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Isolation of plastic degrading and L-Asparaginase Enzyme Producing Bacteria from the Mangrove Environment of Guptapara, South Andaman

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Abstract: ***The present study reports a new insight on the isolation of plastic degrading and commercially important L-asparaginase enzyme producing bacteria from the pristine mangrove ecosystem of Guptapara, South Andaman. Seven out of 38 isolated bacterial strains exhibited significant plastic degradation in a period of 3 months. Bacterial strains MJ42, MJ102, MJ21, MJ22, MJ121, MJ172 degraded 0.002 g and MJ101, 0.003 g of weights from actual weight of plastics. On testing for antibacterial activity against different human pathogens these isolates did not show any positive results but all the isolates were positive to L-asparaginase enzyme production. Interaction of these isolates was demonstrated by cross streaking method and found the growth inhibition of strains MJ93 and MJ101 by strains MJ142, MJ143, MJ171, MJ172 and MJ173. In conclusion, results of this study infer the biotechnologically important bacteria from pristine mangrove environment for further in-depth research applications.***

Keywords*: Plastic degrading bacteria, L-asparaginase, Andaman.*

I. INTRODUCTION

Mangroves are salt tolerant plants found in coastal and estuarine region, distributed in both the tropical and subtropical intertidal parts of the world (Ghizelini et al., 2012). These mangroves are known to adapt to distinct characteristics of high temperatures, salinity, high pH, high concentrations of organic matter, low aeration and moisture provide interesting substrate conditions conducive for the development of diverse microbial communities (Holguin et al., 2001). Mangrove detritus are rich in nutrients hence supporting the mangrove food web that spans protozoa and metazoan, and many commercially important organisms such as fish and shrimp (Bhagwat and Ingale, 2013). A large active microbial population that decomposes plant components such as cellulose, pectin, hemicellulose, lignin, and the animal components such as chitin and keratin are also abundant in mangrove environment (Agate et al., 1988). Microscopic investigation on the decomposition of mangrove leaves reveals a complex community composed of fungi, bacteria, protozoa, and micro-algae (Odum and Heald, 1975).

Several microbial communities such as nitrogen-fixing bacteria, phosphate solubilizing bacteria, sulphate reducing bacteria, photosynthetic anoxygenic bacteria and methanogenic bacteria are the efficient nutrient cyclers (Mohanraju et al., 1997; Sahoo and Dhal, 2009). These microorganisms are known to participate in bio-remediation of organic matter and biotransformation of minerals (Gupta et al., 2009). It was estimated that 91% of the total microbial biomass comprised of bacteria and fungi in tropical mangroves (Alongi, 1988). About 71% of bacteria are found to produce commercially important enzymes such as L-asparaginase.

A halophilic bacterium (Halococcus sp.) isolated from the mangrove sediments produced antitumor activity exhibiting L-Asparaginase enzyme (Savitri and Azmi, 2003). Lee et al. (2014) investigated the mangrove forest of Malaysia and observed mangrove environment as a rich reservoir for the discovery of microorganisms that produced antimicrobial secondary metabolites such as Streptomyces, Mycobacterium, Leifsonia, Microbacterium, Sinomonas, Nocardia, Terrabacter, Streptacidiphilus, Micromonospora, Gordonia and Nocardioides. Plastic degradation (thermoplastic polymer-BOPP film) activities of several bacterial strains and actinomycetes have been recently investigated by Kimi (2011).

Andaman and Nicobar Islands constitutes 13% of the total mangrove area in India (Forest Survey of India, 2013). Coastal areas of these islands are inhabited with abundant mangrove cover consisting of 33 species of 19 genera (Singh, 2012). Most of the mangrove areas have not been explored for microbial diversity with reference to plastic degradation and L-asparaginase enzyme producing bacteria. Hence the present study was focused to isolate industrially important bacteria such as potential plastic degrading and L-asparaginase enzyme producing bacteria associated with mangrove environment.

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II. MATERIALS AND METHODS

A. Study Area

Guptapara is a muddy coastal shoreline with good mangrove vegetation in South Andaman (Longitude N 11˚31.004´ and Latitude E 092˚39.214´) (Fig. 1).

