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Isolation, screening and characterization of gelatinase and protease producing microorganisms from organic kitchen waste

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ABSTRACT

The present study was aimed at isolation, screening and identification of gelatinase and protease producing bacteria from the kitchen waste. The study was also focused on the potential of isolates to produce enzyme protease and gelatinase. The total of 157 bacterial isolates were obtained from the organic kitchen waste collected from various nearby sources. The primary screening was done to screen the potential of these isolates for their enzymatic potentials on selective media. The 57 promising bacterial isolates were found to have enzymatic potentials for both gelatinase and protease. The screened isolates were subjected to secondary screening, amongst which five isolates showed maximum production of both gelatinase and protease. These five isolates viz. KW91, KW104, KW121, KW128, KW98 were the most promising isolates showing high degree of milk and gelatin hydrolysis which may be potent in faster degradation of domestic kitchen waste.

Key words: *Protease, Gelatinase, Producing bacteria, Serratia marcesens, Bacillus*

Introduction

An average Indian city produces about 0.8 to 1 kg solid wastes per capita per day. These wastes are collected and dumped into the landfills, causing major pollution (Sarkar *et al.*, 2011).

One of the methods that can be followed for the degradation of kitchen waste is composting wherein microbes degrade the waste (Sarkar *et al.*, 2011). The kitchen waste comprises of high concentration of gelatinous and proteinous contents which are acted upon by bacteria owing to their potential abilities in enzyme production. A consortium of effective microorganisms contains many species of microorganisms capable of coexisting and bringing out the effective degradation of organic waste. (Higa, 1996). However, traditional composting method takes relatively long time, several days to months. Recently, rapid or fast methods have been developed and are quite attractive and ecofriendly. The composting by conventional methods may take several days to weeks or several months. Also, one of the limitations of these methods is 100% degradation does not takes place at the end of the process and some biodegradation process continues even after application of the end product (fertilizer) to the soil. The period required for degradation is still too prolonged. The increase in the efficacy of the process is therefore desirable. The composting at home can be used as a sound method of kitchen waste management, the waste can be managed at source itself and thereby it is recycled. It results in a sanitized and stabilized

product rich in humic substances that can be used as fertilizer.

Materials and Methods

Collection of food waste samples

Food waste samples were collected from different houses, canteens and cafeterias nearby karad in polythene bags and brought to the laboratory for further use.

Isolation of Bacteria from food waste samples

Isolation of microorganisms is done from kitchen food wastes by enrichment culture technique followed by isolation on solid growth media. The representative well isolated colonies were purified cultures and preserved on agarmedia slants at 4 ^{0}C. The isolated strains were further characterized on the basis of their substrate specificity and gram character.

Primary screening of bacteria for protease and gelatinase production

The selected bacteria from enrichment were grown on milk agar and gelatin agar plates. The colonies showing zones of hydrolysis were further selected for production and characterization.

The gelatinase assay

The promising strain selection was done by Secondary screening method. The potential bacterial strain was freshly inoculated in 100 ml gelatin broth and incubated for 48 h incubation at 37 ^{0}C. Aliquot of 50 ml was taken from the cultured broth and centrifuged at 7000 rpm for 20 min; pellet and supernatant were collected separately. The cell pellet was suspended in 50 ml phosphate buffer and sonicated for 1min. Remaining portion of 50 ml cultured broth was sonicated for 1 min without centrifugation. The gelatinase activity was quantitatively tested according to Tran and Nagano. The reaction mixture contained 0.2% gelatin in water, 0.2 ml of (150 mM) Tris-HCl, and 12 mM $CaCl_2$ with 0.1 ml sample (crude enzyme). The reaction mixture was incubated at 30 ^{0}C for 30 min and stopped by the addition of 0.6 ml of (0.1 N) HCl. The amount of released free amino groups was measured by the Ninhydrin method. The gelatinase activity is expressed mol of leucine equivalent per min/ml of the culture filtrate (Hamza *et al., 2006*). The same mixture except gelatin was used as blank.

The Protease assay

The Protease activity was estimated as follows incubation, the bacterial broth was centrifuged at 7000 rpm for 20 min at 4°c for obtaining the cell free supernatant (CFS). The 1 ml of (CFS) was added to 1ml of 1% (w/v) casein solution in glycine- NaOH buffer of pH 10.5 and allowed to incubate for l0 min at 60^{0}c. The reaction was halted by addition of 4 ml of 5% trichloroacetic acid. The reaction mixture was centrifuged at 3000 rpm for 10 min and to 1ml of supernatant 5 ml of 0.4 M Na_2CO_3 was added followed by 0.5 ml Folin -Ciocaltaeu reagent. The amount of tyrosine released was determined using UV-VIS spectrophotometer at 660 nm against the enzyme blank. The one unit of protease activity was defined as the enzyme required to release l micro-gm of tyrosine per ml per min under standard assay conditions.

Characterization of bacterial isolates

The bacterial isolates showing highest efficiency of producing protease and gelatinase were identified by 16srRNA.

Results and Discussion

Isolation and determination of the metabolic characteristics of bacteria

About 157 bacterial cultures were isolated from the above mentioned sites of which 57 cultures were able to produce both the desired enzymes amongst which 20 best isolates were selected for further study. The clear zone of hydrolysis was seen around the growth. They were subjected to morphological and cultural 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, KW151

From all the 20 best isolates 5 isolates showing maximum enzyme activity for production of both the enzymes were selected and identified on the basis of cultural, morphological, biochemical and 16SrRNA identification.

