Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 22 | No. 2 | pp. 997-1002 | 2023

Original Research Paper

https://doi.org/10.46488/NEPT.2023.v22i02.045

Open Access Journal

Microbial Consortia Preparation for Amylase, Protease, Gelatinase and Lipase Production from Isolates Obtained from Organic Kitchen Waste

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 29-09-2022
Revised: 02-11-2022
Accepted: 12-11-2022

Key Words:
Amylase
Protease
Lipase
Gelatinase
Kitchen waste
Microbial consortium

ABSTRACT

Households, restaurants, canteens, and hotel wastes constitute kitchen waste. Every day our growing cities generate more and more waste, which is overloading our municipal systems. The main aim of the present work was to prepare a microbial consortium that can effectively and rapidly bring about the degradation of kitchen wastes that can be used in agricultural soils. More than 100 different bacterial isolates were obtained from various kitchen waste dumping areas. The bacterial isolates were studied to produce enzymes like amylase, gelatinase, lipase, and protease on respective media plates. The best 20 isolates were subjected to enzyme quantification. The isolates showing maximum production for all four enzymes were selected for consortia preparation. The consortia of isolates were prepared by permutation combinations. Amongst all consortia prepared consortium No. 7 showed maximum enzymatic potential. The bacterial isolates in the best consortium (No. 7) were further characterized and identified as KW104 *Serratia marcescens,* KW37 *Micrococcus luteus,* KW128 *Brevindimonas mediterranea,* KW91 *Bacillus tequilensis,* and KW97 *Exiguobacterium mexicanum.* This consortium showed rapid degradation of waste as compared to others in 15 days duration of time showing good potential for compost formation when applied to plant growth.

INTRODUCTION

Organic kitchen waste comprises to be a major proportion of the waste generated, causing pollution. These wastes are collected and dumped into landfills, causing major pollution (Bouallagui et al. 2004). The traditional composting method takes a relatively long time, several days to months. Recently, rapid or fast methods have been developed and are quite attractive and eco-friendly. Also, one of the limitations of these methods is that 100% degradation does not occur at the end of the process and some biodegradation process continues even after the end product (fertilizer) is applied to the soil. The period required for degradation is still too prolonged. Composting is one of the methods that can be followed for the degradation of kitchen waste, wherein microbes degrade the waste (Sarkar et al. 2011). A consortium of effective microorganisms contains many species of microorganisms capable of coexisting and bringing out the effective degradation of organic waste (Higa 1996). The biological treatment of kitchen waste appears to be the most cost-effective and carries less negative environmental impact (Coker 2006). Kitchen waste contains a high proportion of biodegradable organic material and therefore, microorganisms can play important role in its degradation (Pan et al. 2012). The increase in the efficacy of the process is therefore desirable. Composting at home can be used as a sound method of kitchen waste management, and the waste can be managed at the source itself and thereby recycled.

MATERIAL AND METHODS

Food Waste Samples

Food waste samples were obtained from houses, canteens, and cafeterias nearby Karad and preserved in the laboratory until further use.

Isolation of Bacteria from Food Waste Samples

Microorganisms are isolated from kitchen food wastes in liquid enrichment culture, followed by isolation on solid media. Representative pure isolates were characterized based on their biochemical and morphological properties and preserved at 40°C till further use.

Primary Screening of Bacteria for Amylase, Protease, Lipase 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 Estimation

The good strain selection was made by secondary screening method. The best bacterial strain obtained was inoculated in 100 mL gelatin broth and incubated for 48 h at 30°C. The Aliquot of culture broth was centrifuged at 7000 rpm for 20 min, and pellet supernatant was separately obtained. The pellet was added to the buffer and suspended andsonicated for one min and the remaining broth was used without centrifugation.

Estimation of Gelatinase Enzyme

The Tran & Nagano (2002) method was used for the gelatinase assay. The gelatinase units are expressed as expressed mol of leucine equivalent per min.mL^{-1} of the culture filtrate

Estimation of Protease

The enzyme protease was estimated by using the Begetal method. The unit of protease activity was defined as the enzyme required to release l micro -gm of tyrosine per mL per min.