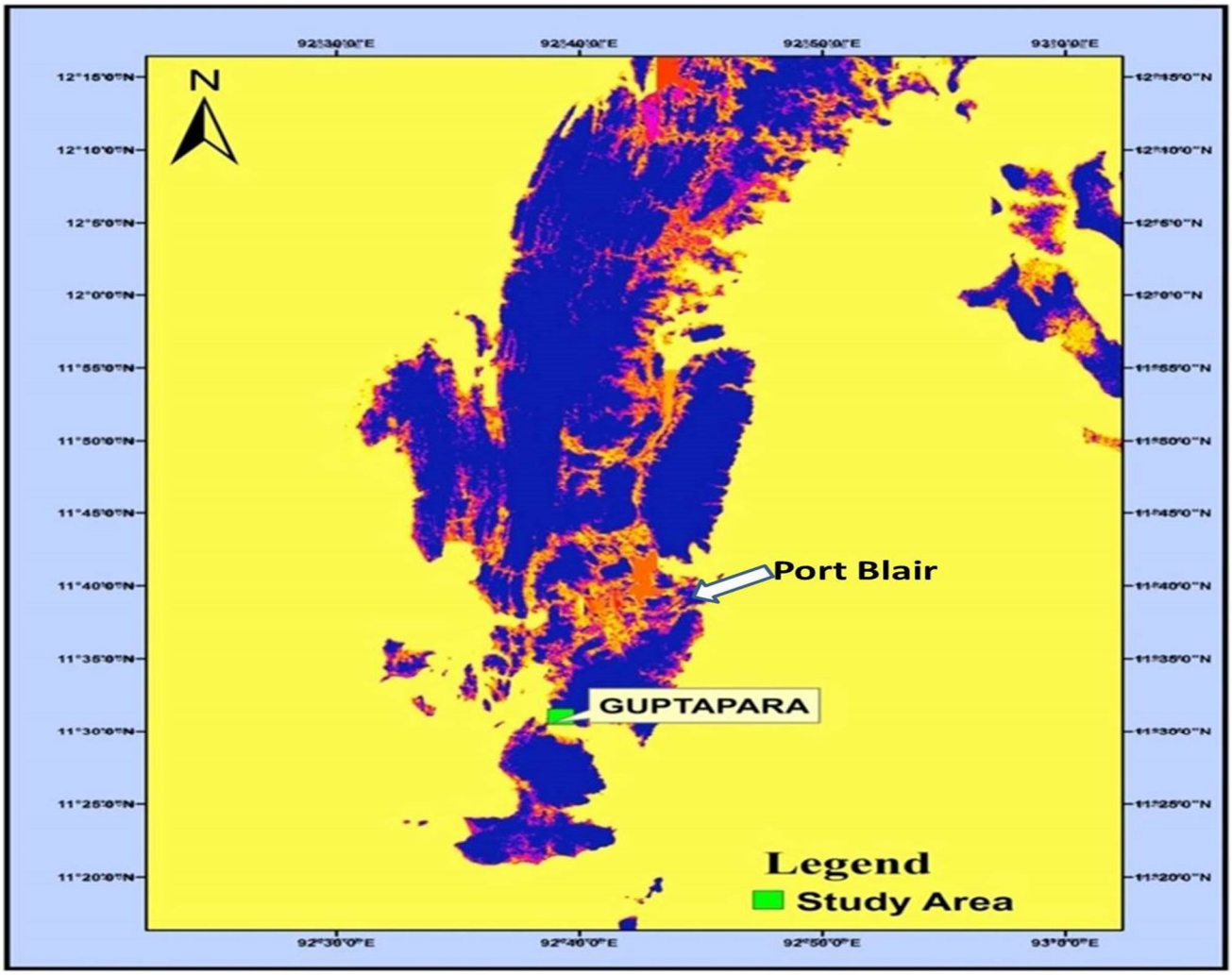

Fig. 1. Study Area- Guptapara in South Andaman.

B. Collection Of Samples

Samples were categorized into four groups such as 1. Floral samples, 2. Faunal samples, 3. Plastic samples, and 4. Sediment samples. Floral samples collected consisted of stilt roots of mangrove *Avicenia alba*; faunal samples included gastropod *Cerithidea cingulate*, crab *Uca* sp., siphonophore (*Muggiaea* sp.), Bryozoan, juvenile Mullet fish *Mugil cephalus*, unidentified nudibranch egg case, polychaete (unidentified); plastic samples collected were plastic cover (Polypropylene), thermocol (Polystyrene), polythene cover, black plastic fiber, plastic nylon thread (Polyamide), plastic glass (Polyactide), and white cover (PETG- Polyethylene terephthalate); and mangrove sediment composed of iron rust. All the samples were collected during low tide from the study area (Fig. 1), and transported to the laboratory for further microbiological analysis under refrigerated condition without any delay.

C. Preparation Of Sample Inoculums

Collected samples of flora, fauna and plastics were washed gently with sterile seawater to remove surface debris and loosely bound epibionts. Then samples were cut into small pieces, placed in separate 30 mL glass vials containing autoclaved alkaline peptone water and incubated for 1 hour at room temperature. On observing the increase in turbidity, these were used as sample inoculums for bacterial isolation. For sediment samples, sediment aliquots were prepared by diluting 1 gm of sediment in 9 ml of sterile seawater (w/v) (Benson, 2001).

