Isolation and Identification of Lipase Producing Bacteria from Vermicompost



A thesis submitted to Bahria University, Islamabad in partial fulfillment of the requirement for the degree of BS in Environmental Sciences

 \mathbf{BY}

RABIA SHAH

USMAN YAQOOB

DANIYAL RAZA

SUPERVISED BY

SYED UMAIRULLAH JAMIL

Department of Earth and Environmental Sciences Bahria University, Islamabad

2014

INDEX

ABSTRACT	I
ACKNOWLEDGMENT	II
CHAPTER 1	
1: INTRODUCTION	
1.1 : Applications of Lipases	2
1.2 :Lipases in Fat and Oil Processing:	2
1.3: Lipases in Food Industry:	3
1.4 : Lipases in Detergents:	3
1.5 : Lipases in Pulp and Paper Industry:	4
1.6 :Lipases in Environmental Management:	4
1.7 : Lipases as Diagnostic Tools:	4
1.8 :Lipases as Biosensors:	4
1.9: Lipases in Tea Processing:	5
1.11 : Use of Lipase in environmental biotechnology	6
1.12 :Compositing in Environmental Biotechnology	7
1.13 : Bacteria	7
1.15: Compost Microorganisms	8
1.16: Lipase producing Bacteria	9

Chapter 2:

LITERA	ATURE REVIEW11	
CHAPTER	.3	
MATER	RIAL & METHODS	
3.1:	Sample collection:	18
3.2	:Materials:	18
3.3:	Isoltion of lipase producing bacteria	18
3.4:	Enrichment	18
3.5:	Identification of Bacterial Isolates	18
3.7	: Preparation of Inoculum	20
3.8:	Incubation	20
3.9	: Analysis of Lipase Production	20
3.11	l : Lipase Agar Assay	21
3.13	3 : Methylorange	22
3.14	4 : Spectrophotometry	22
3.14	4.1 : Determination of Molar Absorption Coefficient	22
CHAPTER	.4	
RES	ULTS:	
4.1:	Identification of Bacterial Isolates	23
4.2	: Agar Plates Assay	24

4.4 : Methylorange as Indicator	25
4.5 : Spectrophotometer	26
Conclusions:	29
Recommendations	29
References	

List of Figures

1.15: Compost Tempera	ture	8
1 1		
4.6: UV Spectroscopy		27

List of Tables

1.10: Industrial Applications of Lipases	5
3.6: Composition of Medium	19
3.10: Bacterial Colonies Count	20
3.12: CFU/ml in the presenance of Substrate	21
4.3: Lipase Assay Formation	25
4.6: Weekly Spectroscopy Results	27

ABSTRACT

Lipases are enzymes that catalyze the hydrolysis of fat and perform essential role in digestion, transport, and processing in dietary lipids. These biocatalyst are interesting substitute for chemical catalyst because of their fast rate of reaction and better specificity. Lipases are produced by various plants, animals and microorganisms. Lipases such as triacylglycerol hydrolyses are important group of enzyme related to biotechnology. The present study was aimed to isolate microorganisms having the ability to produce lipase, from vermicompost. Isolated strains were identified as Staphylococcus sp, Bacillus sp, Pseudomonas sp, and Corynebacterium sp. Detection of lipase enzyme was carried out using three different methods. Isolated strains were grown on agar plates augmented with olive oil as lipid substrate. Formation of transparent rings around bacterial colonies shows hydrolysis of lipids was due to production of extracellular lipases. Methyl orange was used as indicator to detect acidic changes in the medium. Bacterial isolates are grown on agar plates with olive oil as substrate, color change of methyl orange suggested conversion of lipid substrate into fatty acids confirming lipase activity. Production of lipase was also confirmed by UV- spectrometry. UV-spectrometry was carried out after every 7 days for 5 weeks at 470nm; increase in absorption shows presence of lipase. The study was concluded on the note that vermicompost is the potential source of lipase producing bacteria and should be considered for production of bacterial lipases in environmental biotechnology.

ACKNOWLEDGEMENT

We are highly grateful to Almighty Allah, the most beneficent, the most merciful, for giving us strength, courage and resources to complete this research. Putting together a thesis is a challenging task not just for the student but the supervisors who are constantly guiding the students despite their other commitments. We have not had the chance to thank them yet, so we would take this opportunity to thank the individuals who helped us in the completion of our thesis. Foremost, our parents, for always are our pillar of support and a true inspiration in our life, for believing in se more than we believed in ourselves and for teaching us perseverance. Mr. Umair Ullah Jamil Lecturer, Department of Earth and Environmental Sciences at Bahria University, Islamabad, for trusting us as students with individuality and allowing us to develop our own research methodology. Dr. Tariq Sultan; Assoc. Professor, NARC, deserves to be thanked for many reasons, his belief in our ability as capable students, giving us a research direction, encouragement and periodic time restriction reminders which kept us focused throughout the thesis. It is very rare to find supervisors who let you broaden your understanding through your own trials and still provide guidance, we feel fortunate to have such supervisors. We would like to express our gratitude to Dr. Mohammad Zafar, Head of Department Earth and Environmental Sciences, Bahria University, Islamabad. Also, we are grateful to the faculty for providing us with extremely important feedback. Despite the challenges and obstacles, we feel we have grown a lot through this process both personally and academically. As the environment operates as an interconnected Eco-system similarly our journey here could not have been accomplished without the help and support of all those mentioned.