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World Yeast Congress

May 14-15, 2018 | Montreal, Canada

Cannabinoids biosynthesis using recombinant cannabinoid synthase enzymes expressed from industrial *yeast Pichia pastoris*

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The cannabinoid synthase enzymes Tetrahydrocannabinolic acid (THCA) synthase and Cannabidiolic acid (CBDA) synthase were recombinantly produced in yeast *Pichia pastoris* Mut+ strain. The coding regions of THCA synthase and CBDA synthase genes were codon optimized for *Pichia* expression. Both synthase genes were operably linked to the methanol inducible AOX1 promoter, an N-terminal alpha mating factor secretion signal, and a C-terminal 6x His-tag. These elements provide for inducible expression of the genes and simple processing/purification of the encoded enzymes. Each synthase construct was cloned into Invitrogen's pPIC3.5K plasmid. The recombinant plasmids were transformed into *Pichia* strain GS-115. *Pichia* clones transformed with multiple copies of each construct were selected based on their resistance to varying amount of geneticin concentrations. Gene copy numbers were further verified with RT-PCR. Fermentation conditions were optimized by investigating the impact of pH, temperature, methanol feed, and fermentation medium composition on cell growth and enzyme yield. The fermentation conditions were further optimized in a pilot scale, 14-liter fermenter. Based on these results, production was successfully scaled up to 500-liter fermenters. The Teewinot enzyme production system produced active THCA synthase and CBDA synthase enzymes. The THCA synthase converts chemically synthesized CBGA into ∆9-THCA and CBCA or chemically synthesized Cannabigerovarinic acid (CBGVA) into Tetrahydrocannabivarin (THCVA) and CBCVA in a bioreactor. The ratio of ∆9-THCA to CBCA and THCVA to CBCVA is dependent on reaction conditions including pH. The CBDA synthase enzyme converted chemically synthesized CBGA into CBDA, CBCA, and THCA or chemically synthesized CBGVA into CBDVA, CBCVA, and THCVA. Once again, the molar ratios of CBDA, CBCA, and THCA or the molar ratios of CBDVA, CBCVA, and THCVA produced in the bioreactor were dependent on reaction conditions such as pH. Each of the biocatalytically-produced cannabinoids was purified to greater than 99.5% purity. The identity and structure of each biocatalytically-produced cannabinoid was confirmed by HPLC, mass spectral, and NMR analysis.

Speaker Biography

Mingyang Sun has completed his Master's Degree in Synthetic Biology from Concordia University, Montreal. He is a Co-inventor of several US patents on cannabinoid biosynthesis and the Vice President of Teewinot Laboratories Inc, subsidiary of Teewinot Life Sciences Corporation.

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