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Isolation and identification of lipase producing bacteria from coal mines of Darra Adam Khel, Khyber Pakhtoonkhwa, Pakistan

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Abstract

Lipase producing bacteria are widespread in nature and play a key role in our daily lives. These bacteria have the capability to produce lipase enzyme which have many industrial applications. Among the various lipases, bacterial are the most significant as compared to animal and fungal lipases. The aim of current study was to isolate and to identify lipase producing bacteria. Different coal samples were collected from different areas of Darraa Adam Khel Kohat, Khyber Pakhtunkhwa, Pakistan. A total of 14 isolates were recovered from these coal samples. Samples were serially diluted and were cultured for further processing. Identification of lipolytic isolates was carried out on 1 percent tri-butarine added into the Luria Brutenia (LB) medium. Lipase producing was identified having clear zones around their colonies. Identified isolates were subjected to the gram staining and biochemical test like Oxidase Test, Catalase Test, Motility Indol Ureas (MIU) Test, Triple sugar iron (TSI) Test and Simmon Citrate Test.

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Introduction

Human demand to variety of products gradually increases as the process of civilization and industrialization increases (Kumar *et al.,* 2012). Natural products pulled more human attention as compared to industrial synthetics because they are easily degradable and do not deteriorate our environment (Akanbi *etal.,* 2010).The major group of biocatalyst are Lipases that catalase the hydrolysis of insoluble triacylglycerols to glycerol mono, diacylglycerol, and free fatty acids and subsequent synthesis of esters. Lipases do not require co-factor for catalytic activity and remain active in organic solvent (Araviandan *etal.,* 2006).

Lipases are water soluble enzymes which have the capability of hydrolyzing glycerol acting upon acylglycerols to liberate fatty acids and glycerol. Lipases are serine hydrolases and consist of harmony sequence G–X1–S–X2–G as the catalytic moiety, where G= glycine, S = serine, X1 = histidine and X2 = glutamic or aspartic acid (Svendsen, 1994). a major group of biocatalysts i.e Lipases have immense biotechnology applications and perform very specific chemical transformation (biotransformation) which make them very popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries (Joseph *etal.,* 2008; Park *et al.,* 2005; Gupta *etal.,* 2007; Grbavcic *etal.,* 2007).

Microbial lipases today occupy importance among biocatalysts, because they have the capacity to catalyze a great variety of reactions in aqueous and non-aqueous media (Saxena *etal.,* 2003). Extracellular lipases are known as potential of many microorganisms, including bacteria, fungi, and yeast (Veerapagu *etal.,* 2103). Different classes of lipolytic enzyme produced by bacteria such as carboxylesterases (EC 3.1.1.1), which hydrolyse small ester containing molecules at least partly soluble in water, true lipases (EC 3.1.1.3), which display maximum activity towards water insoluble long-chain triglycerides, and various types of phospholipase (Arpigny *etal.,* 1999). Bacterial lipases are important in food and dairy industry for the hydrolysis of cheese ripening, milk fat, flavour improvement and lipolysis of butter fat and cream (Falch, 1991).Knowledge about bacterial lipolytic enzymes is increasing at a rapid and exciting rate. Looking forward to the enormous market demand we have attempted the isolation of bacteria producing lipases. The aim of the research study was to isolate and to identify lipase producing bacteria from different coal samples of Darra Adam Khel Kohat, Khyber Pakhtunkhwa, Pakistan.

Material and methods

Coal Sample Collection

Coal Sample collection was performed from Feb to March (2014) and sampling was done randomly from different areas of Darra Adam Khel. Around 7 coal samples were collected from different coal mines. Samples were collected aseptically in 250 ml sterilize auto cleavable glass bottles and were carried out to the laboratories. Prevent from dust, soil, or other pollutants from contacting the sample container during transportation in the laboratory. All the equipments used in the experimental work were sterilized at 121°C temperature for 15 minutes at pressure of 15-lb/inch2 (psi) by autoclaving.

