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Improving the Efficiency of Homologous Gene Replacement by Disrupting the NHEJ Pathway for Gene KusA in the Oleaginous Fungus Mortierella alpina¹ KATHLEEN KRUEGER, Cal Poly State University SLO, ZIYU DAI, Pacific Northwest National Laboratory, UGER UZUNER, Texan A&M, PNNL COLLABORATION — Mortierella alpina, a oleaginous filamentous fungus, is one of industrial fungal strains known for the production of arachidonic acid. It is also of particular interest for hydrocarbon biofuel production since it is able to produce up to 50% of its mass in rich, long-chain polyunsaturated fatty acids [PUFA's]. In addition to high fatty acid production, M. alpina like many other oleaginous fungi, already have mechanisms for accumulating significant concentrations of hydrophobic compounds making it a naturally equipped candidate to handle potential toxic concentrations of hydrocarbons. The goal of this study was to develop an efficient transformation method for this strain, hence allowing researchers to further manipulate these fungi for further improvement of lipid production. Included was optimization of best culture medium for growth and maintenance, optimal conditions for protoplast generation, and replacement of the homologous KusA gene. A successful deletion of KusA gene within biotechnologically important M. alpina could enable homologous recombination of other genes of interest in a higher frequency. This capacity may also improve the advancing the production of microbial oils for bioenergy and arachidonic acid human health applications.

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