Cultural characteristics

Gene sequencing for the promising isolates
EzBioCloud(www.ezbiocloud.net)
Reference: Yoon *et al.,* 2017. Introducing Ez Bio Cloud: Ataxonomically united data base of

Table 1. The protease and gelatinase producing abilities of bacterial isolates

Sr. No	Isolate no.	hydrolysis on milk agar (protease producers)	hydrolysis on gelatin agar (gelatinase producers)
1.	KW-2	+	+
2.	KW-21	++	+
3.	KW-25	+	+
4.	KW-37	+++	++
5.	KW-81	++	++
6.	KW-91	++	+
7.	KW-92	++	+++
8.	KW-97	++	+
9.	KW-104	++++	+++
10.	KW-110	++	++
11.	KW-115	+	+
12.	KW-120	+	+
13.	KW-121	+++	++
14.	KW-128	++	++++
15.	KW-131	++	+
16.	KW-155	+	+
17.	KW-135	++	+
18.	KW-137	+	+
19.	KW-150	++	+
20.	KW-151	++	+

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

Table 2. Production of protease and gelatinase from the selected bacterial isolates (quantitative study/ assay)

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

16SrRNA and whole genomeassemblies. *Int J Syst Evol Microbiol.* 67: 1613-1617.

It is evident from Tables 1,2 and Fig. 1 that the kitchen (food) waste samples contained variety of gelatinase and protease producing bacteria. Out of 157 isolates 20 showed all gelatinase and protease activities. The Table 3, 4, 5, 6 showed the promising activities. Out of the promising isolates one was cocci, three were rods and one coccobacillary in nature. The 16 SrRNA gene sequencing studies identified them as *Micrococcus luteus, Bacillus tequilensis, Exiguobacterium mexicanum* and *Serratia marscecens.* These five isolates showed 4.0 and 4.2U/ml, 2.3 and 3.6U/ml, 5.0U/ ml and 4.0U/ml, 3.7 and 2.8U/ml and 4.3U/ml and 1.3U/ml of gelatinase and protease activities respectively.

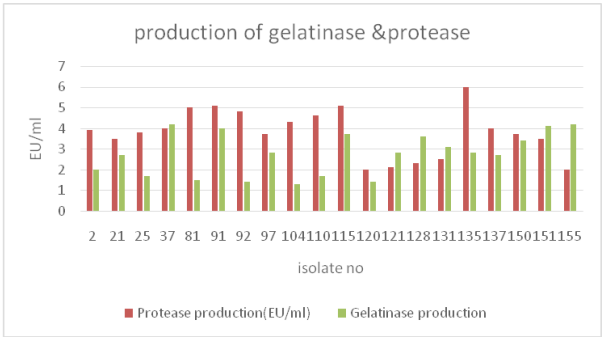

Table 3. Colony characteristics of five promising isolates

Isolate no	Size	Shape	Color	Margin	Elevation	Opacity	Consistency
KW-37	2mm	Circular	Red	Entire	Low convex	Opaque	Moist
KW-128	1mm	Circular	White	irregular	Flat	translucent	Dry
KW-91	2mm	Circular	white	irregular	flat	translucent	Moist
KW-97	1mm	Circular	white	irregular	flat	translucent	Moist
KW-104	2mm	Circular	orange	Entire	Convex	Opaque	Moist

Table 4. Gram nature and motility property of the promising isolates

Sr. No.	Isolate no	Gram Nature	Motility
1.	KW-37	Gram positive, cocci shaped.	Motile
2.	KW-128	Gram Positive, rod shaped	Motile
3.	KW-91	Gram positive rods	Motile
4.	KW-97	Gram positive rods	Motile
5.	KW-104	Gram negative, coccobacilli/short rods	Motile

Table 5. Biochemical Characteristics of the promising isolates

Test	KW-37	KW-128	KW-91	KW-97	KW-104
Sugars :-Glucose	Negative	Positive	Positive	Positive	Positive
Sucrose	Positive	Positive	Positive	Positive	Positive
Fructose	Negative	Positive	Positive	Positive	Positive
Arabinose	Negative	Positive	Positive	Positive	Positive
Lactose	Positive	Positive	Positive	Positive	Positive
Mannitol	Positive	Negative	Negative	Negative	Negative
L tryptophan (indole production)	Negative	Positive	Positive	Positive	Positive
Sodium pyruvate (vogesproskauer)	Negative	Positive	Positive	Positive	Positive
Methylene red	Positive	Positive	Positive	Positive	Positive
Urease	Positive	Negative	Negative	Negative	Negative
Catalase	Negative	Positive	Positive	Positive	Positive
Citrate	Positive	Positive	Positive	Positive	Positive
Gelatine hydrolysis	Positive	Positive	Positive	Positive	Positive
Starch hydrolysis	Positive	Positive	Positive	Positive	Positive

Table 6. Summary of the closest neighbor (s):

Strain No.	Closest Neighbour*	
	Taxonomic Designation	Accession No.*(NCBI)
KW-37	*Micrococcus luteus* NCTC2665 (T)	OP482489
KW-128	*Brevundimonas mediterranea* V4.BO.10(T)	OP482496
KW-91	*Bacillus tequilensis* KCTC13622(T)	OP482499
KW-97	*Exiguobacterium mexicanum* 8N (T)	OP482500
KW-104	*Serratia marcescens* ATCC13880(T)	OP482501

Conclusion

These promising isolates have potential to be used in a consortium for fast degradation of food (kitchen waste).

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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