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Genetically Modified Fuel-Ethanol Industrial Yeast Strains as Biocontrol Agents

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Abstract : Industrial fuel-ethanol fermentations are carried out under non-sterile conditions, which favors the development of microbial contaminants, leading to huge economic losses. Wild yeasts such as Brettanomyces bruxellensis and lactic acid bacteria are the main contaminants of industrial bioethanol fermentation, affecting Saccharomyces cerevisiae performance and decreasing ethanol yields and productivity. In order to control microbial contaminations, the fuel-ethanol industry uses different treatments, including acid washing and antibiotics. However, these control measures carry environmental risks such as acid toxicity and the rise of antibiotic-resistant bacteria. Therefore, it is crucial to develop and apply less toxic and more environmentally friendly biocontrol methods. In the present study, an industrial fuel-ethanol starter, S. cerevisiae Ethanol-Red, was genetically modified to over-express AMPs with activity against fuel-ethanol microbial contaminants and evaluated regarding its biocontrol effect during mixed-culture alcoholic fermentations artificially contaminated with B. bruxellensis. To achieve this goal, S. cerevisiae Ethanol-Red strain was transformed with a plasmid containing the AMPs-codifying genes, i.e., partial sequences of TDH1 (925-963 bp) and TDH2/3 (925-963 bp) and a geneticin resistance marker. The biocontrol effect of those genetically modified strains was evaluated against B. bruxellensis and compared with the antagonistic effect exerted by the modified strain with an empty plasmid (without the AMPs-codifying genes) and the non-modified strain S. cerevisiae Ethanol-Red. For that purpose, mixed-culture alcoholic fermentations were performed in a synthetic must use the modified S. cerevisiae Ethanol-Red strains together with B. bruxellensis. Single-culture fermentations of B. bruxellensis strains were also performed as a negative control of the antagonistic effect exerted by S. cerevisiae strains. Results clearly showed an improved biocontrol effect of the genetically-modified strains against B. bruxellensis when compared with the modified Ethanol-Red strain with the empty plasmid (without the AMPs-codifying genes) and with the non-modified Ethanol-Red strain. In mixed-culture fermentation with the modified S. cerevisiae strain, B. bruxellensis culturability decreased from 5×104 CFU/mL on day-0 to less than 1 CFU/mL on day-10, while in single-culture B. bruxellensis increased its culturability from 6×104 to 1×106 CFU/mL in the first 6 days and kept this value until day-10. Besides, the modified Ethanol-Red strain exhibited an enhanced antagonistic effect against B. bruxellensis when compared with that induced by the non-modified Ethanol-Red strain. Indeed, culturability loss of B. bruxellensis after 10 days of fermentation with the modified Ethanol-Red strain was 98.7 and 100% higher than that occurred in fermentations performed with the non-modified Ethanol-Red and the empty-plasmid modified strain, respectively. Therefore, one can conclude that the S. cerevisiae genetically modified strain obtained in the present work may be a valuable solution for the mitigation of microbial contamination in fuel-ethanol fermentations, representing a much safer and environmentally friendly preservation strategy than the antimicrobial treatments (acid washing and antibiotics) currently applied in fuel-ethanol industry.

Keywords : antimicrobial peptides, fuel-ethanol microbial contaminations, fuel-ethanol fermentation, biocontrol agents, genetically-modified yeasts
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