Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [1] January 2023 : 140-143.

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Isolation, Screening and Characterization of lipolytic, Proteolytic and Gelatinolytic microorganisms from organic kitchen waste

Snehal A. Masurkar and Girish .R. Pathade
Krishna Institute of Allied Sciences,
Krishna Institute of Medical Sciences "Deemed to be University",
(Malkapur) Karad-415539, M.S., India.
Email: snehalmasurkar2882@gmail.com

ABSTRACT

Kitchen waste containing organic matter comprises to be a major area of concern for the municipal solid waste disposal. It forms a major percentage of waste generated containing fats, lipids, cellulosic compounds. This study was aimed at isolation of lipolytic, proteolytic & gelatinolytic microorganisms that can rapidly degrade organic kitchen waste. Kitchen waste samples were collected from different waste dumping areas. All samples were screened for their potential for lipase, protease and gelatinase production on tributyrin agar, casein agar & gelatin agar plates respectively. Total 157 isolates were obtained & designated as KW1 to KW 157. Amongst them 10 promising isolates showing maximum zones of hydrolysis for three enzymes were quantitatively assayed for their ability to produce respective enzymes. It was found that KW37, KW91, KW97, KW104 & KW128 were producing enzymes as lipase 7.0, 4.2, 5.6, 4.0 & 6.0 units/mL protease as 4.3, 4.0, 2.3, 5.1& 3.7units/mL &gelatinase as 1.3, 4.2, 3.6, 1.5 & 2.8 respectively. The 16s rRNA identification by EzBiocloud (taxonomically united database) showed that these isolates were KW104 Serratia marcescens, KW37-Micrococcus luteus, KW128-Brevindimonas mediterranea, KW91–Bacillus tequilensis, KW97-Exiguobacterium mexicanum All these isolates showed good potential of lipase, protease & gelatinase activity. Serratia marcescens showed maximum ability of enzyme production. All these isolates are highly promising and can be used for enzyme optimization studies.

Key words: *kitchen waste, lipase, protease & gelatinase , screening*

Received 22.10.2022 Revised 22.11.2022 Accepted 21.12.2022

INTRODUCTION

According to the UN Environment Programme (UNEP) around 931 million tonnes of food go to waste each year which is about 17% of the total food used. Food waste is an unwanted raw or cooked food thrown away during or after food preparation that is no longer fit for consumption or desirable (1). Kitchen waste is characterized by a high organic content containing soluble sugars, lipids, proteins, and other compounds that are readily biodegradable, and generally contain few compounds that inhibit bacteria (2). Inoculation of biodegradable waste with various beneficial microorganisms, such as bacteria, fungi, and actinomycetes for the synthesis of extracellular enzymes, such as lipase, pectinase, protease, amylase, and cellulase, enhances the organic waste degradation rate (3). The biological treatment of kitchen wastes appears to be most cost effective and carry a less negative environmental impact (4). Thus the present study aimed at isolation of bacteria capable of producing extracellular lipase, protease & gelatinase capable of degrading organic kitchen wastes.

MATERIAL AND METHODS

Collection of food waste samples

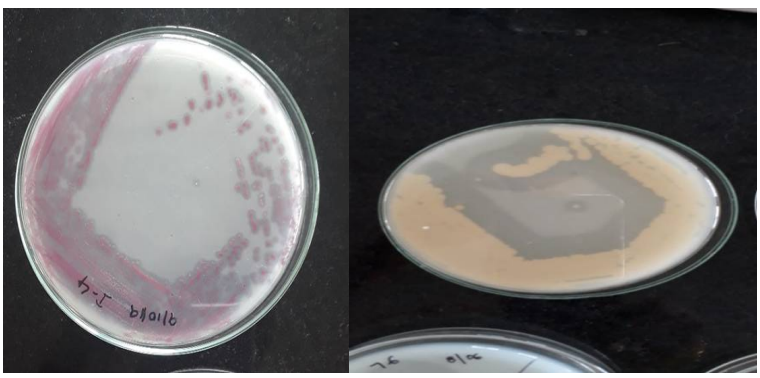
Food waste samples were collected from different dumping areas nearby Karad in polythene bags and brought to the laboratory for further use.

