## SCREENING AND OPTIMIZATION OF MYCODEXTRANASE-PRODUCING MICROORGANISMS FOR VARIOUS RARE SUGARS PRODUCTION

Phichamon Noisuwan<sup>1,3</sup>, Goro Takata<sup>2</sup> and Saisamorn Lumyong<sup>1</sup>

<sup>1</sup>Department of Biology, Faculty of Science, Chiang Mai University,
Chiang Mai 50200, Thailand

<sup>2</sup>Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa 761-0795, Japan

<sup>3</sup>Division of Biology, Department of Science, Faculty of Science and Technology, Rajamangala
University of Technology Krungthep, Bangkok 10120, Thailand

Thirty-nine isolates including 5 bacteria and 34 actinomycetes from soil samples and stock cultures at Chiang Mai, Thailand were screened for mycodextranase production using nigeran agar assay incubated at 28°C, 37°C and 45°C for 2 to 12 days. Optimal condition for the enzyme production was 37°C and 45°C for 7 days, the bacteria isolate CMUP1 showed distinctive clear zone-producing isolate. Enzyme activity at 28°C, 37°C and 45°C of this isolate using LB medium revealed equally with *Bacillus circulan* (positive mycodextranase producer) of 0.062 U/ml. However, CMUP1 was an isolate that giving highest activity of the enzyme in most media and incubation conditions. The isolate was preliminarily identified as *Bacillus* sp. Nigerose was a main component after hydrolysis of mycodextran by isolated mycodextranase, which was gave glucose is a main component of nigerose degradation by  $\alpha$  (1-3) glucosidases, and glucose can be starting material to produce rare sugar such as L-fructose, L-psicose and allitol(D-,L-allitol) via Izumoring parthway. The enzyme activity of some selected isolates compared with the positive strain varied depending on the media components based on Packett-Burman experimental design.

## 1. INTRODUCTION

Novel and rare disaccharides have been currently discovering and used in various fields including food industry, medical, pharmacy, cosmetic, and agriculture. Some of them such as nigerose can be used immunopotential substances and it can also be used as a substrate of D-glucose production for further conversion to other rare sugars such as D-psicose or D-allose. Specific enzyme for nigerose production is mycodextranase (EC 3.2.1.61), which hydrolyses the polymeric form of mycodextran (nigeran) to generate nigerose (Okazaki *et al.*, 1992). This enzyme can be produced by some bacteria, actinomycetes and fungi (Okazaki *et al.*, 1995). Mycodextranase (EC 3.2.1.61) is an enzyme specifically hydrolyzed mycodextran to generate nigerose and tetrasaccharide by an endo-type action, indicating that it is cleaving only the  $\alpha$ -1,4-glucosidic bonds in mycodextran (Okazaki *et al.*, 1995). Furthermore, mycodextranase has the synonym as 1,3-1,4- $\alpha$ -D-glucan 4-glucanohydrolase that it is not hydrolyed of alpha-D-glucans containing only 1,3- or 1,4-bonds (Tung and Nordin, 1968).

With the aim to find the novel sources of mycodextranase-producing microbes, this work has been focused on discovery of these microbes from various soils in Thailand. Moreover, the polysaccharides (mycodextran) was cultured by using of agricultural waste for microbial growth in the industrial scale is value added for the organic waste in agricultural country as Thailand.