EVALUATION OF BIODEGRADABILITY OF *PSEUDOMONAS AERUGINOSA* AND *BACILLUS SUBTILIS* IN BIOREMEDIATION OF KITCHEN WASTES

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Abstract: In the present study, *Pseudomonas aeruginosa* and *Bacillus subtilis* played major role in remediation of kitchen wastes for composting in environmental cleanup strategy. These bacterial isolates with their concomitant enzymatic activity were found to be effective in degradation of kitchen wastes. Kitchen wastes were made up of mainly starch, protein, lipids and cellulose which are also degradable by the pathogenic microbes which, however, contaminate the whole environment. The said efficient bacterial isolates were checked by gram staining, colony characterization, biochemical characterization, and their extracellular hydrolytic enzyme production. These two successful bacterial isolates were eligible to produce all of these enzymes (amylase, protease, lipase and cellulase) in a special media, and are responsible for degradation of kitchen wastes. Production of all these enzymes is mandatory for the bacterial isolates wastes for biodegradation. From the present investigation, it can be concluded that useful bacteria can be isolated from the surrounding environment for ecofriendly bioconversion of solid kitchen wastes, as compost, which is a novel approach.

Index Terms - Pseudomonas aeruginosa, Bacillus subtilis, kitchen wastes, extracellular hydrolytic enzymes, biochemical characterization, Bioremediation and bioconversion.

I. INTRODUCTION

The major chemical components of kitchen wastes are starch, protein, fat, cellulose, and others. In most developing countries, Municipal Solid Waste (MSW) management is an extremely ignored part of the whole environmental management. Improper handling of Municipal Solid Waste with its consequences is now a serious concern of developing nations (Sharholy *et al*., 2008).

1.1 Sources of kitchen wastes-

Kitchen waste is the dumped and surplus organic matter from households, lodges and restaurants (Lie, *et al*., 2009). The deterioration of kitchen waste produces large amounts of toxins and foul odors, such as NH_3 and H_2S. Ammonia (NH_3) has a strong, pungent odor and can cause serious burns to the skin, eyes and respiratory tract (Chowdhury *et al*., 2014). Gases cause serious water and air pollution. Therefore, the efficient and environmentally responsible disposal of kitchen waste is important.

This study aimed to investigate optimum pH, temperature and incubation period for enhanced Kitchen waste biodegradation by utilizing biodegrading bacteria isolated from kitchen waste. This study has been designed with objectives of isolation and screening of kitchen wastes degrading bacterial isolate and these bacterial isolates play a major role to enhance in biodegradation of Kitchen waste.

II. MATERIALS AND METHODS

2.1 Isolation of Bacteria-

Kitchen wastes samples were collected from five different areas dumped with kitchen wastes in Gwalior M.P., India. The different bacterial isolates were isolated using standard serial dilution procedure and spread plate method according to Williams and Cross (1971). The isolated bacteria were further characterized on the basis of their substrate specificity and gram character.

2.2 Identification of enzyme producing bacteria-

For determination of enzyme producing activity (amylase, protease, lipase and cellulase) of the isolates screening tests are conducted. In order to observe enzymatic activities of bacteria, pure cultures of bacteria were inoculated in selective media (Mazzucotelli *et al*., 2013).

2.3 Different Enzymatic Assay-

For enzymatic assays of two bacterial isolates were centrifuged at 10,000 rpm for 10 min. The supernatant was used for enzymatic assay-

2.3.1 Amylase assay:

Amylase was determined by spectrophotometric method as described by Fisher and Stein (1961).

2.3.2 Protease assay:

Protease activity was determined by a modified method of Folin and Ciocalteu (1927).

2.3.3 Lipase assay:

Lipase activity was assayed according to the method of Hoshino *et al.* (1992).

2.3.4 Cellulase assay:

Cellulase activity was assayed using dinitrosalisic acid (DNS) reagent (Miller, 1959) and estimation of reducing sugars, released from carboxymethyl cellulose (CMC) solubilized in 0.05 M phosphate buffer at pH 8 was done according to Bailey *et al.* (1992).

2.4 Biochemical characterization and identification-

Biochemical characterization of bacterial isolates was done by various biochemical tests like Methyl Red test, Voges Proskauer test, Simmons citrate utilization test, urease test, gelatin hydrolysis, starch hydrolysis, Skimmed milk hydrolysis, lipid hydrolysis, cellulose hydrolysis, catalase, urease and nitrate reduction test were performed according to (Vashit *et al.*, 2013).

2.4.1 Isolation of genomic DNA from bacterial isolates-

The genomic DNA was extracted according to the method of Chen and Kuo (1993) with slight modifications.

