CHARACTERIZATION OF XYLANASE FROM *MICROBULBIFER* SP. CL37 FOR INDUSTRIAL APPLICATIONS

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DEDICATION

This dissertation is dedicated to my beloved parents for their endless eternal love, encouragement and support.

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ABSTRACT

Xylan is the most abundant sugar in hemicellulose and can be found in plant biomass. Xylanase produced by microorganisms such as bacteria can be used in industries such as paper and pulp for deinking process. A halophilic bacterium, *Microbulbifer* sp. strain CL37, was previously isolated from mangrove sediment and its extracellular xylanase was characterized in this study. Strain CL37 is a motile Gram-negative bacterium with rod shape, catalase, and oxidase positive. Strain CL37 also can hydrolyse xylan, casein, gelatin, Tween 20, Tween 40, Tween 60 and Tween 80. Cells are sensitive to gentamicin, tetracycline, polymyxin B, doxycycline, minocycline and rifampicin. The xylanase exhibited maximum activity at 70 ℃, pH7, and absent of NaCl. The xylanase remained activity up to 14% (w/v) NaCl indicates it is halotolerant xylanase. The xylanase activity was enhanced in the presence of Al^{3+}, Ca^{2+}, Co^{2+}, Cu^{+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} (112-175% relative activity), stable in K^{+}, Na^{+}, and Ni^{2+} (>80% relative activity), but reduced in the presence of Mg^{2+} (59% relative activity). The xylanase activity also enhanced in the presence of acetone (127% relative activity) and remains stable (>70% relative activity) in most of the tested detergent constituents. Xylanase is also compatible with commercial detergents such as Top®, Dynamo®, Sunlight®, Glo®, Breeze® and Dixan®. Evaluation of the enzymatic deinking activity demonstrated that xylanase from strain CL37 has the ability to detach the adsorbed ink particle from the surface of paper. Collectively, xylanase from *Microbulbifer* sp. strain CL37 could have potential in various applications, such as detergent formulation, lignocellulolytic biofuel production and paper deinking.

ABSTRAK

Xilan merupakan gula paling banyak dijumpai di dalam hemiselulosa dan boleh diperoleh daripada biomas tumbuhan. Xilanase yang dihasilkan oleh mikroorganisma seperti bakteria boleh digunakan dalam industri seperti kertas dan pulpa untuk proses membersihkan dakwat. Bacteria halofilik, *Microbulbifer* sp. strain CL37 telah dipencilkan daripada sedimen bakau dan xilanase telah dicirikan dalam kajian ini. Strain CL37 adalah Gram-negatif bakteria berbentuk rod, positif dalam penghasilan katalase dan oksidase. Strain CL37 juga boleh menghidrolisis xilan, casein, gelatin, Tween 20, Tween 40, Tween 60 dan Tween 80. Selain itu, sel juga sensitif kepada gentamicin, tetracycline, polymyxin B, doxycycline, minocycline dan rifampicin. Xilanase tersebut mempamerkan aktiviti maksimum pada 70℃, pH7, dan ketiadaan NaCl. Xilanase tersebut mengekalkan aktiviti sehingga 14% (w/v) NaCl dan ini menunjukkan ianya merupakan halotoleran xilanase. Seterusnya, aktiviti xylan dapat dipertingkatkan dalam kehadiran Al^{3+}, Ca^{2+}, Co^{2+}, Cu^{+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, dan Zn^{2+} (112-175% aktiviti relatif), stabil dalam K^{+}, Na^{+}, dan Ni^{2+} (>80% aktiviti relatif), tetapi dikurangkan dalam kehadiran Mg^{2+} (59% aktiviti relatif). Xilanase aktiviti juga dipertingkatkan dalam kehadiran acetone (127% aktiviti relatif) dan stabil (>70% aktiviti relatif) dalam kebanyakkan konstituen detergen yang diuji. Xilanase juga serasi dengan detergen komersial seperti Top®, Dynamo®, Sunlight®, Glo®, Breeze® dan Dixan®. Penilaian aktiviti pembersihan dakwat enzimatik menunjukkan xilanase daripada strain CL37 mempunyai kebolehan untuk melepaskan zarah dakwat yang terserap daripada permukaan kertas. Secara kolektif, xilanase daripada *Microbulbifer* sp. strain CL37 mempunyai potensi dalam pelbagai aplikasi, seperti dalam formulasi detergen, pengeluaran biofuel daripada bahan lignoselulosa dan membersihkan dakwat daripada kertas.

