

Study of the tolerance of *Cutaneotrichosporon mucoides* to phenolic compounds aiming to its use to produce biosurfactants in lignocellulosic biorefineries

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Introduction

Phenolic compounds are the main inhibitors in bioprocesses when sugarcane bagasse hemicellulosic hydrolysate (SBHH) is used as substrate. Phenolics found in SBHH are derived from the lignin present in the lignocellulosic biomass, obtained during acid hydrolysis at high temperature. The phenols and derivatives commonly found in lignocellulosic hydrolysates are syringaldehyde, syringic acid, 4-hydroxybenzaldehyde, gallic acid, protocatechic acid, vanillin, vanillic acid and ferulic acid (Rossel, 2006). Microbial production of interesting molecules from lignocellulosic biomass can be impaired by inhibitor compounds that interfere on cell growth and in the fermentative process. The toxicity of these compounds to microbial cells is dependent on the molar mass, where the phenolics of lower mass are considered more toxic (Klinke et al., 2004). These compounds are harmful to cells because they can disrupt the cellular membrane by affecting their selective barrier ability, the inactivation of enzymatic matrices that are fundamental for cell function and the destruction or inactivation of genetic material (Palmqvist; Hahn-Hägerdal, 2000a; 2000b). Despite the various detoxification methods described in the literature, some phenolics can remain in the medium and this step turn the global process more expensive. Therefore, the search for microorganisms tolerant to these compounds in the bioprocesses is an interesting approach, with potential to eliminate the necessity of detoxification step. The present study had as main objective to evaluate the tolerance of the yeast *Cutaneotrichosporon mucoides*, producer of biosurfactants, to various concentrations of phenolic compounds present in a semi-synthetic medium with xylose as carbon source.

Material and methods

In the experiments, the effects of addition, separately, of phenol, vanillin and tannic acid, on the Kitamoto medium (g/L: potassium dihydrogenphosphate 2.0; yeast extract 1.0; ammonium nitrate 11.0; magnesium sulfate heptahydrate 2.0; xylose 40.0) and concentrations of phenolic compounds in the experiments ranged from 0.1 to 3.0 g/L. The components of culture medium were sterilized separately in autoclave to 121 °C, 1 atm by 15 minutes. The pH of medium was adjusted to 5.0 – 5.5. The inoculum was grown for 48 hours in shaker at 30 °C and 200 rpm. After this step, the cells were aseptically separated by centrifugation 2000 \times g for 10 minutes and supernatant discarded. Thus, the cells were standardized by spectrophotometry using McFarland scale modified and inoculated approximately 1.0×10^7 total cells/mL in Erlenmeyer flasks of 50 mL with 10 mL of medium. Fermentations were carried out for approximately 68 hours, at 30 °C and 200 rpm. Subsequently, after stopping the fermentation, the cells were separated by centrifugation at 2000 \times g for 10 minutes and cell growth was evaluated by spectrophotometry. The pH of supernatants was measured by potentiometric method using potentiometer and glass electrode.

Results and discussion

In experiments in the medium containing phenol and vanillin in the concentration range from 0.1 to 0.5 g/L, 2.50×10^9 - 3.42×10^9 total cells/mL and a final pH of 3.6 were observed. Cells were not able to grow in experiments in which culture media was supplemented with concentrations above 1.0 g/L of phenol or vanillin. When tannic acid was added to the medium at concentrations varying between 0.1 - 3.0 g/L, about 1.6×10^9 cells/mL and a final pH value of 3.6 were observed. The analysis of the obtained data showed the tolerance of the yeast *C. mucoides* to phenolic compounds of low molar mass, such as phenol and vanillin, in concentrations lower than 1.0 g/L. In addition, according with literature, vanillin in concentration between 55 – 175 mg/L adversely affected cell growth and ethanol production by *Scheffersomyces (Pichia) stipitis* (Zeferino, 2013). Higher tolerance was verified when tannic acid was used, indicating this high molecular mass phenolic compound results in lower toxicity to the cells (Rossel, 2006).

Conclusion

These results are interesting for the development of fermentative processes in lignocellulosic biorefineries, considering the use of microorganisms tolerant to inhibition compounds prevents the necessity of detoxification step, thus reducing process costs.

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