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MICROBES FROM THE MARINE COMPOST AND THEIR ENZYME PRODUCTION

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Abstract

Treating trash fish and processing waste is one of the more conservative and naturally safe techniques for reusing waste created by the purchaser society. Because of the intricacy of substrates and middle of the road items, microbial decent variety and the progression of populaces is an essential to guarantee total biodegradation. In the present work, we examined the progression of microbial population amid fertilizing the soil procedure of natural division of marine junk fish and handling waste and some physical and synthetic parameters were pursued. Amid the procedure, the moisture content was kept up at 50-60% and the temperature was checked every day. The outcomes demonstrated that the substrate was colonized in significant extent by bacteria (45.5%), actinomycetes (31.8 %) and in lower number by fungi (22.7%), predominantly spoken to by *Streptomyces, Pseudomonas, Bacillus* and *Actinomyces.*

Key words : Marine environment, trash fish, processing wastes, microbes, compost.

Introduction

Fish processing industries deliver significant measure of waste including substandard muscles, viscera, heads, skins, balances, outlines, trimmings, shellfish and scavenger shell waste every year. These by-products from fish processing plants have been considered of low esteem and are disposed (Turchini *et al*., 2009; Kuo, 1995; Guerard *et al.,* 2010). With the expansion in fish production, there is an expansion in the generation of the amount of fish processing waste. It is additionally ending up increasingly more basic to take care of the issue of natural pollution by using these waste or maybe disposing them (Turchini *et al*., 2009). Fermentation of this waste is a pressing issue for a considerable lot of these enterprises as landfill destinations close, as administrators confine the measure of waste that can be brought into landfill locales, and as directions have wiped out a portion of the recently utilized transfer rehearses (Kuo, 1995). Much of the time, composting can give a reasonable elective strategy for overseeing natural squanders (Cournoyer, 1996).

Fish squander has for some time been viewed as an administration issue in light of its commonly high smell levels. In any case, this nitrogen-rich waste can be overseen adequately through composting as it creates a correction that can be brought into the soil as a supplement source furthermore, soil conditioner, giving improved developing situations for plants (Kuo, 1995; Cournoyer, 1996; Schaub and Leonard, 1996) Composting is the organic deterioration of biodegradable strong waste under controlled overwhelmingly vigorous conditions to an express that is adequately steady for annoyance free stockpiling and dealing with and is satisfactorily developed for safe use in farming (Dias *et al.,* 2010). Composting is a productive technique for waste transfer, empowering reusing of natural issue. Composting is a standout amongst the most fermentation advancements for strong waste treatment. The natural substrates in strong waste can be biodegraded and balanced out by composting and the last manure items could be connected to arrive as the compost or soil conditioner. Composting includes a dynamic progression of a few gatherings of organisms successively connecting with the substrate (Dias *et al.,* 2010). It relies upon giving a situation positive to microbiological deterioration (Turchini *et al*., 2009).

The procedure is started by method for the breakdown of the perplexing particles in the crude substrate to less complex structures by organisms

indigenous to the substrate" (Kuo, 1995; Dias *et al.,* 2010; Yumei *et al*., 2013). Composting of the natural material is accomplished by billions of microbes normally present in leaf and yard squander, including microscopic organisms, actinomycetes, and fungi. These microorganisms duplicate quickly in the natural material, utilizing it as a wellspring of nourishment, and deliver heat, carbon dioxide and water vapour (Anon, 2002). Bacteria win early phase of composting, fungi are available amid the whole process yet can be very dynamic when moisture levels fall underneath 35% and are idle at temperatures more noteworthy than 60°C (Paul, 2014). Amid the development stage Actinomycetes prevail, and together with fungi are prepared to do degrade exceptionally safe polymers.

The key creatures decide the rate and degree of treating trash fish, since they have an enzymatic complex that licenses them to attack, degrade and use the natural issue in crude waste (Dias *et al*., 2010). Microbial and enzymatic access to the substrates quickens deterioration of squanders (O'riordan, 2014). Microbial growth amid fermentation procedures results in the generation of an assorted variety of metabolites including enzymes (Omafuvbe, 1999; Omafuvbe *et al.,* 2002). Amylases are among the most vital industrial enzymes and are of extraordinary essentialness in present-day Biotechnology (Selvakumar *et al*., 1996; Pandey *et al*., 2000; Qader *et al*., 2006). Amylases can be gotten from a few sources, for example, plants, animals and microorganisms. The microbial amylases meet industrial demands (Pandey, 1999 and 2000; Kathiresan and Manivannan, 2006).

