

Optimization Of Capsaicin Acylase Production From *Streptomyces Mobaraensis* In Bench-Top Reactor

Crognale S., Barghini P., Di Matteo P., Federici F., Ruzzi M.

Department of Agrobiological and Agrochemistry, University of Tuscia

Via C. de Lellis, snc – 01100 Viterbo (Italy)

crognale@unitus.it; ruzzi@unitus.it

Capsaicin, the major pungent principle in hot pepper fruit, can be hydrolyzed enzymatically to vanillylamine (a natural precursor of vanillin) using a specific acylase from *Streptomyces mobaraensis*. Production of this enzyme using strain DSM40847 was studied under batch fermentation conditions in stirred tank (STR) and airlift (AR) bioreactors. The process performance in both fermentation devices was different with respect to biomass, enzyme concentration and specific yield (enzyme activity/biomass content); in particular the specific yield was lower in the AR (5.7 mU/g of biomass) than in the STR (6.25 mU/g of biomass). Experiments carried out in STR bioreactors at controlled (DO = 20% of saturation) and uncontrolled dissolved oxygen concentration, and at constant stirrer speeds (300, 450 and 600 rpm) demonstrated that the DO level has no remarkable effect on the production of the capsaicin-hydrolyzing enzyme, which is mainly produced in a cell-associated form.

1. Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla, which is one of the most widely used and important flavouring ingredient worldwide. Since current production of natural vanilla is not sufficient to meet the increasing demand for this flavour compound, vanillin has been a target for biotechnological production by several approaches: use of enzymes to release or generate vanillin from *Vanilla* and other plant material, development of tissue cultures, genetic modification and, finally, use of microbial cultures (Priefert *et al.*, 2001; Walton *et al.*, 2003). Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the major pungent principle in hot pepper fruit (*Capsicum annuum* L.), is a potentially attractive and cheap feedstock for the production of vanillin. Capsaicin can be hydrolyzed enzymatically to vanillylamine (4-hydroxy-3-methoxybenzylamine), a natural precursor of vanillin, by either specific bacterial acylases (Koreishi *et al.*, 2006; Flagan and Leadbetter, 2006) or mammalian carboxylesterases (Oi *et al.*, 1992). So far, the only one bacterial acylase, which efficiently hydrolyzes capsaicin and has been characterized, is the capsaicin acylase from *Streptomyces mobaraensis*. This enzyme is a heterodimeric protein that consists of two dissimilar subunits (61 and 19 KDa) and has a specific activity toward capsaicin 100-10000 times higher than that of capsaicin-hydrolyzing enzymes found in mammalian (Koreishi *et al.*, 2006). The vanillin production from capsaicin as natural source using a bi-enzymatic process (with mammalian and bacterial enzymes) has been