ISOLATION AND IDENTIFICATION OF A 3-CHLOROPROPIONIC ACID (3CP) DEGRADING *ASPERGILLUS ACULEATUS* STRAIN MBS_179

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To my beloved father, mother, brothers, sisters, wife, and my daughters

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ABSTRACT

The halogenated organic compounds which are used in agricultures and industries consider as xenobiotics. They cause the major group of environmental pollutions. They are potential toxicity and carcinogenicity effect and they pose health risks to humans and animals. In this study, the main objectives are to isolate and identify fungus from bread that has the ability to utilize 3-chloropropionic acid as a sole source of carbon and energy. The 3-chloropropionic acid (3-CP) is a synthetic halogenated compounds and classified as of ß-chlorinated alkanoic acid (ß-chloro substituted haloalkanoates). This compound is used as intermediates for the synthesis of pharmaceuticals, herbicides and pesticides. The MBS_179 fungus which was isolated has been identified by using conventional and molecular analysis techniques. The MBS_179 showed the ability to grow on minimal medium containing only 3-chloropropionic acid as a sole carbon source and energy. The growth was measured after inoculated into 20 mM 3-chloropropionate liquid minimal media (pH 7) and incubated at 30°C in a rotary incubator at 120 rpm over 20 days period. Sample was taken at 2 days intervals. The molecular analysis depended on the amplified 18S rRNA gene sequence of the MBS_179 and the phylogenetic relationship was constructed using MEGA4 software® in order to verify their evolutionary distance. From our current study, analysis of 18S rRNA gene and the evolutionary relationship described the subspecies and characteristic of the MBS_179 which had 100% identity to the *Aspergillus aculeatus*. This study demonstrates the ability of this MBS_179 to utilize 3-CP that indicates it may has a significant role to play in the removal of this environmental pollutant.

ABSTRAK

Sebatian organik berhalogen yang digunakan dalam sektor pertanian dan perindustrian adalah dianggap sebagai bahan xenobiotics. Bahan-bahan ini merupakan punca utama pencemaran alam sekitar. Ia berpotensi memberikan kesan kotoksidan dan kekarsinogenan yang memberi risiko kepada kesihatan manusia dan haiwan. Dalam kajian ini, objektif utama adalah untuk mengasingkan dan mengenalpasti kulat daripada roti yang mana mempunyai keupayaan dengan menggunakan 3-chloropropionik sebagai sumber utama karbon dan tenaga. Asid 3-chloropropionik (3CP) merupakan sebatian sintetik berhalogen dan diklasifikasikan sebagai asid β-chlorinated alkonic (β-chloro menggantikan haloalkanoates). Sebatian ini digunakan sebagai perantaraan untuk sintesis farmaseutikal, racun herba dan racun perosak. Kulat MBS_179 yang telah diasingkan telah dikenalpasti dengan menggunakan teknik konvensional dan molekul. MB_179 menunjukkan keupayaan untuk tumbuh dalam medium yang minimum yang hanya mengandungi asid 3-chloropropionik sebagai sumber tunggal kepada karbon dan tenaga. Pertumbuhan diukur berdasarkan penggunaan asid 3-chloropropionic dalam medium yang minimum. Analisis molekul bergantung kepada kekuatan urutan gen 18S rRNA daripada MBS_179 dan hubungan filogeni dibina menggunakan perisian MEGA4 untuk mengesahkan evolusi menunjukkan subspecis dan cirri-ciri MBS_179 yang mana 100% mempunyai identiti sebagai *Aspergillus aculeatus*. Kajian ini menunjukkan keupayaan MBS_179 menggunakan 3CP yang menunjukkan bahawa ia mungkin memainkan peranan penting untuk meningkatkan penyingkiran pencemaran pada alam sekitar.

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CHAPTER 1

INTRODUCTION

1.1 Overview

The dawn of the 20th century, marked the beginning of the use of haloaliphatic compounds in industries and agriculture. After some decades of their discovery, the hazard associated with the use of these compounds started to manifest in the environment and in living organisms. The quest by people for a cleaner lifestyle lead to the use of man-made chemical such as haloaliphatic hydrocarbon compounds as herbicide, pesticide, fungicides, paint, solvents and industrial chemicals which cause harmful damage to the environment via a pollution (Rasul and Chapalamaduqu, 1991).

Halogenated compounds are very essential group of xenobiotics. 3-chloropropionic acid (3CP) that belongs to the class of chlorinated mono carboxylic acid or β-chloro substituted haloalkanotes. They are considered as a possible chemical inclusion in pesticides. This compound has a carcinogenic and genotoxic effect on animals and humans (Jing and Huyop, 2007).