Proteases are a standout amongst the most essential gatherings of modern catalysts and record for almost 60% of the complete chemical deal (Brown and Yada, 1991; Escobar and Barnett, 1993; Manjeet Kaur *et al*., 1998; Dutta and Banerjee, 2006). A large portion of the accessible proteases delivered economically are of microbial source (Oskouie *et al*., 2007). Proteases happen pervasively in a wide assorted variety of sources, for example, plants, animals, bacteria and fungi. Lipases are flexible catalysts that are conveyed all through living beings. It is created by well evolved, animals, bacteria, fungi and plants in expansive sums (Bapiraju *et al*., 2004). Distinctive organisms and microbes have been utilized for cellulase production (Bahkali, 1996; Magnelli and Forchiassin, 1999; Shin *et al.*, 2000).

Chitinases might be utilized to change over chitin containing biomass into helpful segments and they might be used for the control of contagious and creepy crawly pathogens of plants (Brurberg *et al*., 2000). Chitinases are utilized in industry of sustenance, drug store and horticulture. Composting is a fruitful system for the maintainable reusing of natural squanders (Fermor, 1993; Tuomela *et al.,* 2000). The aim of this study was reveal the succession of microbial populations during a whole composting process and the influence of some physical and chemical parameters under microbial concentrations. The composition of microbial communities was investigated by conventional cultured techniques. The aim of this investigation was to uncover the progression of microbial populaces amid an entire composting procedure under microbial fixations. The piece of microbial networks was researched by regular refined systems.

Materials and Methods

Site Selection

The trial was directed in a fitting spot for composting of fish squanders, found far from residence, water bodies and the common pathway.

Collection of Waste

Trash fish and handling squanders were predominantly utilized for composting while wood shavings, sawdust coir substance and fallen leaves were utilized as co-composting material.

Shredding / Size Reduction of Wastes

The size of wastes were decreased to less than 2 inches.

Composting and Compost Production

Composting was done by blending the fish squanders and the bulking agents and by observing the required diverse parameters. Turning was done at required frequencies and tests were drawn specifically from the heaps (in the wake of turning) at an interim of 15 days (for a time of 10 months) and their microbial (bacterial, fungal and actinobacterial) populace were dissected utilizing standard methodology.

Isolation and Identification of Microbial Cultures from Compost

The compost sample (5g) was diluted in 45 mL of buffer (0.06M Na_2HPO_4/NaH_2PO_4) (1/9 v/v), pH 7.6. Serial dilution (10^{-1} to 10^{-5}) was made and aseptically innoculated in Petri plates with various culture media: Potato Dextrose Agar (PDA), Nutrient Agar (NA) and Starch Ammoniacal Agar (SAA) so as to encourage the development of bacteria, fungi and actinomycetes, also, were incubated at 30°C (mesophilic microorganisms) and 50°C (thermophilies) for 72h (PDA), 37°C or 50°C for 24h (NA) and 37°C or 55°C for 120 h (SAA), as per the stage were the separation was done. After hatching, secluded microbes, fungi and actinomycetes were chosen.

Characterisation of Isolates

Regular morphological and biochemical tests were made to pure cultures of bacteria and actinomycetes as per the Bergey's Manual of determinative Bacteriology (1994). The fungal identification was completed by Raper and Fenell (1965); Ellis, (1971, 1976) and Bissett (1991).

Screening for Enzyme Production

The isolates were screened for their production of the following enzymes:

Amylase Activity

Starch agar (Components (g/l): Starch (soluble): 20.0 g; Peptone : 5.0 g; Beef extract : 3.0 g; Agar : 15.0 g) plates were prepared and streaked with the individual test organisms. The plates were incubated at 37°C for 24-48 hours. After incubation, the plates were flooded with iodine solution. Amylase activity was indicated by a clear/white zone around the colonies.