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LIST OF ABBREVIATIONS

Al^{3+}	-	Aluminium ion
$Al_2(SO_4)_3$	-	Aluminium sulfate
BOD	-	Biological oxygen demand
Ca^{2+}	-	Calcium ion
$CaCl_2$	-	Calcium chloride
CLSI	-	Clinical and Laboratory Standards Institute
CMC	-	Carboxymethyl-cellulose
Co^{2+}	-	Cobalt ion
$CoCl_2$	-	Cobalt chloride
COD	-	Chemical oxygen demand
Cu^{+}	-	Copper (I) ion
Cu^{2+}	-	Copper (II) ion
CuCl	-	Copper (I) chloride
$CuSO_4$	-	Copper (II) sulfate
DMSO	-	Dimethyl sulfoxide
DNS	-	Dinitrosalicylic acid
Fe^{2+}	-	Ferum (II) ion
Fe^{3+}	-	Ferum (III) ion
$FeCl_3$	-	Ferum (III) chloride
$FeSO_4$	-	Ferum (II) sulfate
GH	-	Glycoside hydrolase
H_2O_2	-	Hydrogen peroxide
K^{+}	-	Potassium ion
KCl	-	Potassium chloride
$K_3[Fe(CN)_6]$	-	Potassium ferricyanide
MA	-	Marine Agar
Mg^{2+}	-	Magnesium ion
$MgSO_4$	-	Magnesium sulfate
Mn^{2+}	-	Manganese ion
$MnCl_2$	-	Manganese chloride

Na^+	-	Sodium ion
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
Ni^{2+}	-	Nickel ion
$NiSO_4$	-	Nickel sulfate
OD	-	Optical density
ONPG	-	Ortho-nitrophenyl beta-D-galactopyranoside
rpm	-	Rotation per minute
SDS	-	Sodium dodecyl sulfate
SDS-PAGE	-	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sp.	-	Species (singular)
spp.	-	Species (plural)
UV	-	Ultraviolet
Zn^{2+}	-	Zinc ion
$ZnSO_4$	-	Zinc sulfate

LIST OF SYMBOLS

A_{540}	-	Absorbance at 540 nm
α	-	Alpha
β	-	Beta
cm	-	Centimeter
℃	-	Degree celcius
=	-	Equal
g	-	Gram
>	-	Greater than
≥	-	Greater than or equal to
h	-	Hour
kPa	-	Kilo Pascal
<	-	Less than
≤	-	Less than or equal to
µg	-	Microgram
mg/mL	-	Milligram per milliliter
mL	-	Milliliter
mm	-	Millimeter
mM	-	Millimolar
M	-	Molar
nm	-	Nanometer
-	-	Negative
n	-	Number
OD_{540}	-	Optical density at 540 nm
OD_{600}	-	Optical density at 600 nm
%	-	Percent
+	-	Positive
®	-	Registered trademark
×	-	Times
U	-	Units
U/mL	-	Units per milliliter

v/v	-	Volume per volume
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Lignocellulose which is the major component of plant biomass is abundant in our planet and many of them is considered as waste and dispose through burning which can cause environmental pollution (Howard *et al.*, 2003). The lignocellulosic materials can be widely used in many industries such as paper, pulp and biofuel production industries (Chakdar *et al.*, 2016). The use of lignocellulosic materials as sustainable biomass in industries can potentially help to reduce the production cost as well as reducing environmental problems.

