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GENETIC MOLECULAR CHARACTERIZATION OF FUSANTS FROM PROTOPLAST FUSION OF S. CEREVISIAE AND P. STIPITIS ATCC 58785 FOR IMPROVEMENT OF BIOFUEL PRODUCTION

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Protoplast fusion is a common approach used to improve fermentation of industrial yeast strains. Molecular study using DNA content, RAPD, DNA sequences and protein profile were applied to differentiate between *S. cerevisiae and P. stipitis* ATCC 58785 and their fusants from protoplast fusion for improvement of biofuel production from biomass. Fusants were showed higher DNA content than that in the parental strain and DNA concentration obtained for recently generated fusant was generally lower compared to the values expected by the theoretical addition of DNA concentration from the respective parental strains. Fusants were showed new combination of DNA fragment patterns. The DNA sequencing, which includes two non-coding regions designated as the internal transcribed spacers (ITS1 and ITS2) and the 5.8S genes were performed for further confirmation of the fusant nature of fusants. According to genetic similarity and intra-species differentiation, two parent strains and fusants were grouped into two different clusters. S. cerevisiae corresponds to 88% sequence similarity whereas approximately 97% similarity was observed with P. stipitis ATCC 58785. The sequence of the ITS1, ITS2 and the 5.8S gene of each fusants was submitted to Genbank with the NCBI accession no. In general, the obtained new combination of DNA fragment patterns and the presence of new DNA fragment or the absence of existing parental DNA fragments in the fusant strains compared to their parents could be considered as indicator of nuclear fusion of the two parental nuclei in the fused protoplasts, also differences in polypeptide profile on SDS-PAGE analysis were investigated. The polypeptide profile was seen with reference to a protein ladder (GangNam-STAIN™ prestained). SDS–PAGE protein analysis of the selected fusants and their parental strains confirmed that all fusant strains acquired and expressed many specific protein bands from the parental strains.

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