Protease Activity

Skim milk agar (Components (g/l) : Skim milk powder : 100 g; Peptone : 5.0 g; Agar : 15.0 g; pH : 7.2) plates were prepared and streaked with test organisms. They were then incubated at 37°C for 24-48 h. After incubation, the plates were flooded with $HgCl_2$ solution and were observed for zone formation.

Lipolytic Activity

The microbial isolates were single streaked individually on Spirit blue agar plates (Components (g/l) : Hiveg hydrolysate : 10g ; Yeast extract : 5g; Spirit blue : 0.15g; Agar : 17g; pH : 6.8 ± 0.2) and tributyrin agar plates (Components (g/l) : Tributyrin : 10 g; Peptone : 5 g; Agar : 15 g; Rhodoamine B : 0.01 g; pH : 7) with fluorescent dye Rhodomine B (0.001%) and they were incubated at 37°C for 48 h. Lipase positive cultures showed opaque zones around them and when exposed to UV light of 254 nm, an orange fluorescent halo appearance around the colonies was observed.

Cellulase Activity

The colonies were single streaked individually on Carboxy Methyl Cellulose (CMC) agar (Components (g/l) : CMC : 10.0 g; KH_2PO_4 : 4.0 g; Na_2HPO_4; 4.0 g; Tryptone : 2.0 g; $MgSO_4.7H_2O$: 0.2 g; $CaCl_2$: 0.001 g; $FeSO_4.7H_2O$: 0.004 g; Agar : 15.0 g; pH : 7.0) plates and were incubated at 37°C for 24-48 h. The plates were flooded with 0.1% aqueous congored solution and were allowed to stand for 20 min. Then they were thoroughly washed with 1 M NaCl solution and were observed for zone formation against a dark background.

Chitinase Activity

The chitinase detection agar (CHDA) (Components (g/l) Colloidal chitin : 10.0 g; Agar : 20.0 g; Soya bean powder : 20.0 g; Starch : 3.0 g; Peptone : 3.0 g; Yeast extract : 2.0 g; $CACO_3$: 1.0 g; : M9 medium: Na_2HPO_4 : 0.65 g; KH_2PO_4 : 1.5 g; NaCl : 0.25 g; NH_4Cl : 0.5 g; $MgSO_4$: 0.12 g; $CaCl_2$: 0.005 g; pH : 6.5) plates were prepared. The isolated gut microbes were single streaked individually into the CHDA plates and were incubated at 37°C for 72 h. They were then observed for zone formation. The colonies which formed a zone around them were the chitinase positive strains. The positive cultures were then sub cultured regularly for further study.

Results

The microbial activity along the entire composting process was monitored. During the initial phase, bacteria and fungi predominated (10.4×10^5 CFU/g and 7.6×10^2 CFU/g) while the actinobacterial population was minimum (6.3×10^3 CFU/g). In the mesophilic phase, the microbial population increased exponentially (Bacteria 12.7×10^5 CFU/g; actinobacteria 6.9×10^3 CFU/g and fungi 7.0×10^2 CFU/g). At high temperatures in the thermophilic stage, bacteria were mainly present (19.3×10^5 CFU/g), whereas the fungal population was much less in number (3.0×10^2CFU/g). Moreover, when the temperatures of composting were found to be greater than 50°C the number of bacteria, actionobacteria and fungi increased very well in the second mesophilic phase or cooling or stabilisation phase as there was a decrease in the temperature slowly to a constant (Bacteria 17.5×10^5 CFU/g; actinobacteria 10.3×10^3 CFU/g and fungi 15.1×10^2 CFU/g). Finally in the maturation phase, the bacterial population declined (11.3×10^5 CFU/g). The isolates were identified to be *Bacillus* sp, *Micrococcus* sp, *Pseudomonas sp*, *Azotobacter* sp, *Azospirillum.* sp, *Streptomyces* sp, *Actinomyces* sp, *Trichoderma* sp, *Alternaria* sp, *Penicillium* sp, *Ulocladium* sp and *Aspergillus* sp. respectively Table 1.

Enzymatic characterization of identified bacterial species

All the identified microbes showed amylolytic, proteolytic, lipolytic, cellulolytic and chitinolytic positive activities, except the *Pseudomonas* species which did not show any cellulolytic activity Table 2.