D. Isolation And Purification Of Marine Bacteria

Standard microbial techniques were followed for media preparation and isolation of bacteria. Seawater agar (Himedia, Mumbai) (SWC) containing 2% Sodium chloride (NaCl) was used as medium for the isolation of bacterial isolates. To freshly prepared Seawater agar plates, 100 µl of each sample inoculums prepared earlier were transferred to the plates and spread onto the surface of the media following spread plate technique as described by Benson (2001). All the petri plates were incubated at 37º C for 24 hours. After the incubation period morphologically different individual colonies were selected from all the plates and restreaked onto fresh plates and purified to obtain single isolated colonies. The isolated colonies were checked for single morphology under a microscope.

E. Storage Of Bacterial Isolates

Pure isolated single bacterial colonies obtained were maintained on Nutrient agar slants containing 2% NaCl, and stored at room temperature or under refrigerated conditions.

F. Antagonistic Activity (Interaction Of Environmental Isolates)

Antagonistic activity was investigated to understand the microbial war between two or more related or distant bacterial species. Antagonistic activities of 38 isolates were tested by cross streaking method (Benson, 2001). Briefly, all the isolates were cross streaked with each other and incubated at 37º C for 24 hours and observed to check any organism is inhibited or the other is unaffected.

G. Preparation Of Inoculums Of Pathogens

One loop full of each of the following pathogenic bacteria (*Vibrio fluvialis* IDH strain 02036, *Vibrio parahaemolyticus* serovar O3: K6 strain K5030, Enteropathogenic *Escherichia coli* serotype O114 strain 11044, Shiga toxin producing *E. coli* serotype O157:H7 strain VT3, *Aeromonas hydrophilla* IDH strain 1585, *Vibrio cholera* O139 strain SG24, *V. cholera* O1 Ogawa strain C0835, *Shigella dysenteriae* type 5 strain NK2440, Enterotoxigenic *E. coli* serotype O115 strain 1571, *Salmonella enterica* serovar Typhi strain C6953, *Salmonella enterica* serovar Typhimurium strain B12101, and *Shigella boydii* type 1 strain NK2379) were inoculated into 5 ml test tubes containing sterile Nutrient broth and incubated at 37º C for 18 hours (Benson, 2001). These cultures were used as inoculums suspension for antibacterial assay.

H. Antibacterial Activity

Antibacterial activities of all the 38 isolates were tested against twelve human pathogenic bacteria following the cross streaking method as detailed above (Benson, 2001). Plates were incubated at 37º C for 24 hours and examined for growth inhibition zones.

I. Plastic Degradation Activity

Three different types of plastics i.e. black (Polyethylene), translucent (HDPE- High Density Polyethylene), and white or transparent (PETG- Polyethylene terphthalate) were tested to understand the biodegradation potentiality of the bacterial isolates. These plastic materials were cut into small, equal size, weighed carefully and aseptically transferred to 30 ml glass tube containing sterile Nutrient broth with 2% NaCl. The tubes were inoculated with the various isolated bacterial strains and incubated on a shaker for 2 months at room temperature. After the incubation period the plastic pieces were removed and washed thoroughly using distilled water, shade-dried and then weighed for final weight. The weight loss of the plastics and polythene bags was calculated as described by (Kathiresan, 2003).

J. Screening For Production Of L-Asparaginase

The isolates were grown in Nutrient agar containing 1% L-asparagine as sole source of carbon to study the L-asparaginase activity (Ramaiah and Chandramohan, 1992).

K. Biochemical Test

The isolated bacterial strains were presumptively identified to genus and species level on the basis of biochemical tests. Biochemical test were performed according to Benson (2001) and the results obtained were used for identification of bacteria using ABIS Online software.

L. Results

A total of thirty eight pure bacterial strains were isolated from different samples to understand their microbial activities (Table 1).

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Table 1. List of strains isolated from different samples.

S. No	Sample name	No. of isolates	Strain Codes
1.	Mangrove sediment	2	MJ11 and MJ12
2.	Sediment with iron rust	3	MJ21, MJ22 and MJ23
3.	Stilt root of mangrove Avicenia alba	1	MJ3
4.	Siphonophore (Muggiaea sp.)	2	MJ41 and MJ42
5.	Gastropod Cerithidea cingulate	2	MJ51 and MJ52
6.	Bryozoan	2	MJ61 and MJ62
7.	Juvenile Mullet fish	2	MJ71 and MJ72
8.	Nudibranch egg case	3	MJ81, MJ82 and MJ83
9.	Crab Uca sp.	3	MJ91, MJ92 and MJ93
10.	Polychaete (unidentified)	3	MJ101 MJ102 and MJ103
11.	Plastic cover (Polypropylene)	2	MJ111 and MJ112
12.	Thermocol (Polystyrene)	2	MJ121 and MJ122
13.	Polythene cover	2	MJ131 and MJ132
14.	Black plastic fiber	3	MJ 141, MJ142 and MJ143
15.	Plastic nylon thread (Polyamide)	1	MJ15
16.	Plastic glass (Polyactide)	2	MJ161 and MJ162
17.	White cover (PETG- Polyethylene terphthalate)	3	MJ171, MJ172 and MJ173