2.4.2 Agarose gel electrophoresis -

The DNA bands in the gel was visualized using short wave ultraviolet light provided by a transilluminator and photographed using Dolphin-DOC gel documentation system (WEALTEC).

2.4.3 16s rRNA sequencing and phylogenetic analysis-

16S rRNA sequence and phylogenetic analysis were performed for molecular based identification of selected isolates.

III. RESULTS AND DISCUSSION

3.1 Isolation and identification-

In the present study 2 bacterial isolates namely *Pseudomonas aeruginosa* and *Bacillus Subtilis* (B4 and B6) were isolated from different sites of kitchen wastes samples.

3.2 Amylase producing bacteria-

During experiment, B6 bacterial isolates showed positive test for amylolytic activity as clear hydrolysis zone formation around an individual colonies whereas B4 bacterial isolates showed negative test for no amylolytic activity.
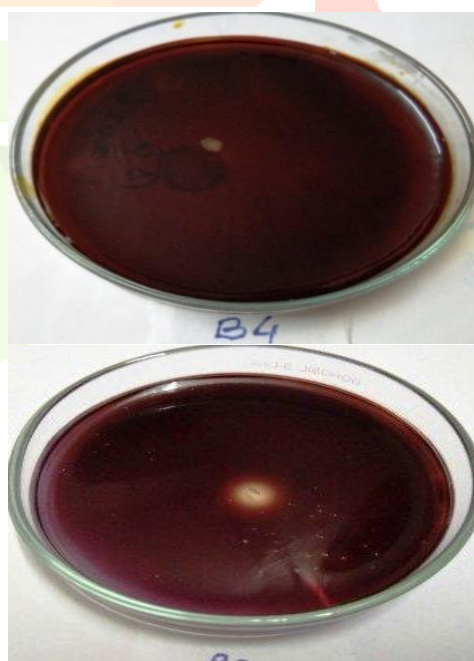

Fig- 1- Showing hydrolytic zones produced by amylase producing bacterial isolates.

3.3 Protease producing bacteria-

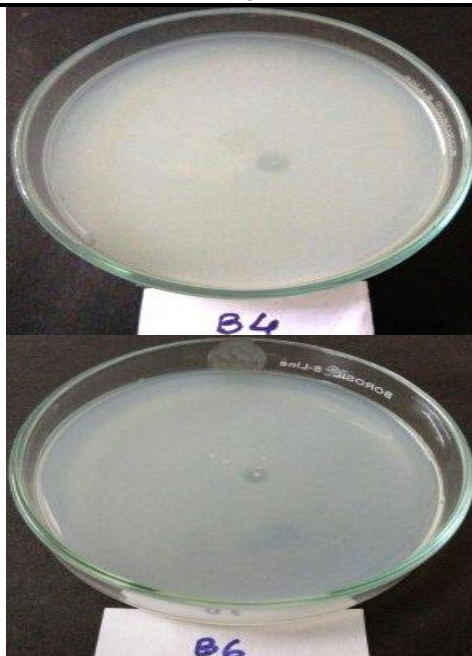During experiment, all bacterial isolates showed positive test for proteolytic activity.

Fig- 2- Showing hydrolytic zones produced by protease producing bacterial isolates.

3.4 Lipase producing bacteria-
During experiment, these bacterial isolates showed positive test for lipolytic activity.
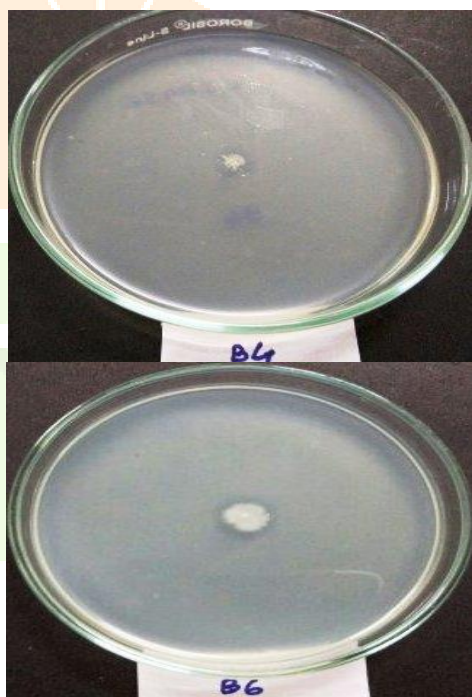

Fig- 3- Showing hydrolytic zones produced by lipase producing bacterial isolates.

