts.

MATERIAL AND METHODS

Yeast strains and growth conditions

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. Cultures were grown aerobically to late exponential phase in YMB medium (DIFCO, Detroit, USA), with pH adjusted at 4.5 (except where noted) and incubated at 25ºC. Cellular growth was monitored spectrophotometrically (at 660 nm) and 4EP concentration was determined by GC-FID using the method described by Bertrand (1981).

Influence of *p*-coumaric acid on growth of *D. anomala* and *D. bruxellensis*

Cultures were grown in YMB medium containing *p*-coumaric acid at 0, 100, 200 and 500 mg l^{-1}. Each individual assay was made in triplicate and incubated at 25ºC. Yeast growth was monitored daily.

Kinetics of of 4-ethylphenol production during growth of *D. anomala* and *D. bruxellensis*

Cultures were grown in YMB medium supplemented with *p*-coumaric acid at 20 mg l^{-1} and pH adjusted to 4.5. Yeast growth and 4EP concentration were measured daily.

Yield of production of 4-ethylphenol by *D. anomala* and *D. bruxellensis* at different levels of *p*-coumaric acid concentration

Cultures were grown in YMB growth medium containing *p*-coumaric acid at 0, 50, 75 and 100 mg l^{-1}. Yeast growth and 4EP concentration were measured daily.

Influence of pH on production of 4-ethylphenol by *D. anomala* and *D. bruxellensis*

Cultures were grown in YMB medium with adjusted pH between 3.0 to 5.0 and supplemented with *p*-coumaric acid at 25 and 50 mg l^{-1}.

Influence of SO2 concentration on growth and 4-ethylphenol production of *D. anomala* and *D. bruxellensis*

Cultures were grown in YMB medium at pH 4.0, with different initial SO_2 concentrations (adjusted with sodium bisulfite) and supplemented with 50 mg l^{-1} *p*-coumaric acid

RESULTS

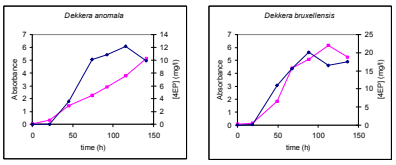

Figure 2 – Production of 4-ethylphenol (4EP) by *D. anomala* PYCC 5153 and *D. bruxellensis* PYCC 4801 during growth in YMB medium (pH 4.5) supplemented with 20 mg l^{-1} *p*-coumaric acid; —◆— 4EP concentration, —■— corrected absorbance (at 660 nm).

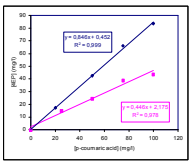

Figure 3 – Production of 4-ethylphenol (4EP) by —◆— *D. anomala* PYCC 5153 and —■— *D. bruxellensis* PYCC 4801 in YMB medium (pH 4.5) with different initial concentrations of *p*-coumaric acid.

Table 1 – Influence of pH on 4-ethylphenol (4EP) production of *D. anomala* PYCC 5153 and *D. bruxellensis* PYCC 4801 in YMB 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

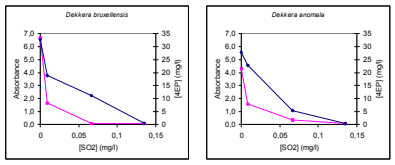

Figure 4 – Production of 4-ethylphenol (4EP) by *D. anomala* PYCC 5153 and *D. bruxellensis* PYCC 4801 during growth in YMB medium (pH 4.0) supplemented with 50 mg l^{-1} *p*-coumaric acid and different initial molecular SO_2 concentrations; —◆— 4EP concentration, —■— maximum corrected absorbance (at 660 nm).

DISCUSSION

- Growth of both *D. anomala* and *D. bruxellensis* was affected negatively by *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 2).
- 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 3).
- 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).
- At low molecular SO_2 concentrations (<0.15 mg l^{-1}), yeast growth was more affected than 4EP production. Higher SO_2 concentrations inhibited completely cell growth and 4EP production (Figure 4)

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