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Hyper cellulase-producing fungus *Talaromyces pinophilus* EMM development through random mutagenesis and genetic engineering

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Talaromyces pinophilus UTA1 and EMM are cellulase hyper-producing mutants that originated from *T. pinophilus* OPC4-1 through UV irradiation and chemical mutagenesis by NTG and EMS. Full genome sequencing of these two mutants and the parent strain was conducted and 73 genes were identified with either SNPs or InDels. Functions of the 73 genes were identified using NCBI GenBank database. Among the 73 genes, 3 transcription factors were identified. They might be responsible for the enhancement of cellulase activity in mutant strains, UTA1 and EMM. Genes encoding the 3 transcription factors were successfully cloned to further confirm their enhancement in cellulase and hemicellulase production in mutant strains. Further genetic engineering of the mutant strain EMM was conducted to further enhance its enzyme production. A uracil auxotroph strain *T. pinophilus* EMU was isolated through random mutagenesis. A wild-type *pyrF* gene encoding orotate phosphoribosyl transferase (OPRTase, EC 2.4.2.10) isolated from *T. pinophilus* OPC4-1, the parent strain can be used as the selection marker for genetic engineering of strain *T. pinophilus* EMM. A marker recycle system was developed and was used for the knock-out of creA gene, the gene mediating catabolite repression. A creA gene knock-out strain, A creA 21 was successfully isolated. It demonstrated enhanced cellulase and xylanase production and higher resistance to the increased glucose concentration. The genetic engineering tools were successfully developed for strain *T. pinophilus* EMM and disruption of creA gene in strain EMM was effective for enhanced enzyme production.

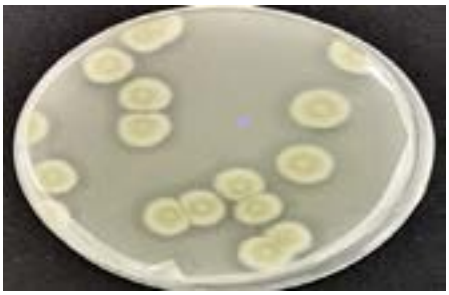

Figure 1: Zone of clearance generated by creA knock-out mutant of *T. pinophilus* EMM

Biography

Anli Geng is currently an Assistant Director of Life Sciences and Chemical Technology of Ngee Ann Polytechnic. She currently holds the President position in BioEnergy Society of Singapore (BESS). She is also the Co-founder and Director of Sunvisiae Biotech Pte Ltd, a Singapore-based industrial biotechnology company. Prior to joining Ngee Ann Polytechnic, she was working at Institute of Environmental Science and Engineering (IESE) as a Research Scientist. She has more than 25 years of R&D experience, working extensively in environmental biotechnology, green energy technology and industrial biotechnology. She has more than 30 journal publications and her work has been presented in many international conferences. Her current research focuses on developing novel microorganisms to produce industrial enzymes, chemicals and fuels, novel nutraceuticals and cosmetics ingredients at Ngee Ann Polytechnic. She obtained Ngee Ann Polytechnic Staff Excellence Award and IChemE Award on Sustainable Technology in 2012.

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