Isolation of Lipase Producing Bacteria

LB Agar was used for determination of Lipase producing bacteria. All tubes in which Lipase producing bacteria were cultured then subculture by streaking on LB Agar plate's ad incubated at 35C for 18-24 hrs. Positive plates contained typical colonies with metallic sheen were inoculated for morphological and biochemical tests. For screening of lipase enzyme, 1% tributyrine was used along with LB agar.

Identification of Bacteria

The lipase producing bacteria were identified and confirmed by gram staining technique and further confirmed via different biochemical tests including Oxidase Test, Catalase Test, Motility Indole Urease (MIU) Test, Triple sugar iron (TSI) Test and Simon Citrate Test (Nadeem *et al*., 2015).

Statistical Analysis

The analysis was done by using the statistix software for windows.

Results and discussions

Isolation

Research was focusing on lipolytic bacteria present in coal mines. Samples were collected from coal mines of Darra Adam khel. Samples were inoculated on LB media and were incubated for 24 hours. Total 14 species were isolated. Some of them produce white colonies and some produce yellow colonies. Colony morphology of some isolated were rounded and mucoid. Some rounded colonies were also gave rough appearance. Irregular shaped colonies were also observed. Rohit Sharma (2001) *et al.*,work on lipase but the results were not consistent to the present work.

Table 1. Showing the results.

Isolates	Gram Staining	Oxidase	Catalase	Simmon Citrate
G	Gram Positive and rod shaped	Positive	Negative	Negative (Green)
Y	Gram Positive and rod shaped	Negative	Negative	Positive (Blue)
S	Gram Positive and circular shaped	Positive	Negative	Negative (Green)
R	Gram Positive and rod shaped	Positive	Negative	Negative (Green)

Screening of Lipase Producers

All the 14 isolates were screened for lipolytic activity. Among them 4 isolates were detected positive for lipid degradation. Culturing on media containing lipids the lipolytic isolate produced clear zones as shown in figure 1. While 10 isolates doesn't produce zone which shows that these isolates were unable to degrade lipids. The work ofFariha Hasan (2010) *et al.*, is not consistent with present work.

Identification of Bacteria

Gram staining results showed some of isolates were cocci and some were rod shaped. In rounded shaped isolates were observed in the form of bunch, chain and pair. Some rounded shaped isolates were also observed as single cell and the same were observed for rod shaped lipolytic isolates as shown in table 1. Wei Fenga *et al.*, (2001) work is consistent to present work.

Table 2. Shows the results of MIU and TSI tests.

Isolates	M	I	U	TSI
G	Positive	Positive	Positive	H_2S present Gas positive
Y	Positive	Positive	Negative	H_2S present Gas positive
S	Positive	Positive	Positive	H_2S present Gas positive
R	Positive	Positive	Negative	H_2S present Gas positive

All positive isolates were further processed for biochemical characterization. From theoxidase test, G, R and S labeled isolates shows positive oxidase reaction while Y shows negative. R. Gupta (2004) *et al.*, work on lipase but not consistent with present work. Then the catalase tests were done which resulting the bacteria were catalase negative. Simmon citrate test were also performed for lipolytic isolates

and the isolate showed citrate positive reaction. M. Shakila Begam's (2012) work is not consistent with present result as shown in the table 1.

Fig. 1. Lipase producing bacteria.

Table 2 showing the results of MIU and TSI tests which were also performed for all 4 lipolytic isolates. Isolate G were positive for all MIU profile and also for H_2S gas production. Y isolate were positive for motility and Indole while negative for Urease. It was positive for H_2S gas production. Isolate S were observed positive for MIU reactions and also for H_2S gas. R isolate were positive for M and Indole while negative for Urease. It was observed positive for H_2S gas production.

Conclusion

The present study highlighted that the coal mines that were present in Darra Adam Khel are rich in lipase producing bacteria. Further study on molecular characterization of lipases and lipases producing indigenous bacterial flora is needed for novel discovery which can be further used in many newer areas where they can serve as potential biocatalysts.

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