M. Antagonistic Activity (Interaction Of Environmental Isolates)

Antagonistic activity was observed in five out of 38 strains- MJ142, MJ143, MJ171, MJ172 and MJ173. These five bacterial strains inhibited the growth of strains MJ93 and MJ101 (Fig. 2).

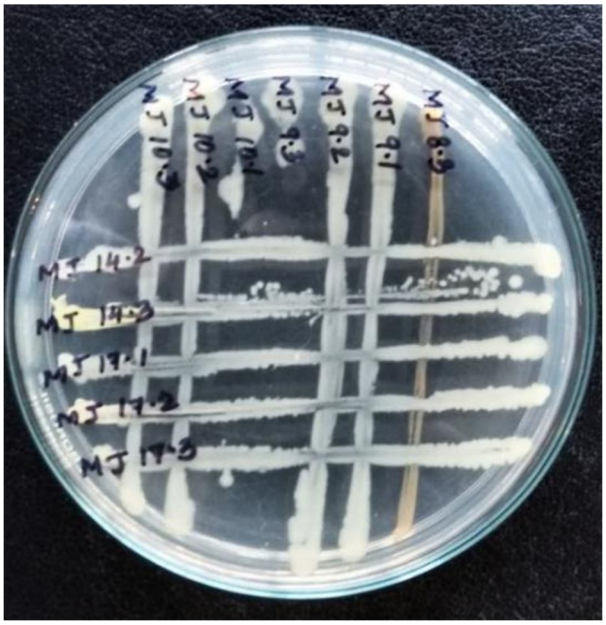


Fig. 2. Interaction of mangrove isolates.

N. Plastic degradation activity

Plastic degradation activities were found in 31 strains isolated from different samples (Table 1). Strains MJ11, MJ12, MJ3, MJ41, MJ42, MJ51, MJ52, MJ61, MJ62, MJ71, MJ92, MJ93, MJ103, MJ112, MJ15, MJ161, MJ23, MJ121, MJ122, MJ141, MJ142, MJ143, MJ171 and MJ173 degraded 0.001 gm weight from the actual weight; MJ42, MJ102, MJ21, MJ22, MJ121, MJ172 with 0.002 gm weight degradation and MJ101 degraded 0.003 gm weight (Figure 3-5).

Fig. 3. HDPE degradation activity by 38 strains.