A major process through which xenobiotic chemicals such as chloroaliphatic compounds is removed from the environment by microorganisms is called biodegradation (Sinha *et al*., 2009). The microbial degradation of a contaminant usually occurs because microorganisms derive support for growth through the use of the contaminant as an electron donor and carbon source. Hydrolytic dehalogenases typifies the major step in the degradation of haloaliphatic compounds. These enzymes propel the cleavage of halogen-carbon bonds by nucleophilic substitution, and change the halogen ion by a hydroxyl group obtained from water (Schwarze *et al*., 1996). Field and Alvarez (2004) noted that by using the dehalogenase enzyme, compounds containing chlorine are being metabolized and chlorine substituent are enzymatically removed to form non-halogenated compounds.

The decontamination of these substances can be done by using biological or non-biological degradation process. Since the biological methods are reasonable, harmless, and environmentally friendly, they are favored (Janssen *et al*., 2005). Bioremediation is a process that involves the use of biological methods like microbial or enzymatic biocatalysts (Janssen *et al*., 2001). Many years ago, studies on microbial degradation of harmful waste started, due to growing of xenobiotic compounds in industrial and agricultural uses. Microbial degradation of xenobiotic chemicals such as chloroaliphatic compounds involves the ways and processes of biodegradation that assist in removing these compounds from the environment. There are so many studies had been done on the microbial catabolism of dehalogenase (Olaniran *et al*., 2001, 2004; Jing and Huyop, 2007; Jing *et al*., 2008). Most of the degradation pathway of halogenated compounds includes dehalogenation, the cleavage of the carbon-halogen bond to release halogen, catalyzed by 'dehalogenase' (Fetzner and Lingens, 1994; Janssen *et al*., 2005).

The ability of the enzyme to catalyze the dehalogenation of various halogen-substituted organic acids was investigated and the highest activity was found with 3-chloropropionic acid as a sole carbon source in the growth medium

(Jing *et al*,.2008). There are many different microorganisms (like bacteria and fungi) which are capable of utilizing halogenated compound as the only carbon source and consequently, are supposed to have a significant role in their natural detoxification (Janssen *et al*., 2001; Song *et al*., 2003; Park *et al*., 2003), and is a major step in aerobic mineralization pathways of many halogenated compounds that occur as environmental pollutants (Janssen *et al*., 2005). A number of these microorganisms were originally isolated from polluted soil and sewage oxidation ponds (Olaniran *et al*., 2001, 2004). So far, fungi had been found to be isolated to degrade halogenated compound particle (α-haloalkanoic acid).

The current study focuses on the isolation and identification of other environmental microorganisms to dissociate the presence of 3-chloropropionic acid. In detail, the aim of this study is identification of the fungus isolated from the bread that can degrade 3-chloropropionic acid based on the 18S rRNA molecular approach. This will be a genotypic dependable approach to characterize the isolated fungi, compared to conventional methods like morphological procedures.

1.2 Problem statement

The widespread usage of chloroaliphatic hydrocarbon compounds in industries has resulted in extensive pollution of environment and the ground water (Fliermans, 1989). Chlorinated aliphatic compounds form one of the most vital groups of industrially produced chemicals. Several of these compounds are weakly degraded in the environment (Reineke, 2001). The 3-chloropropionic acid which is one of these compounds, considered as a possible chemical inclusion in certain herbicide and carcinogenic and genotoxic to the animal and human. The uses of herbicides in general (2,2DCP and 3CP) are the main contributor of the environmental pollution (Jing *et al*., 2005). Aerobic biodegradation by using microorganisms such as fungi, to

degrade the chloroaliphatic hydrocarbon compounds to carbon dioxide, water, and hydrogen chloride is more efficient than anaerobic or chemical processes. Therefore, the aerobic biodegradation by using microorganism such as fungi is preferable as it is costly and biologically effective.

1.3 Goals and Objectives

The main goals of the current research were to isolate and identify 3-chloropropionic acid degrading fungi. Therefore, to achieve these goals, the following objectives were considered:

(i) To isolate of a single fungus from the Bread that can degrade 3CP.

(ii) To identify and characterize the isolated fungus by determining the morphological appearances and genetic sequence analysis.

(iii) To study the growth capability of isolated fungus on 3CP.

1.4 Research Scope

The scope of this research includes the molecular identification of the fungus that isolated from bread, which has ability to degrade 3-chloropropionic acid by amplifying the ITS region, whereas the universal ITS primers (ITS1 and ITS4) used to run the PCR (White *et al*., 1990). In addition, the conventional methods that used for further identification and characterization.

1.5 Research Significance

The outcome of this study tends to identify fungal species from bread capable of degrading 3CP compounds. Novel fungal species was isolated, which has the ability of degrading the chloroaliphatic compounds and this new strain of fungus, which is previously unknown for its reaction of chloroaliphatic compounds.

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