The Amylase Estimation

The amylase assay was done by the DNSA method. The one unit of enzyme activity was measured as the amount of enzyme that releases 1µmole of reducing sugar (glucose) per minute under assay conditions (U.mL^{-1}.min^{-1}).

The Estimation of Lipase

For the quantitative estimation of lipase titrimetric method was used. The unit of enzyme is defined as the amount of enzyme that releases 1 µmole of fatty acid per minute under assay conditions and calculated as

$$\text{Lipase activity} = \frac{(\text{Volume of titrant alkali, mL}) \times (\text{Normality of NaOH})}{(\text{Time of incubation}) \times (\text{Volume of enzyme solution, mL})}$$

Preparation of Bacterial Consortium

The best 20 isolates were selected for consortia by permutation and combination. The ten different combinations were prepared. The antagonism assays and maximum enzyme production of the bacterial isolates at certain intervals. (24 h, 48 h, 72 h, 4 days, 5 days) was studied.

Characterization of Bacterial Isolates Obtained from the Best Consortium

16srRNA gene sequencing studies identified the members from the best consortium.

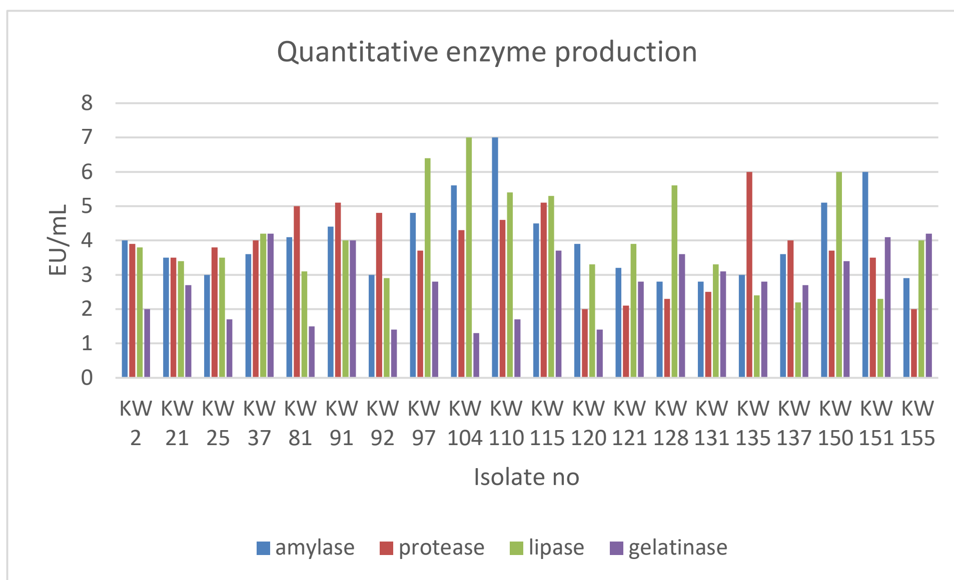

Fig. 1: Quantitative enzyme production.

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Table 1: Quantitative enzyme assays for 20 promising isolates.

Sr. No.	Quantitative enzyme production by obtaining the best isolates expressed in EU.mL^{-1}				
	Isolate No.	Amylase	Protease	Lipase	Gelatinase
1.	KW 2	4	3.9	3.8	2
2.	KW 21	3.5	3.5	3.4	2.7
3.	KW 25	3	3.8	3.5	1.7
4.	KW 37	3.6	4	4.2	4.2
5.	KW 81	4.1	5	3.1	1.5
6.	KW 91	4.4	5.1	4	4
7.	KW 92	3	4.8	2.9	1.4
8.	KW 97	4.8	3.7	6.4	2.8
9.	KW 104	5.6	4.3	7.0	1.3
10.	KW 110	7	4.6	5.4	1.7
11.	KW 115	4.5	5.1	5.3	3.7
12.	KW 120	3.9	2	3.3	1.4
13.	KW 121	3.2	2.1	3.9	2.8
14.	KW 128	2.8	2.3	5.6	3.6
15.	KW 131	2.8	2.5	3.3	3.1
16.	KW 135	3	6	2.4	2.8
17.	KW 137	3.6	4	2.2	2.7
18.	KW 150	5.1	3.7	6	3.4
19.	KW 151	6	3.5	2.3	4.1
20.	KW 155	2.9	2	4	4.2