Discussion

The expansion in bacterial and fungal concentrations confirm amid the mesophilic stage, was impacted in a general sense by temperature and pH. Amid starting period of composting, the substrate is at surrounding temperature, the pH is generally marginally acidic and are accessible effectively natural mixes. The high

Table 1: Microbial Population during Composting of trash Fish.

Sampling (days)	Bacteria (CFU/g)	Actino bacteria (CFU/g)	Fungi (CFU/g)
0	10.4×10^5	6.3×10^3	7.6×10^2
15	10.6×10^5	6.5×10^3	7.7×10^2
30	11.4×10^5	6.7×10^3	7.8×10^2
45	12.7×10^5	6.9×10^3	7.0×10^2
60	12.9×10^5	6.9×10^3	6.4×10^2
75	14.2×10^5	7.3×10^3	4.6×10^2
90	14.9×10^5	7.7×10^3	4.7×10^2
105	16.2×10^5	7.8×10^3	4.5×10^2
120	16.8×10^5	7.9×10^3	4.1×10^2
135	17.9×10^5	8.3×10^3	4.9×10^2
150	19.3×10^5	8.6×10^3	3.0×10^2
165	19.5×10^5	8.9×10^3	5.8×10^2
180	18.4×10^5	9.3×10^3	5.9×10^2
195	17.9×10^5	9.5×10^3	6.7×10^2
210	17.7×10^5	9.9×10^3	10.3×10^2
225	17.5×10^5	10.3×10^3	15.1×10^2
240	15.2×10^5	12.8×10^3	13.7×10^2
255	14.3×10^5	13.5×10^3	14.6×10^2
270	13.2×10^5	14.7×10^3	11.9×10^2
285	12.7×10^5	15.2×10^3	9.7×10^2
300	11.3×10^5	15.9×10^3	7.7×10^2

Table 2: Enzymatic activities of identified bacterial strains.

Sl. No.	Bacterial strains	Amylolytic activity	Proteolytic activity	Lipolytic activity	Cellulolytic activity	Chitinolytic activity
1.	*Bacillus* sp	+	+	+	+	+
2.	*Micrococcus* sp	+	+	+	+	+
3.	*Trichoderma* sp	+	+	+	+	+
4.	*Azotobacter* sp	+	+	+	+	+
5.	Streptomyces sp	+	+	+	+	+
6.	*Pseudomonas* sp	+	+	+	-	+
7.	*Aspergillus* sp	+	+	+	+	+
8.	Actinomyces. sp	+	+	+	+	+
9.	*Penicillium*. sp,	+	+	+	+	+
10.	Azospirillum. sp	+	+	+	+	+
11.	*Alternaria*. sp	+	+	+	+	+

+ = Positive enzyme activity

– = Negative enzyme activity

proportion of bacteria permits a fast exchange of dissolvable substrate into a cell. In any case, actinomycetes are normally distinguished as one the primary gatherings in charge of organic matter conversion amid last phases of composting and thermophilic stage (temperatures of 45 to 55°C) as indicated by Chopra (2004) and Velasco *et al*., (2004).

Mesophilic fungi and bacteria are the prevailing dynamic degraders of organic wastes. Sustenance squander containing vegetable build-ups frequently have a low beginning pH (4.5-5), which invigorates the proliferation of fungi (Ryckeboer *et al*., 2003). High temperatures supported gradation and elimination of pathogenic microorganisms. Actinomycetes compete with others organisms for nutrients and can inhibit microbial growth due to the production of antibiotics, lytic enzymes or even by parasitism. their enzymes enablethem to degrade tough debris.

High temperatures bolster debasement and disposal of pathogenic microorganisms. Actinomycetes contend with other microbes for supplement and can restrain microbial development because of the generation of anti-microbials, lytic proteins or even by parasitism. Their catalysts empower them to degrade intense flotsam and jetsam. The genera Micrococcus, Bacillus, Streptomyces, Actinomyces, Azotobacter, Aspergillus, Penicillium and Trichoderma have also been examined by Ryckeboer *et al*., (2003); Velarde *et al*., (2004) and Martínez (2013) in various composts.

Thus it may be inferred from this study that, high organic matter and nutrient content of trash fish only stimulated the growth of various microbes like bacteria, actinobacteria and fungi and the subsequent synthesis of enzymes, what's more, the ensuing combination of compounds.

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