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Outlining Metabolic Versatility of a Commercial Waste Composting Consortium in Fish Waste Management

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Abstract

Effective and sustainable management of fish waste produced in markets and processing industries, is a major challenge faced by the coastal communities. Composting is an environment friendly method for fish waste disposal, and for the production of organic fertilizers. However, quality of compost depends on the use of proficient microbial consortia to mediate the bioconversion processes. Accordingly, as a first step in developing a competent fish waste composting strategy, suitability of a commercial organic waste composting consortium was assessed. For this, various selective media were used for outlining the metabolic activities required for waste degradation such as production of hydrolytic enzymes *viz*., chitinase, lipase and protease (both in standard media and fish waste component embedded media), ammonia oxidization, organic and inorganic phosphate solubilisation, nitrite oxidization and sulphur oxidization. There were potential producers for all organic waste degrading enzymes with highest enzymatic index (EI) as 1.8, 2.9 and 1.5 for protease, lipase and chitinase respectively. Among these, 10 microbial consortia were able to degrade fish waste components with the highest EI as 1, 2.4 and 0.2 for protein, chitin and oil respectively; indicating that efficacy of fish oil degraders in the evaluated consortia was very less. Four isolates were found efficient for denitrification. Highest organic and inorganic phosphate solubilisation efficiency was 1.9 and 7 respectively. Despite the presence of some AOB (ammonia oxidizing bacteria), NOB (nitrite oxidizing bacteria) and SOB (sulphur oxidizing bacteria), their efficacy was less. Potential isolates were characterized as *Lysinibacillus xylanilyticus, Halotalea* sp, *Bacillus oryzaecorticis, Cronobacter condiment* and *Bacillus megaterium*. As fish waste is rich in nitrogen and sulphur, inclusion of efficient AOB, NOB and SOB can improve the quality of final compost. Similarly, exclusion of denitrifiers can avoid the loss of nitrogen during composting. Consequently, while there were efficient organic matter degrading enzyme producers including fish protein and chitin, absence of efficient AOB, NOB, fish oil degraders and presence of denitrifiers recommends an improvisation of the evaluated consortium before application in fish waste composting.

Keywords: Fish waste, composting, bio-fertiliser

Introduction

Fishery sector contributes about 0.9% to National GDP and 5.17% to agriculture GDP (DADF, 2016). This contribution could improve, if the fish wastes and byproducts had also been effectively utilized (Jayathilakan et al., 2012). Effective and sustainable management of fish waste produced in markets and processing industries is a major challenge faced by coastal communities. As these wastes are rich in nutrients, unless effectively utilized, is likely to be dumped in the environment making pollution and health related complications (Selvi et al., 2014). Additionally, non-utilization of these by-products can lead to increased cost of waste disposal. The most cost effective, environment friendly method for waste disposal will be biological management (composting); as it can lead to the production of valuable organic fertilizers besides simple waste disposal. Composting practices for organic waste are broadly classified into two namely "Traditional" and 'Rapid' methods; based on the practices being adopted. The active composting period in traditional processes (Eg. Indian Bangalore method) may

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range from one to two years. Rapid methods include the processes that accelerate the composting process either through individual or combined application of treatments such as, shredding and frequent turning (Eg. Indian Indore method), addition of mineral nitrogen compounds (Eg. Berkley Rapid Composting' and 'North Dakota State University Hot Composting), use of effective microorganisms (EM based Quick Compost Process), use of worms (Vermicomposting), mechanical forced aeration (Aerated Static Pile) and mechanical forced aeration and accelerated mechanical turning (In-vessel composting) (Misra & Roy, 2003). Of these, two methods (aerated static pile composting and in-vessel composting) were tested in fish waste (Kinnunen et al., 2005). However, exploration of composting processes through the proper utilization of metabolic versatility of beneficial microbes has not been tested in fish waste management. Use of beneficial microbes will be useful in composting methods, as the actual number of degraders during natural composting represents only 5-10% of the total microbial community (Sayler et al., 1984). Therefore, conventional composting without using efficient microbial consortium tends to be very tedious, less efficient and may result in less valuable fertilizer.