RESULTS AND DISCUSSION

Identification of Bacteria

Out of 157 isolates, 20 best isolates were selected for further study based on the larger zones of hydrolysis. 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, KW15.All these isolates were subjected to enzyme production quantitatively (Table 1 and Fig. 1).

All these 20 were selected for preparing 10 consortia using permutation combination with five isolates in each consortium (Table 2).

After this, all these consortia were subjected to compatibility tests. From the compatibility studies, it was

Table 2: Types of consortia.

Consortia	Isolate Composition/combination				
1	KW2	KW81	KW110	KW131	KW155
2	KW21	KW115	KW135	KW151	KW25
3	KW37	KW97	KW121	KW150	KW91
4	KW92	KW104	KW120	KW128	KW137
5	KW2	KW115	KW110	KW151	KW155
6	KW21	KW97	KW135	KW150	KW25
7	KW37	KW104	KW97	KW128	KW91
8	KW92	KW115	KW120	KW151	KW137
9	KW2	KW97	KW110	KW150	KW155
10	KW21	KW104	KW135	KW128	KW25

Table 3: Quantitative enzyme production from six consortia.

Sr. No.	Consortia No.	Quantitative enzyme production by consortia EU.mL^{-1}			
		Amylase	Protease	Lipase	Gelatinase
1	1	10	15	15	11
2	3	12	20	18	13
3	6	9	10	16	15
4	7	18	22	18	19
5	9	10	15	13	16
6	10	13	16	12	12

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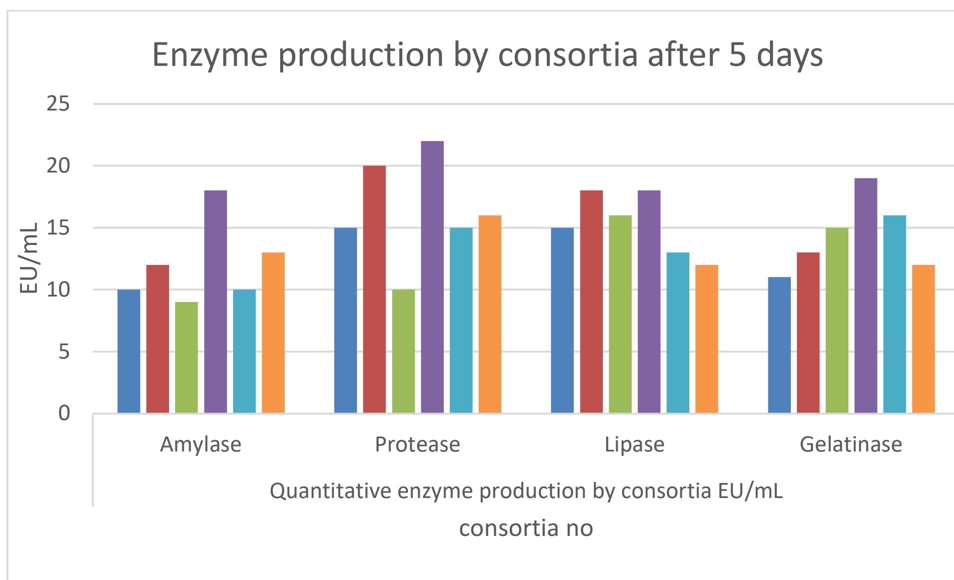

Fig. 2: Enzyme production by consortia after 5 days.