3.5 Cellulase producing bacteria
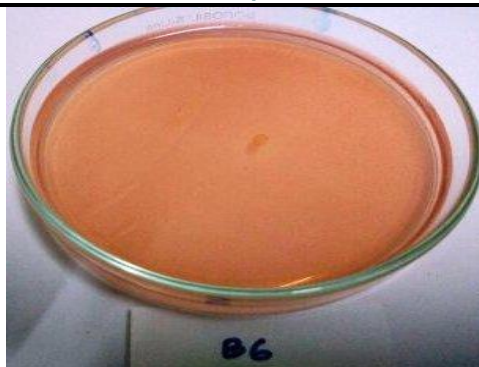During experiment, these bacterial isolates showed positive test for cellulolytic activity.

Fig- 4- Showing hydrolytic zones produced by cellulase producing bacterial isolates.

3.6 Different enzymatic assay-
3.6.1 Amylase enzyme assay:-
In amylase assay, Bacterial isolates viz. B4 did not show amylase activity whereas B6 showed amylolytic activity. Then, B6 showed highest amylolytic activity.
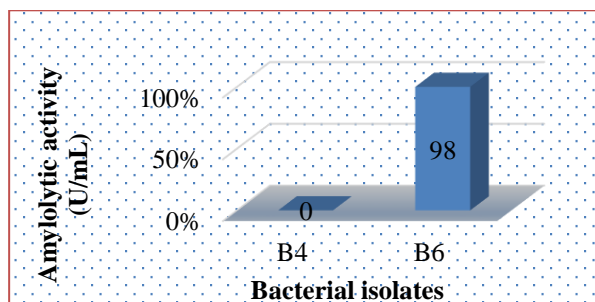

Fig:- 5- Enzyme assay for amylase producing bacterial isolates.

3.6.2 Protease enzyme assay:-
In protease assay, these two bacterial isolates showed proteolytic activity and B4 bacterial isolate had maximum proteolytic activity than B6.
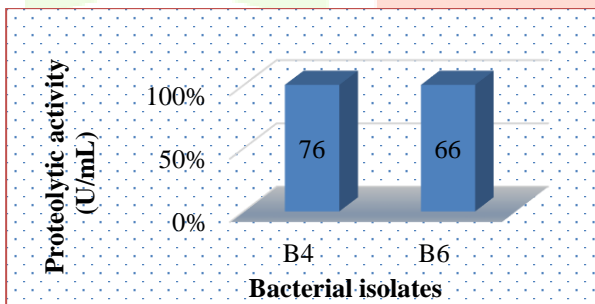

Fig:- 6- Enzyme assay for protease producing bacterial isolates.

3.6.3 Lipase enzyme assay:-
In lipase assay, these two bacterial isolates showed lipolytic activity, and B6 bacterial isolates had maximum lipolytic activity than B4.
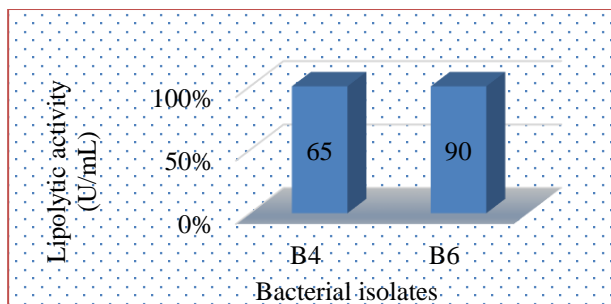

Fig:- 7- Enzyme assay for lipase producing bacterial isolates.

3.6.4 Cellulase enzyme assay:-

In the assay for cellulase, these bacterial isolates showed cellulolytic activity, and B6 bacterial isolates had maximum cellulolytic activity than B4.
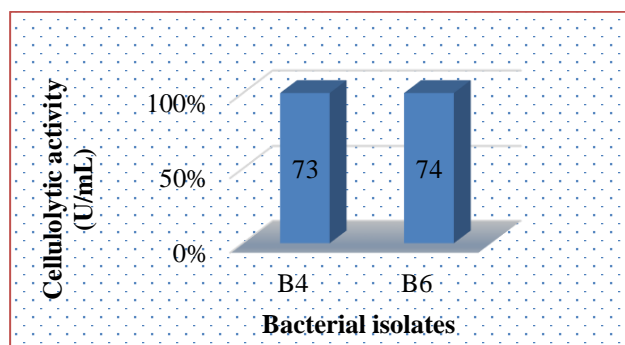

Fig:- 8- Enzyme assay for cellulase producing bacterial isolates.

3.7 Biochemical characterization-

Table- 1– Showing biochemical characterization of bacterial isolates–

S.N.	Characteristic / Bacterial Isolates	B4	B6
1.	Gram's staining	-	+
2.	Shape	Rod	Rod
3.	Motility test	+	+
4.	Methyl Red test	+	+
5.	Nitrate Reduction test	-	-
6.	Gelatin liquefaction hydrolysis	-	-
7.	Catalase test	+	-
8.	Skimmed milk hydrolysis	+	+
9.	Starch hydrolysis	-	++
10.	Lipid hydrolysis	+	++
11.	Cellulose hydrolysis	++	++
12.	Urea hydrolysis	+	+
13.	Vogus -Proskauer test	-	-
14.	Simmon citrate utilization test	+	-

3.8 Isolation of genomic DNA from bacterial isolates-

The genomic DNA was extracted according to the method of Chen and Kuo (1993) with slight modifications.