Isolation of Bacteria from Food waste samples:

Isolation of microorganisms was done from kitchen food wastes initially pulverized and by enrichment culture technique in nutrient broth at 30^0C for 10 days & then isolated on solid growth media. Representative well isolated colonies were purified, preserved on agar slants at 4^0C. The isolated strains were further characterized on the basis of their substrate specificity and gram character.

Primary screening of bacteria for lipase, protease and gelatinase production:
The selected bacteria from enrichment were grown on tributyrin agar, casein agar and gelatin agar plates at 30^0C for 48-72 h. The colonies showing positive results as zones of hydrolysis were further selected for production and characterization.
Potential isolate selection by enzyme assay (Secondary screening):
The potential bacterial isolates were freshly inoculated in 100mL tributyrin broth, gelatin broth & casein broth and incubated at 30^0C for 48-h centrifuged broth was (10,000 rpm for 10') quantitatively assayed for enzyme production by standard procedures, protein assay , & fatty acid release assay(5) .
Characterization of bacterial isolates :
The bacterial isolates having highest efficiency of lipase, protease and gelatinase were studied for morphology, biochemical & cultural characteristics and then identified by 16SrRNA gene sequencing & then accession numbers from NCBI were obtained.

RESULTS AND DISCUSSION
Isolation and determination of the metabolic characteristics of bacteria :
The total of 157 bacterial cultures was isolated from kitchen waste samples, out of which 20 were found to produce all three enzymes effectively. The clear zones of hydrolysis were seen around the growth. They were subjected to morphological, cultural & biochemical characteristics. They were designated as KW-2,KW-21, KW25, KW37, KW81, KW81, KW91, KW92, KW97, KW104, KW110, KW115, KW120, KW121, KW128, KW131, KW155, KW135, KW137, KW150 and KW151. (Photoplate 1& 2)
Out of total 20 isolates (table -1) showed high activity of lipase, protease and gelatinase enzymes & from these 20 isolates best promising isolates were selected.
All 20 isolates were grown in respective broth media &cell free supernatants were assayed for lipase, protease & gelatinase activities quantitatively. On the basis of extent and enzyme activity & number of enzymes produced (three) by isolates five isolates were selected for further studies (Table 2). These five isolates were subjected for further identification on the basis of cultural , morphological , biochemical & 16SrRNA identification .KW-37, KW-128, KW-91, KW-97, KW-104 were the best promising isolates showing maximum enzymatic abilities for all three enzymes.
Gene sequencing for the promising isolates
At a molecular level, five potential bacterial isolates viz.KW37, KW128, KW91, KW97 and KW104 were identified by analyzing 16S rRNA gene sequence. BLAST was used to analyze the obtained sequences
The 16 SrRNA gene sequencing studies identified five isolates as (KW37, KW128, KW91, KW97 and KW104) *Micrococcus luteus, Brevundimonas mediterranea, Bacillus tequilensis, Exiguobacterium mexicanum & Serratia marscecens* respectively. All these isolates have ability to produce all the enzymes extracellularly which are effective to be acted upon organic kitchen waste substrates to convert them into mineralised detoxified products (6).Lipase producing organisms using tributyrin agar from kitchen waste contaminated site and subsequent optimization of culture parameters showed 2 potential isolates with lipolytic potentials(7).Vegetable waste degrading bacteria using enriched medium of raw vegetable peels as substrate were isolated and eight cultures producing extracellular enzymes were found effective(8). Microbial consortium that can effectively and rapidly bring about the degradation of the kitchen wastes and help in the process of rapid conversion of kitchen wastes into the fertilizer that can be applied to soil to increase soil fertility(9). The activity of gelatinase enzyme recovered at 60 % ammonium sulfate saturation fraction and it was 3.6 U/ml which were comparable with our isolates (10).