Compared to other organic wastes, fish waste contains a large amount of chitin (shell residues of shell fish) and readily digestible protein comprised of abundant nitrogen and sulphur. Therefore, removal of chitin, nitrogen and sulphur from fish waste is of extreme environmental importance (Selvi et al., 2014). Consequently, when fish waste is composted, these relevant features need to be considered, which may result in unique composting performance and superior quality organic fertilizer. Accordingly, selection of efficient microbes and development into a successful consortium that can concomitantly degrade different components of fish wastes in a less span of time and detoxify nitrogenous/ sulphur compounds into easily absorbable plant nutrients without producing any foul odour; forms the first step in developing a competent fish waste composting strategy. Hence, a commercial organic waste composting consortium was microbiologically profiled in the present study to check its suitability for fish waste management. In other words, the reason for the failure of uniform results and poor performance of organic waste composting consortium on fish waste composting was assessed in the present study.

Materials and Methods

A commercial organic waste composting consortia which are regularly used by the agricultural farmers of Kochi, Kerala for organic waste composting, was purchased from Coimbatore, Tamil Nadu. As per the feedback obtained from farmers regarding the failure of uniform results and poor performance of this consortium on fish waste composting, the reason for the failure was evaluated. Ten gram of the consortia embedded in coir pith was crushed aseptically and tenfold diluted with sterile normal saline. Resultant mixture was incubated at room temperature for 30 min with vigorous shaking. The mixture was used for isolation of microbes by inoculating into various media such as nutrient agar (NA), a non-selective media (Himedia), Sulfur-oxidizer medium (SOM) for the isolation of sulphur oxidizing bacteria (Behera et al., 2014), Nitrifier media 1 (NM-1) for cultivation of ammonia and nitrite oxidizers (Alexander & Clark, 1965) and Pikosovasky agar (PKA) for isolation of phosphate solubilising bacteria (Himedia). The plates were incubated at room temperature and inspected daily for 7 days. Morphologically unique colonies were randomly picked up and purified, and each pure culture was stored as agar slants and glycerol stocks.

Each isolate was screened for production of enzymes required for organic waste degradation (chitinase, lipase and protease) by spotting onto respective substrate embedded agar plates and incubating at room temperature for 7 days. Zone diameter was measured and enzymatic index (EI) [(Total Diameter-Colony Diameter)/Colony diameter] was calculated (Fungaro & Maccheroni, 2002).

Each isolate was further screened for production of degradative enzymes of major fish waste components namely, protein, lipid and chitin. For this, pure culture was spotted onto respective substrate embedded agar plates namely, crude fish-protein powder (20%) (Belchior & Vacca, 2006), fish oil (1%) and chitin (0.5%) extracted from the shell of shrimps (Kumari & Rath, 2014) in such a way that the mentioned substrate will be the sole carbon or energy source for bacteria. Proximate composition of crude fish-protein powder (on dry matter basis) was analysed by the methods of AOAC (2003) before its final incorporation into media which revealed that it contained crude protein (61.59%), crude fat (5.73%), crude ash (9.70%), crude fibre (0.56%), acid insoluble ash (0.02%) and nitrogen free extract (22.42%). Zone diameter in each media was measured after 7 days of incubation and EI was calculated (Fungaro & Maccheroni, 2002).

Micro-well-plate assay was used (Zhao et al., 2013) for screening AOA and NOA. AOA was screened by checking the rate of disappearance of ammonia from the medium while; NOA was evaluated by checking the rate of disappearance of nitrite from the medium, after growing each isolate in recommended medium. Sulphur oxidizing activity (SOA) was screened by measuring the formation of sulphate ions (barium chloride based colorimetric method), after growing in recommended medium (Behera et al., 2014). For evaluating organic and inorganic PSA, culture was spotted onto PKA and Yolk medium respectively, and phosphate solubilisation index (PSI) [(Total Diameter-Colony Diameter)/Colony diameter] was calculated after 7 days of incubation (Nguyen et al., 1992). Denitrifying activity of each bacterium was checked by spotting onto bromothymol blue media (Takaya et al., 2003). Potential isolates having desired characteristics was identified using conventional microbiological methods and 16*SrRNA* gene sequencing.

