IC-069 PRODUCTION OF XYLANOLYTIC ENZYMES USING AGRICULTURAL RESIDUES:
I. STUDY ON THE OPTIMUM CONDITIONS FOR PRODUCTION OF XYLANASE FROM
ALKALIPHILIC Bacillus sp. K-1.

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Recently in Thailand there was a problem about water pollution from pulp and paper industries.
To apply the alkalophilic xylanolytic enzymes in pulp prebleaching process for reducing chloride and
adsorpbable organic compounds from these industries, we isolated alkalophilic xylanolytic bacteria from
many sources. We found that the alkalophilic Bacillus sp. K-l isolated from a wastewater treatment plant
of pulp and paper industry in Thailand is the most potent organism for using in pulp prebleaching. The
strain K-l produced extracellular cellulase-free alkaline xylanolytic enzymes containing xylanases (one is
small size xylan-binding endoxylanase), p-xylosidase, arabinofuranosidase and acetyl esterase when
grown in alkaline xylan medium (Ratanakhanokchai, et al, 1999). The xylanolytic enzymes were active
and stable in alkaline condition. Due to the binding ability of xylan-binding region of the xylan-binding
endoxylanase to insoluble xylan, xylanolytic enzymes from the strain K-l hydrolysed insoluble xylem,
lignocellulosic materials and xylan in kraft pulps effectively (Ratanakhanokchai, et al, 2000).

Xylanolytic enzymes from alkalophilic Bacillus sp. K-l could hydrolyze xylan from pulps without
purification but for large scale enzyme production in pulp and paper industries, there is a problem about
the price of xylan (Sigma Co.) (946 $/kg) as sole source of carbon. If instead of xylan, we can use other
carbon sources such as agricultural wastes. We can reduce the price of enzyme and compete the chemical
process which causes waste pollution. In this project, we will use agricultural wastes for production of
alkaline xylanolytic enzymes from the strain K-l. As Thailand is an agricultural country, every year we
have these agricultural wastes more than 45 million ton/year.

In this presentation, the optimum conditions for production of xylanolytic enzymes from
alkalophilic Bacillus sp. K-l was performed in shake flasks. It was found that 1% of corn hull was the best
carbon source for production of xylanolytic enzymes when compared with rice bran and the other
agricultural residues such as rice straw, corn cob and sugarcane bagasse. The SDS-PAGE of culture
supernatant showed that protein which was induced from corn hull, had three more protein bands (49, 39
and 33 kDa) than those from the other carbon sources. These proteins bands might be xylanolytic
enzymes because it had the highest xylanase activity. The best nitrogen source was 0.40% urea when
compared with ammonium nitrate, ammonium sulfate, sodium nitrate and potassium nitrate. When the
bacterium was grown in the alkaline mineral salt medium using 1% of corn hull and 0.40% of urea as
carbon source and nitrogen source, respectively, 1.60 U/ml xylanase activity was detected in the culture
supernatant. The various concentrations of mineral salts such as CaCl2, FeSO4, MgSO4, MnSO4, CdSO4,
CoSO4 and ZnCl2 had no effect on xylanase production.

Xylanase activity also increased when agitation speed increased from 250 to 400 rpm. In addition, when aeration rate increased
from 0.25 to 1.25 vvm, it was found that 1.00 vvm at 400 rpm gave the highest xylanase activity.