World Academy of Science, Engineering and Technology
International Journal of Biological and Ecological Engineering
Vol:16, No:10, 2022

Bioconversion of Antifungal Antibiotic Derived from Aspergillus Nidulans

Authors : Savitha Janakiraman, Shivakumar M. C

Abstract : Anidulafungin, an advanced class of antifungal agent used for the treatment of chronic fungal infections, is derived from Echinocandin B nucleus, an intermediate metabolite of Echinocandin B produced by Aspergillus nidulans. The enzyme acylase derived from the fermentation broth of Actinoplanes utahensis (NRRL 12052) plays a key role in the bioconversion of echinocandin B to echinocandin B nucleus. The membrane-bound nature of acylase and low levels of expression contributes to the rate-limiting process of enzymatic deacylation, hence low yields of ECB nucleus and anidulafungin. In the present study, this is addressed through novel genetic engineering approaches of overexpression and heterologous expression studies, immobilization of whole cells of Actinoplanes utahensis (NRRL 12052) and Co-cultivation studies. Overexpression of the acylase gene in Actinoplanes utahensis (NRRL 12052) was done by increasing the gene copy number to increase the echinocandin B nucleus production. Echinocandin B acylase gene, under the control of a PermE* promoter, was cloned in pSET152 vector and introduced into Actinoplanes utahensis (NRRL12052) by a ϕC31-directed site-specific recombination method. The resultant recombinant strain (C2-18) showed a 3-fold increase in acylase expression, which was confirmed by HPLC analysis. Pichia pastoris is one of the most effective and versatile host systems for the production of heterologous proteins. The ECB acylase gene was cloned into pPIC9K vector with AOX1 promoter and was transformed into Pichia pastoris (GS115). The acylase expression was confirmed by protein expression and bioconversion studies. The heterologous expression of acylase in Pichia pastoris, is a milestone in the development of antifungals. Actively growing cells of Actinoplanes utahensis (NRRL 12052) were immobilized and tested for bioconversion ability which showed >90% conversion in each cycle. The stability of immobilized cell beads retained the deacylation ability up to 60 days and reusability was confirmed up to 4 cycles. The significant findings from the study have revealed that immobilization of whole cells of Actinoplanes utahensis (NRRL 12052) could be an alternative option for bioconversion of echinocandin B to echinocandin B nucleus, which has not been reported to date. The concept of co-cultivation of Aspergillus nidulans and Actinoplanes utahensis strains for the production of the echinocandin B nucleus was also carried out in order to produce echinocandin B nucleus. The process completely reduced the ECB purification step and, therefore, could be recommended as an ingenious method to improve the yield of the ECB nucleus.

Keywords : acylase, anidulafungin, antifungals, Aspergillus nidulans
Conference Title : ICMMF 2022 : International Conference on Mycology, Mushrooms and Fungi
Conference Location : New York, United States
Conference Dates : October 06-07, 2022