Results and Discussion

Awareness on the identities and metabolic functions of microbial communities in a consortium is crucial to determine whether that can be used as an inoculum for enhancing bioconversion processes in specific compost. Accordingly, as a first step for developing a competent fish waste composting strategy, suitability of a commercial organic waste composting consortium/reason for the failure of uniform results and poor performance of organic waste composting consortium on fish waste composting, were tested in the present study. A total of 42 morphologically unique bacterial isolates were obtained through isolation attempts using 4 different media (one non-selective and 3 selective).

When the metabolic versatility of these 42 isolates were assayed, there were positive isolates for all the major organic components of fish waste namely, protein, lipids and chitin. The obtained EI value and enzymatic versatility on standard screening media were depicted in Fig. 1. The highest EI achieved for protease, lipase and chitinase were 1.83, 2.9 and 1.5 respectively. As the isolates showing EI value ≥ 1 are potential enzyme producers for that enzyme (Fungaro & Maccheroni, 2002), it was concluded that the evaluated consortia contained 8, 4 and 3 different potential protease, lipase and chitinase producers respectively. Therefore, the action of these potential isolates might be the reason for the good performance of evaluated consortia on organic wastes.

As the activity of most enzymes are substrate specific, there is a chance for different degrading potential on fish waste components compared to the substrates contained in standard screening media. Hence, in order to check the degradative ability of isolates on fish waste, the isolates were further screened on media containing fish waste components (Belchior & Vacca, 2006). Results revealed that all the isolates found to be efficient in standard screening media were not efficient in the degradation of corresponding fish waste component suggesting the necessity for the inclusion of specific substrate in screening media depending on the target of study. Nevertheless, there were positive isolates for all fish waste specific degrading enzymes, and the results were depicted in Fig. 2. There

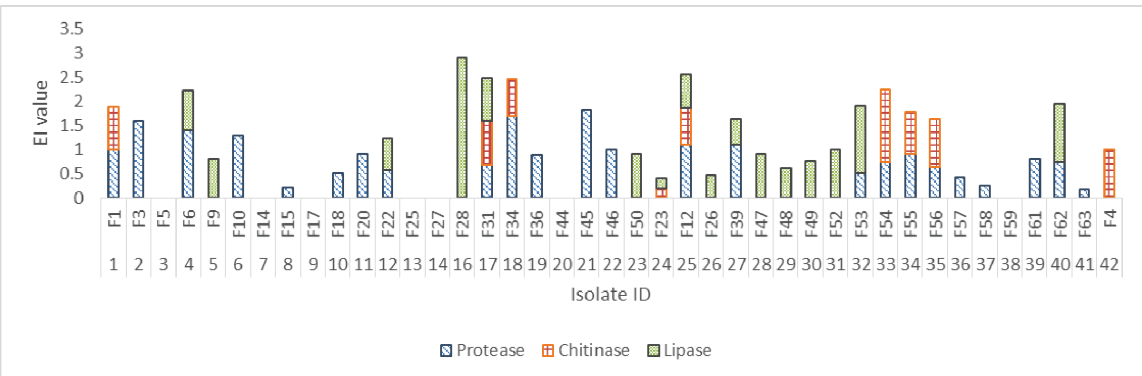

Fig. 1. Evaluation of waste degrading enzyme production

were 3 (namely F18, F31 and F56) and 2 different bacterial isolates (namely F25 and F12) having good potential for fish protein and fish chitin degradation (EI value ≥ 1) respectively. On contrast, none of the isolates revealed good fish oil degrading efficacy (Highest EI=0.2). Therefore, fortification with efficient fish oil degrading bacteria is necessary before the final application of this consortia into fish waste management.