3.9 Agarose gel electrophoresis -

The DNA bands in the gel was visualized using short wave ultraviolet light provided by a transilluminator and photographed using Dolphin-DOC gel documentation system (WEALTEC).
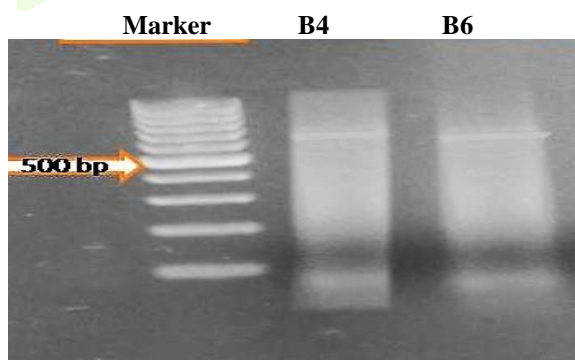

Fig:-9-- Agarose (2%) gel electrophoresis illustration.

3.10 Molecular identification of kitchen wastes degrading bacterial isolates as B4 and B6 -

DNA of kitchen wastes degrading bacterial isolates were isolated and amplified for 16s rRNA gene of 1.5 kb length on agarose gel (Fig- 9). DNA ladder used in experiment was 500 bp to 3 Kbp. Sequencing was done by Sanger di deoxy method, (Sanger *et al*., 1977). Sequence analysis of 16s rRNA of two bacilli (B4andB6isolates) were compared with GenBank data base using BLAST algorithm available on NCBI (www.ncbi.nlm.nih.gov).

The B4 bacterial isolate was identified as *Pseudomonas aeruginosa* as it shows 99 percent similarities with *Pseudomonas aeruginosa* strain GTJPR41186.

The B6 bacterial isolate was identified as *Bacillus* sp. as it shows 99 percent similarities with *Bacillus subtilis* strain CABCDRDA-a.

3.11 Polymerase Chain Reaction (PCR):-

PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template.

PRIMER DETAILS

| Primer Name	Sequence Details	No. of Base
27F	AGAGTTTGATCMTGGCTCAG	20
1492R	TACGGYTACCTTGTTACGACTT	22

Denaturation
The DNA template is heated to 94°C for 3 minutes.

Annealing
The mixture is cooled to anywhere from 94°C for 30 sec, 50°C for 60 sec, and 72°C for 60 sec.
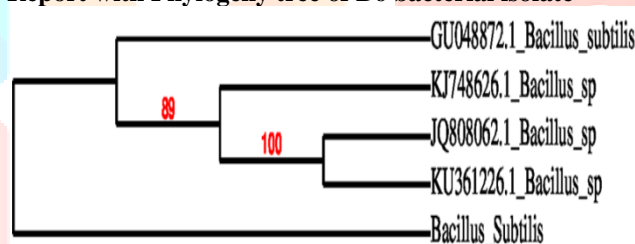
Extension
The reaction is then heated to 72 °C for 10 mins, the optimal temperature for DNA polymerase to act.
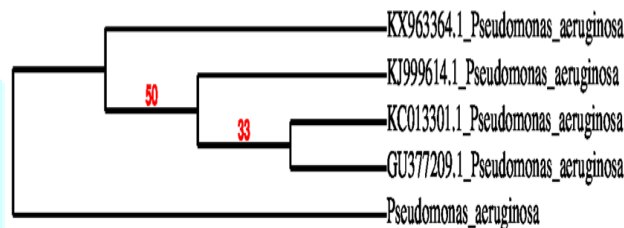
Result: *Pseudomonas aeruginosa*

Fig- 10- Bioinformatic Summary Report with Phylogeny tree of B6 bacterial isolate-

Result- *Bacillus subtilis*

Fig- 11- Bioinformatic Summary Report with Phylogeny tree of B6 bacterial isolate.

IV. CONCLUSION

The degradation of organic wastes by the bacterial isolates are highly significant. It reduces the time of degradation and produces no foul odour. The use of effective bacterial isolates generated through natural selection or improvement of the performance of these bacteria in organic kitchen waste degradation through genetic manipulation, may be the best option for the efficient treatment of domestic kitchen wastes in the near future. The pretreatment of kitchen waste can be used for biological solubilization and mineralization in garbage disposal system which is a novel approach.

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