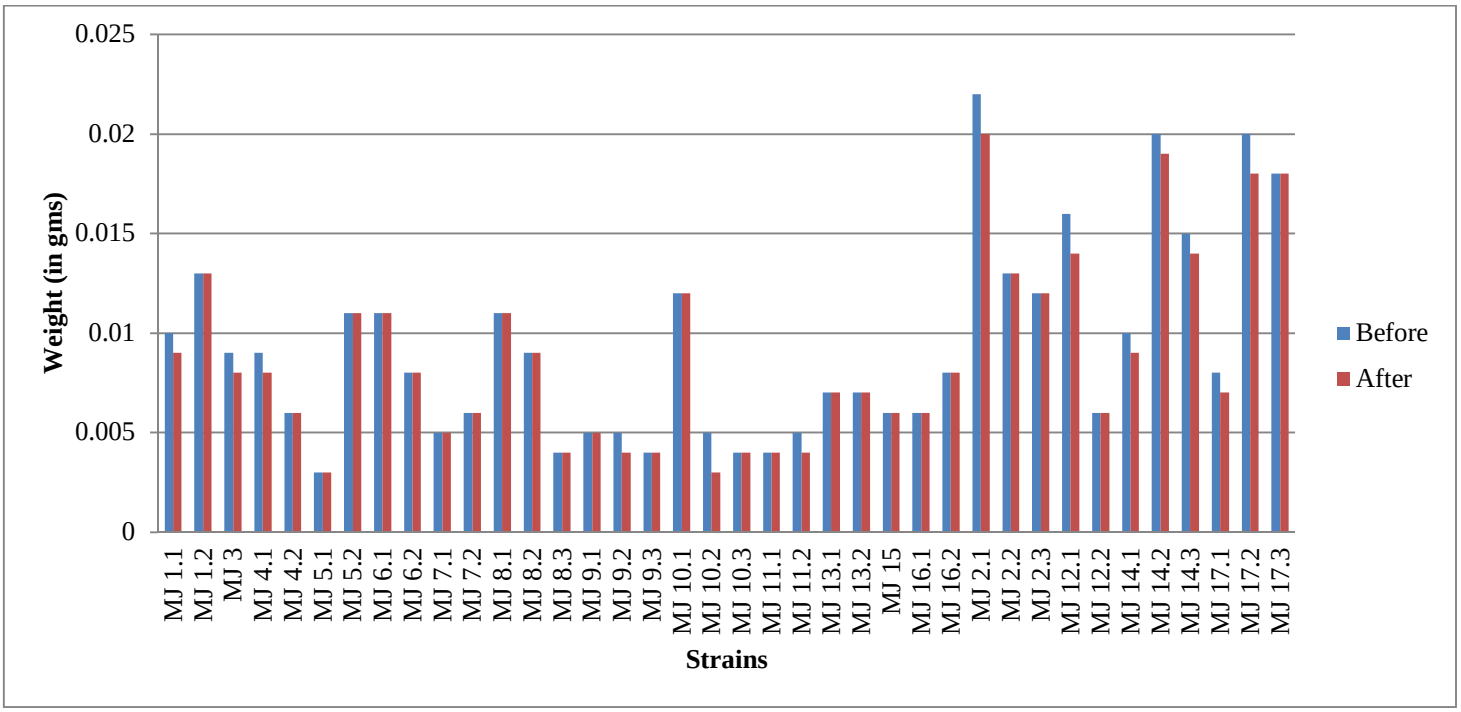


Fig. 4. PETG degradation activity by 38 strains.