As the presence of Phosphate solubilizing bacteria (PSB) can also add the value of final compost product as a plant fertilizer, evaluation of organic and inorganic phosphate solubilisation activity (PSA) was followed and results were shown in Fig. 3. The highest inorganic and organic PSI was 7 and 1.9 respectively, evidencing the presence of highly efficient inorganic PSB and medium efficient organic PSB. Thus, the results suggested that consortia can improve the available P content (the second most essential macro nutrients for plant growth) of the final compost product.

Next step was to evaluate AOA, NOA and SOA activity of the isolates. Among 42 isolates screened, only 2, 1 and 3 isolates showed positive AOA, NOA and SOA respectively. Unfortunately their efficacy was very less. Removal of nitrogenous compounds from fish waste using AOB and NOB is of extreme environmental importance to avoid devastating eutrophication (Selvi et al., 2014). Conversion of ammonia to nitrate (nitrate can be easily absorbed by plants) through action of AOB and NOB (nitrifiers) are essential for efficient bio-fertilizer production (Kowalchuk et al., 1999). Similarly, presence of efficient SOB is required to remove the odour and to improve the nutrient content of the compost product as a plant fertilizer (Selvi et al.,

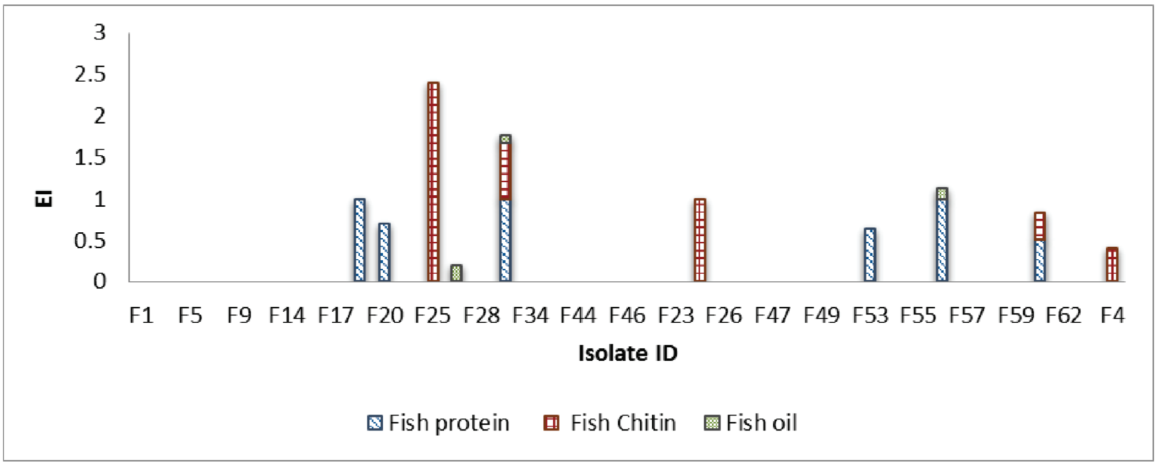

Fig. 2. Relative abundance of bacteria producing waste degrading enzymes

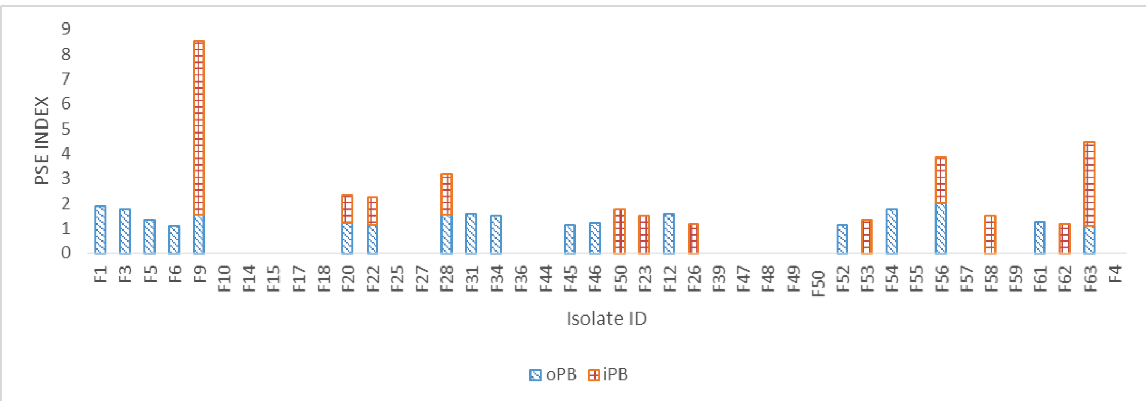

Fig. 3. Evaluation of phosphate solubilizing activity
oPB: organic PSB; iPB: inorganic PSB

Table 1. Identification of bacteria having desired activities

Strain	Genus	*P	*L	*Ch	*oPB	*iPB	*SOB	*AOB	*NOB	*FP	*FO	*FC
F9	*Lysinibacillus* sp.	-	+	-	+	+	+	+	-	-	-	-
F18	*Paenibacillus* sp.	+	-	-	-	-	-	-	-	+	-	-
F25	*Bacillus* sp.	-	-	-	-	-	-	-	+	-	-	+
F28	*Halotalea* sp.	-	+	-	+	+	+	-	-	-	-	-
F31	*Bacillus* sp.	+	+	+	+	-	+	+	+	+	+	+
F45	*Bacillus* sp.	+	-	-	+	-	-	-	-	-	-	-
F50	*Cronobacter* sp.	-	+	-	-	+	+	-	-	-	-	-
F54	*Bacillus* sp.	+	-	+	+	-	-	+	+	-	-	-
F56	*Bacillus* sp.	+	-	+	+	+	-	-	-	+	+	-

* Protease (P), lipase (L) and chitinase (Ch), organic PSB (oPB), inorganic PSB (iPB), Sulphur oxidizing bacteria (SOB), Ammonia oxidizing bacteria (AOB), Nitrite oxidizing bacteria (NOB), Fish protein degrader (FP), Fish oil degrader (FO), Fish chitin degrader (FC)