Lignocellulose consists of three types of polymers namely cellulose, lignin and hemicellulose. Cellulose is the main component of lignocellulose and can be found in the protective cell wall of plants (O'sullivan, 1997). Lignin is the component mainly found in the cell wall of woody tree species to provide structural support and resistance against microbial attack (Pérez *et al.*, 2002; Duval and Lawoko, 2014; Norgren and Edlund, 2014). Hemicellulose is heterogeneous polymers of sugar acids, pentoses and hexoses. Xylan is found abundant sugar in hemicellulose and it gets high attention today due to its applications in many industries (Coughlan and Hazlewood, 1993).

Xylanase is the enzyme used to degrade xylan in industrial processes such as biopulping of wood and biofuel production. Many organisms have been reported to produce xylanase (Polizeli *et al.*, 2005). Bacterial xylanase has been more attractive than fungal xylanase to be used in industries because bacterial xylanase has optimum pH in 7-9 while pH optimum for fungal xylanase is in acidic range (pH 4-6). Many xylanase using industries such as paper and pulp industry normally operate in neutral to slightly alkaline condition. This means that low pH requirement for optimum

activity of fungal xylanase is an extra steps in industrial processes, which directly increase the production cost thus making fungal xylanase less attractive (Chakdar *et al.*, 2016).

Members from genera of *Arthrobacter*, *Bacillus*, *Cellulomonas*, *Microbacterium*, *Micrococcus*, *Paenibacillus*, *Pseudoxanthomonas*, *Rhodothermus* and *Staphylococcus* have been reported as xylan degrading bacteria (Beg *et al.*, 2001; Gupta *et al.*, 2001; Chapla *et al.*, 2012; Chakdar *et al.*, 2016). The extremophilic bacterial xylanases have advantage in industrial application, which these xylanases could be obtained from thermophilic, alkaliphilic and halophilic bacteria. Characterization on new xylanase producing bacteria and exploration on their xylanase with extraordinary properties are always in demand for researches and applications.

1.2 Problem Statement/Significance of Study

Halophilic bacteria produce unique enzymes that could be applied in various industries. For example, xylanase could be used in paper deinking and lignocellulosic waste degradation. Most of the current commercial xylanases are originated from fungus origin. These xylanases are active in acidic condition that are not suitable to be used in paper and pulp industry, which the working pH of this industry is usually in neutral or slightly alkali condition. Many xylanases produced from bacteria are found to be active in neutral and alkaline pH. Characterization on xylanase producing bacteria have been studied such as genera *Streptomyces*, *Glaciecola* and *Gracilibacillus* (Guo *et al.*, 2009; Giridhar and Chandra, 2010; Liu *et al.*, 2013). However, no study was reported on characterization of xylanase from genus *Microbulbifer*. In this study, a xylanase producing halophilic bacterium, *Microbulbifer* sp. strain CL37 and its crude xylanase were characterized.

1.3 Research Goal

1.3.1 Research Objectives

The objectives of the research are:

i. To characterize *Microbulbifer* sp. strain CL37 from phenotypic aspect.

ii. To determine the effect of pH, temperature and salinity on xylanase activity and stability.

iii. To assess the stability of xylanase in presence of various metal ions, organic solvents and detergents.

iv. To determine the xylanase efficacy in paper deinking activity.

1.4 Scope of Study

The previously isolated halophilic bacterium *Microbulbufer* sp. strain CL37 was streaked from glycerol stock and the extracellular xylanase activity was screened qualitatively. Bacterial phenotype was studied by checking bacterial morphology, physiology and biochemical tests. After that, effects of pH, temperature and salinity on xylanase activity and stability were determined. Xylanase stability in the presence of various metal ions, organic solvents and detergents was assessed. Lastly, the efficiency of extracellular xylanase of *Microbulbifer* sp. strain CL37 in paper deinking activity was analysed by using qualitative and quantitative methods.

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