Table 4: Colony properties of members of the best consortium.

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

clearly understood that 6 consortia were compatible with each other for all enzyme production (Table 3).

The above results indicate that consortium No. 7 was more effective for the production of respective enzymes (Fig. 2). 16SrRNA Sequencing identified the isolates of consortium No. 7.

Cultural, Morphological and Biochemical Characteristics of the Isolates from Consortium No. 7

Table 5: Gram nature and motility property of the promising isolates from consortium No. 7.

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

The cultural, morphological, and biochemical characteristics of the obtained isolates are shown in Tables 4, 5, 6 & 7 respectively. The colony properties of members of all the bacteria present in best consortia are presented in Table 4.

Table 5 shows the gram nature and motility of the promising isolates from consortium No. 7.

The detailed biochemical characterization of the obtained promising isolates from consortium 7 is given in Table 6.

The promising isolates from consortium No. 7 were identified by 16 S rRNA Gene sequencing. The results of gene sequence identification and obtained gene accession numbers are given in Table 7.

A total of 157 bacterial isolates were obtained. In the secondary screening, 20 were found promising and were used to prepare different consortia. Out of 10 consortia, consortia No. (7) was the best consortium. Out of the promising isolates, one was cocci, three were rods and one was coccobacillus in nature. The 16 -SrRNA gene sequencing studies identified them as *Micrococcus luteus*,

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Table 6: Biochemical Characteristics of the promising isolates from Consortium No. 7.

Test	KW-37	KW-128	KW-91	KW-97	KW-104
Sugars :-(fermentation) Glucose	-ve	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve	+ve
Fructose	-ve	+ve	+ve	+ve	+ve
Arabinose	-ve	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve	+ve
Mannitol	+ve	-ve	-ve	-ve	-ve
L-Tryptophan (indole production)	-ve	+ve	+ve	+ve	+ve
Sodium pyruvate (vogesproskauer)	-ve	+ve	+ve	+ve	+ve
Methyl red	+ve	+ve	+ve	+ve	+ve
Urease	+ve	-ve	-ve	-ve	-ve
Catalase	-ve	+ve	+ve	+ve	+ve
Citrate	+ve	+ve	+ve	+ve	+ve
Gelatin hydrolysis	+ve	+ve	+ve	+ve	+ve
Starch hydrolysis	+ve	+ve	+ve	+ve	+ve

Bacillus tequilensis, Exiguobacterium mexicanum, and *Serratia marcescent*. Consortium No. (7) was found to be an effective consortium that can be used for faster degradation of organic kitchen waste as compared with the reports of Anuradha *et. al*(Anuradha.S.Tanksali Sridevi.S.Angadi Asha.Arwikar2014).

It can be used as an effective and fast method for deleting kitchen waste and forming compost. Fang et al. (2001) prepared a consortium of three organisms. Results and therefore present the maiden report.

In contrast, Karnchanawong & Nissaikla (2014), based on their extensive studies, concluded that adding inoculants to facilitate composting household organic waste was not necessary; instead and mature compost can be used as a seed starter to improve composting. Anwar et al. (2017) reported optimum biodegradation of kitchen waste by a consortium of *Serratia s, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis,* and *Bacillus megaterium* at 7.0 pH and 45°C temperature. In view of previous reports, our results are better as we used seven different enzymatic potentials by best consortia.

Table 7: Summary of the closest neighbour(s) for promising isolates in consortium No. 7:

Strain No.	ClosestNeighbour*	
	TaxonomicDesignation	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 obtained in the present study have the potential to be used in a consortium of fast degradation of kitchen waste samples.

ACKNOWLEDGEMENTS

The authors of this study are thankful to Hon. Chairman Dr. Suresh J. Bhosale, Krishna Charitable Trust, and the Research Fund Allocation Committee of KIMSDU for the facilitation of the Ph. D. work.

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