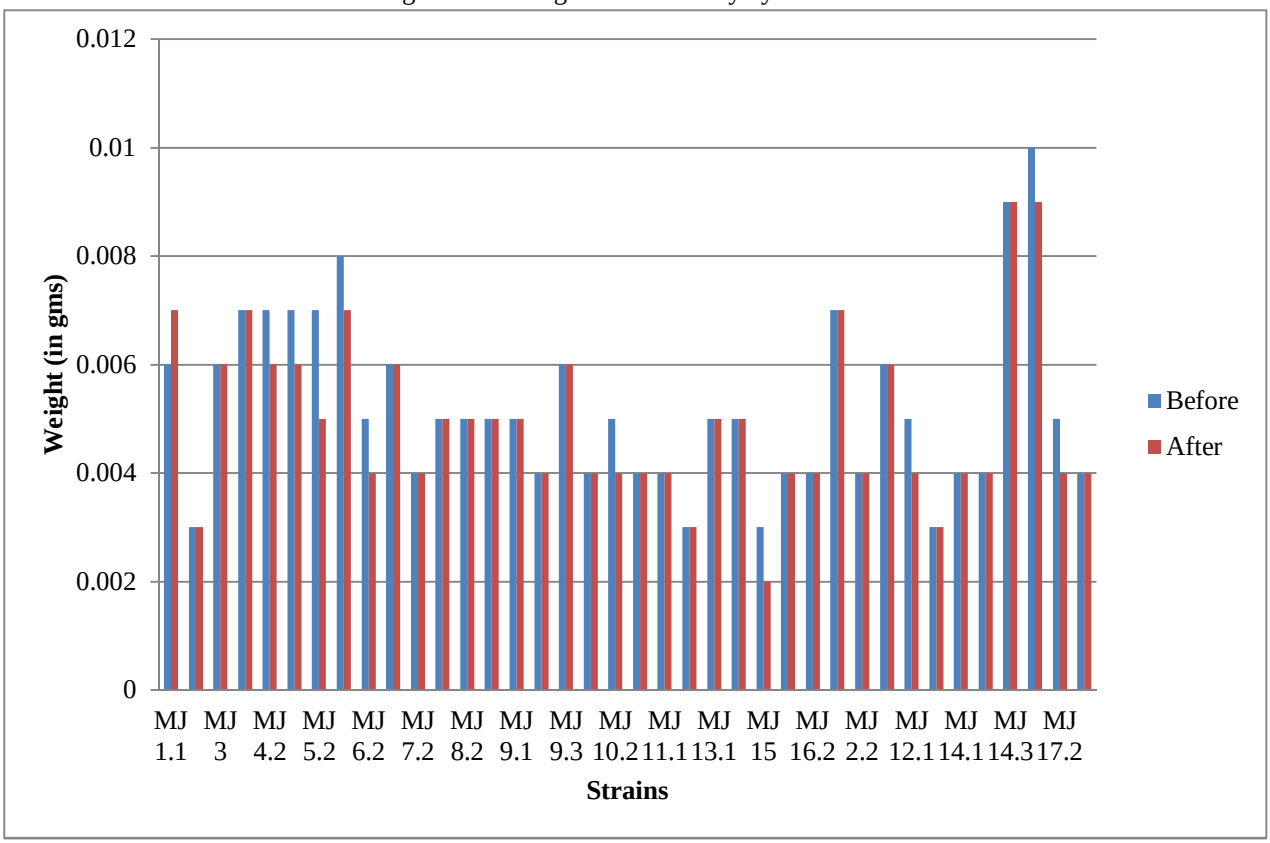
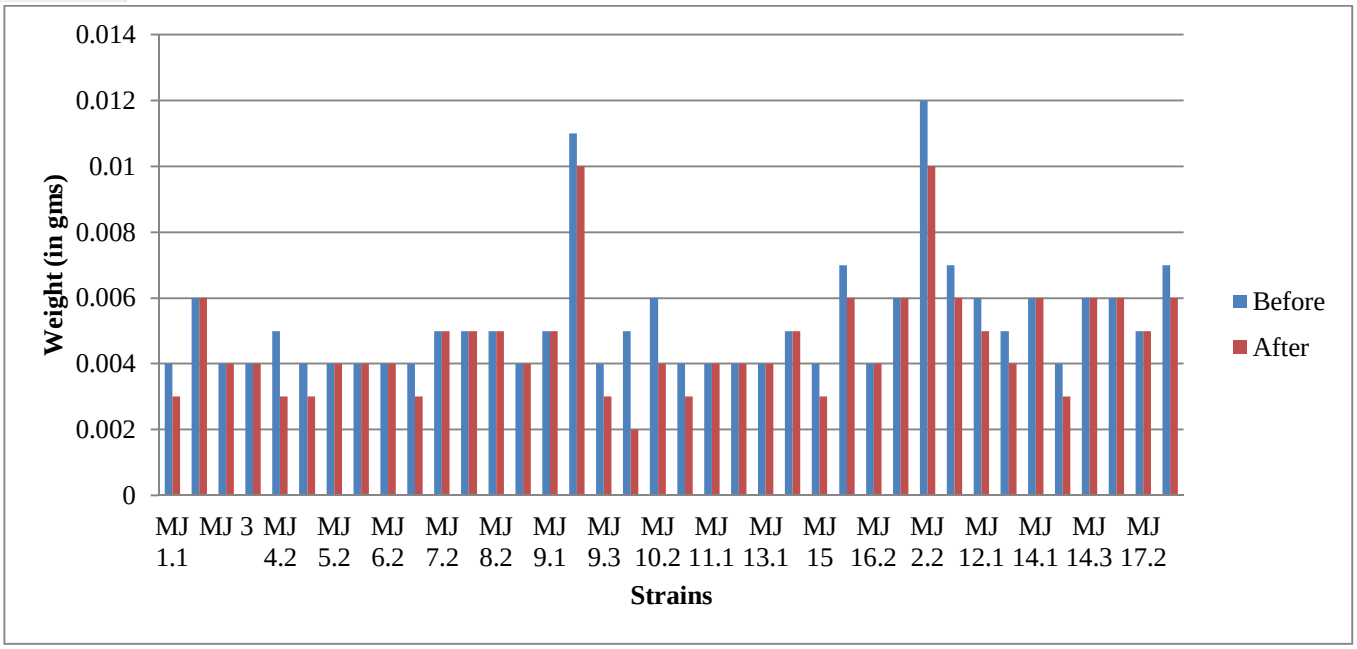


Fig. 5. Black polyethylene degradation activity by 38 strains.



O. Screening For The Production Of L- Asparaginase

Results revealed that all 38 strains were able to utilize L-asparagine. All the strains that grew on this medium were considered as positive to L-asparaginase for further analysis (Figure 6).

Fig. 6. Production of L-asparaginase.

