Multi-copy expression and overproduction of eriodictyol 3’-O-methyltransferase gene: ROMT-9 in Yarrowia lipolytica

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The flavonoid 3’-O-methyltransferase Gene: ROMT-9 (Gen Bank accession number: 29893141) has the ability to transfer the methyl group of S-adenosyl-L-methionine to the eriodictyol to homoeriodictyol with strict specificity. Homoeriodictyol has various biological activities such as antioxidant, anti-virus and anti-bacterial, thus has a great potential value in application of food and pharmaceutical industries. As a bitter-masking flavanones, it was also wanted in coffee, dark chocolate and tea. The ROMT-9 was totally synthesized and cloned into the multi-copy integrative vector of pINA1297 and expressed in Yarrowia lipolytica, under a growth phase synthetic hp4d promoter. We obtained 20 positive transformants, among them, h14 was found to have the highest 3’-O-methyltransferase activity which was 26U/(L*h) in PPB medium in shake flasks after 4 days. After the ammonia sulfate was replaced with malt extract, the recombinant ROMT-9 activity reached 120 U/(L*h), resulted in 4.6-fold increase of ROMT-9 production. As the non-conventional yeast Yarrowia lipolytica has aroused a strong industrial interest for heterologous protein production, we also designed a completed defined medium as the present medium uses complex media which is not convenient for large scale production. When we replaced the yeast extract and ammonia sulfate with 2.64g/L monosodium glutamate, supplemented with 0.93g/L calcium chloride, 80 mg/L boric acid, myo-inositol and biotin, the ROMT-9 activity reached 90 U/(L*h) in shake flaks. Further optimize the medium composition and fed-batch fermentation, the ROMT-9 production may be greatly enhanced. The recombinant protein was purified by affinity chromatography. Electrophoresis separation in native conditions indicated a molecular mass of the recombinant protein slightly higher (5kDa) than that of the mature protein from rice, suggesting a limited excess of glycosylation.

Key words: Homoeriodictyol, 3’OMTs, ROMT-9, Yarrowia lipolytica, Multi-copy expression, Overproduction