2014). Conversely, the tested consortia did not contain any efficient AOB, NOB or SOB. Therefore, the absence of efficient AOB, NOB and SOB in evaluated consortia recommended that the same cannot be an ideal inoculum for fish waste degradation.

As the presence of denitrifiers cause the loss of beneficial nitrogenous compounds to atmosphere, it is considered as an undesirable quality in the compost intended for the production of bio-fertilizer. Accordingly, denitrifying activity of each bacterium in the consortia was checked which revealed 4 efficient denitrifiers in evaluated consortia. The results suggested that all these 4 have to be excluded from the final consortia to avoid the loss of nitrogenous compounds from the compost product. As the final step, the potential isolates in the tested beneficial activities were then identified and the results are presented in Table 1. The most potential isolates were *Paenibacillus* sp, *Bacillus* sp, *Lysinibacillus* sp, *Cronobacter* sp and *Halotalea* sp. In concordance with our observation, the potential of these genera for degrading various organic waste and improving the agricultural quality of the compost product was already described in literature (Ribeiro et al., 2017; Zhao et al., 2017).

In conclusion, suitability of a commercial organic waste composting consortium for fish waste composting was assessed in the present study. Microbiological profiling showed that even though there were efficient organic matter degrading enzyme producers (including fish protein and fish chitin), the evaluated consortia did not contain any efficient AOB, NOB, SOB and fish oil degraders. Additionally, it had 4 efficient denitrifiers, which are not desirable in the consortia intended for bio-fertilizer production. All these observations might explain the reported failure of uniform results and poor performance of organic waste composting consortium on fish waste composting. The results also pointed out the need for the usage of specific consortia for application on fish waste composting rather than the ordinary organic waste composting consortia.

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