P. Biochemical Test

Based on biochemical tests and on the basis of ABIS biochemical identification database (Table 2), all the 38 strains were identified as followed: MJ11 as Bacillus korensis (99%); MJ12, Vibrio pacinii (95%); MJ3, Enterobacter aerogenes (90%); MJ41, Pectrobacterium betavasculorum (90%); MJ42, Paenibacillus macreans (98%); MJ51, Vibrio alginolyticus (89%); MJ52, Bacillus castaneae (95%); MJ61 and 62, Paenibacillus macreans (92%); MJ71, Paenibacillus sputa (91%); MJ72, Stenotrophomonas maltophilia (98%); MJ81, Erwinia psidii (99%); MJ82, Buttiauxella agrestis (99%); MJ83, Paenibacillus wynnii (92%); MJ91, Buttiauxella izardii (99%); MJ92, Burkholderia psudomallei (87%); MJ93, Bacillus luciferensis (98%); MJ101, Paenibacillus macerans (92%); MJ102, Psedomonasa indica (98%); MJ103, Enterovibrio calviensis (99%); MJ111, 112, 131, 161 and 162, Psedomonas fuscovaginae (99%); MJ132, Salinivibrio costicola (96%); MJ15, Enterobacter nimipressuralis (90%); MJ21, Paenibacillus timonensis (91%); MJ22, Paenibacillus glucanolyticus (95%); MJ23, Paenibacillus assamensis (95%); MJ121 and 122, Burkholderia caryophylli (99%); MJ141 and 142, Bacillus decolorationis (98% and 90%); MJ143, Paenibacillus barcinonensis (99%); MJ171, Enterobacter aerogenes (99%); MJ172, Paenibacillus massiliensis (92%); and MJ173, Lysinibacillus fusiformis (96%) (Plate 2).

Table 2. Biochemical tests results of 38 strains.

TEST/ STRAINS	MJ 11	MJ 12	MJ 3	MJ 41	MJ 42	MJ 51	MJ 52	MJ 61	MJ 62	MJ 71	MJ 72	MJ 81	MJ 82	MJ 83	MJ 91	MJ 92	MJ 93	MJ 101	MJ 102	MJ 103	MJ 111	MJ 112	MJ 131	MJ 132	MJ 15	MJ 161	MJ 162	MJ 21	MJ 22	MJ 23	MJ 121	MJ 122	MJ 141	MJ 142	MJ 143	MJ 171	MJ 172	MJ 173
Gram staining	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fat Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP Test	+	-	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	-
Amylase	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	-
Oxidase	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	-	-	+
MR Test	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	+
Urease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulphide production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Blood agar	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+
L- Asparaginase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	*	*	-	*	*	*
Dextrose	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	*	*	-	+	+	+	+	+	*	+	+	*	-	-	+	+	*	+	-	*	*	*	*
4º C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20 º C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-
Lactose	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Arabinose	+	+	+	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	-
Inositol	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Mannose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
Species Identified	B.korensis 99%	V. pacinii 95%	E. aerogenes 90%	P. betavaculorum 90%	P. macreans 98%	V. alginoyticus 89%	B. castaneae 95%	P. macerans 92%	P. macerans 92%	P. sputa 91%	S. maltophilia 98%	E. psidii 99%	B. agrestis 99%	P. wynnii 92%	B. izardii 99%	B. psedomallei 87%	B. luciferensis 98%	P. macerans 92%	P. indica 98%	E. calviensis 99%	P. fuscovaginae 99%	P. fuscovaginae 99%	P. fuscovaginae 99%	S. costicola 96%	E. nimipressuralis 90%	P. fuscovaginae 99%	P. fuscovaginae 99%	P. timonensis 91%	P. glucanolyticus 95%	P. assamensis 95%	B. caryophylli 99%	B. caryophylli 99%	B. decolorationis 98%	B. decolorationis 90%	P. barcinonensis 99%	E. aerogenes 99%	P. massiliensis 92%	L. fusiformis 96%

III. DISCUSSION

Marine bacteria associated with mangrove environment are found to exhibit potential antimicrobial activities and thus offering biotechnologically important bioactive secondary metabolites (Malek et al., 2015). Understanding the interactions of heterotrophic marine bacteria from a specific area would reveal their abilities in inhibiting intra and inter bacterial species, which can be considered for further antimicrobial studies. Though the present study has not found any antibacterial activity against tested human pathogens, yet it is evident that interaction of these thirty isolated strains revealed a new insight as strains MJ142, MJ142, MJ171, MJ172 and MJ173 inhibited the growth of strains MJ93 and MJ101.

Kathiresan (2003) reported that biodegradation of polythene bags were higher (4.21% in 9 months) than plastics cups (0.25% in 9 months) and both gram positive and negative bacteria were associated in degrading the materials. Significantly *Pseudomonas* species degraded 20.54% of polythene (Kathiresan, 2003). Conversely, in the present study more number of gram negative bacteria were obtained and results show that *Pseudomonas fuscovaginae* MJ161, degraded 0.01 g of black polyethylene in two months; and *P. fuscovaginae* MJ112 degraded 0.01 g of HDPE in two months, suggesting that mangrove environment is a good source for polythene and plastics degrading bacteria.