Photoplate 1 Colonial growth of isolate KW 104 showing clear zone of hydrolysis on tributyrin agar
Photoplate 2 Colonial growth of isolate KW 91 showing clear zone of hydrolysis on gelatin agar

Table 1 enzymatic hydrolysis of potential promising isolates on respective Media:

Isolate no	hydrolysis on tributyrin agar (lipase producers)	hydrolysis on milk agar (protease producers)/ (caesinase)	hydrolysis on gelatin agar (gelatinase producers)
KW-2	+	+	+
KW-21	+	+	+
KW-25	+	+	+
KW-37	++	+	++
KW-81	+	++	++
KW-91	+	+++	+
KW-92	+++	+++	+++
KW-97	++	++	+
KW-104	++	+++	++
KW-110	++	++	++
KW-115	+	+	+
KW-120	+	+	+
KW-121	++	++	++
KW-128	+++	++	++++
KW-131	+	+	+
KW-155	+	+	+
KW-135	+	+	+
KW-137	+++	++	++
KW-150	+	+	+
KW-151	+	+	+

(+ ----- low enzyme production ability
++----- moderate enzyme production ability
+++---- high enzyme production ability)

Table : 2 Production of lipase, protease & gelatinase from the selected bacterial isolates (quantitative study/assay)

Sr. No	Isolate no	Lipase production (EU/mL)	Protease production (EU/mL)	Gelatinase production (EU/mL)
	KW 2	3.8	3.9	2
1.	KW 21	3.4	3.5	2.7
2.	KW 25	3.5	3.8	1.7
3.	KW 37	3.2	4	4.2
4.	KW 81	3.1	5	1.5
5.	**KW 91**	**4**	**5.1**	**4**
6.	KW 92	2.9	4.8	1.4
7.	**KW 97**	**2.4**	**3.7**	**2.8**
8.	**KW 104**	**6**	**4.3**	**1.3**
9.	KW 110	5.4	4.6	1.7
10.	KW 115	5.3	5.1	3.7
11.	KW 120	3.3	2	1.4
12.	**KW 121**	**3.9**	**2.1**	**2.8**
13.	**KW 128**	**2.6**	**2.3**	**3.6**
14.	KW 131	3.3	2.5	3.1
15.	KW 135	2.4	6	2.8
16.	KW 137	2.2	4	2.7
17.	KW 150	6	3.7	3.4
18.	KW 151	2.3	3.5	4.1
19.	KW 155	4	2	4.2

Table 3 16 S r RNA sequencing of the best potential isolates and their accession numbers

Strain No.	Closest Neighbour*	
	Taxonomic Designation	Accession No.*(NCBI)
KW-37	*Micrococcus luteus* NCTC 2665(T)	OP482489
KW-128	*Brevundimonas mediterranea* V4.BO.10(T)	OP482496
KW-91	*Bacillus tequilensis* KCTC 13622(T)	OP482499
KW-97	*Exiguobacterium mexicanum* 8N(T)	OP482500
KW-104	*Serratia marcescens* ATCC 13880(T)	OP482501

CONCLUSION

1. The five promising isolates in the present studies have potential to be used in a consortium for fast degradation of food (kitchen waste).
2. These isolates after optimization studies can be used for commercial production of lipase, proteases and gelatinases.
3. The five isolates were genetically identified as KW37, KW128, KW91, KW97 and KW104 *Micrococcus luteus, Brevundimonas mediterranea, Bacillus tequilensis, Exiguobacterium mexicanum & Serratia marscecens* respectively.
4. These five isolates i.e. *Micrococcus luteus, Brevundimonas mediterranea, Bacillus tequilensis, Exiguobacterium mexicanum & Serratia marscecens* were given accession nos. by NCBI as OP482489, OP482496, OP482499, OP482500 & OP482501.

ACKNOWLEDGEMENTS

Authors are grateful to Hon'ble Chairman Dr. Suresh J. Bhosale and Research Fund Allotment Committee for providing financial assistance and facilities for to the Ph. D research project.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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CITATION OF THIS ARTICLE

S.A. Masurkar and G.R. Pathade: "Isolation, screening & characterization of lipolytic, proteolytic & gelatinolytic microorganisms from organic kitchen waste". Bull. Env. Pharmacol. Life Sci., Spl Issue [1]: 2023: 140-143.