Recently, Meena et al. (2015) isolated industrially important L-asparaginase enzyme producing bacteria *Streptomyces griseus* from marine sediments of Phoenix Bay in Port Blair, South Andaman. However, this study is focused only on *Streptomyces* rather than other microorganisms. A study on marine bacteria associated with litter fall and soil samples collected from *Rhizophora*, *Avicenia* and *Nypa* mangroves of Andaman Islands were assayed for bio medically important enzyme L-asparaginase (Shome and Shome, 2001). In which a total of one hundred and eight (54%) of total 200 isolates synthesized L–asparaginase. Only two gram negative

strains appeared to be potent for large-scale production (Shome and Shome, 2001). They concluded that mangrove dominated areas in Andaman Islands found to harbor potential L-asparaginase producing bacteria. In the present study, all the isolated gram negative (31) and gram positive (7) strains were positive to L-asparaginase production, with dominance of gram negative strains. In conclusion, the present study appends a new insight on the diversity of industrially important bacteria from mangrove environment of Guptapara, South Andaman. Further studies are focused on extraction and quantification of L-asparaginase enzyme from these strains for further applications.

IV. ACKNOWLEDGEMENTS

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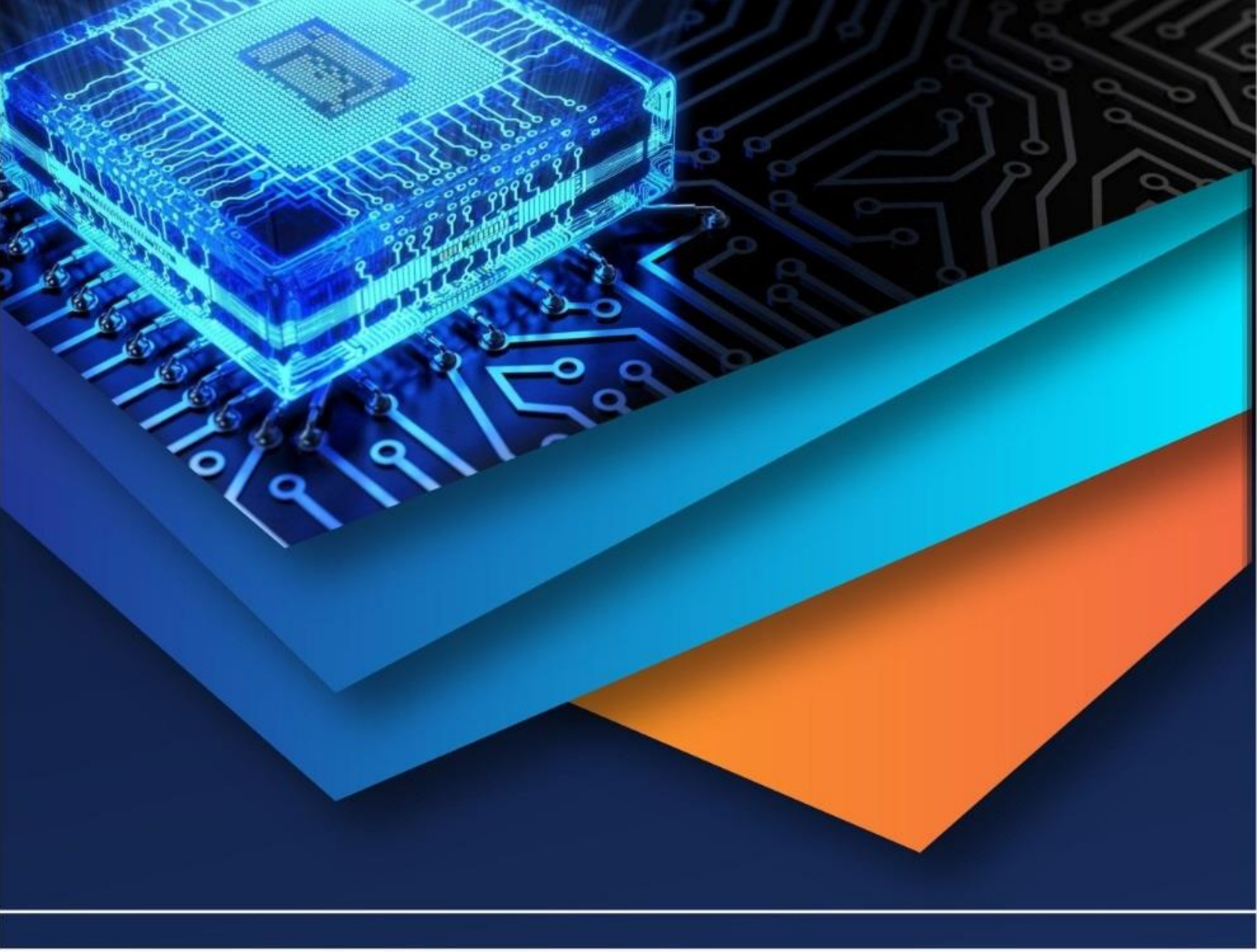

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