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Potential Keratinase-Producing Bacteria Isolated from Pangasius (*Pangasianodon hypophthalmus*) Farms Fed with Poultry Waste

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Abstract

Keratinase-producing bacteria degrade keratin, a tough protein found in feathers, hair, nails, and other keratinized materials. Mud, water, and fish samples from ten poultry waste-fed, *Pangasianodon hypophthalmus* farms in Palakkad and Alappuzha districts of Kerala, India, were screened for keratinolytic bacteria. Among 116 proteolytic isolates, 6.9% showed keratinolytic activity on feather meal agar. Three isolates namely FWKB1, FMKB1 and FMKB2 were identified as having good keratinolytic potential. FMKB1 and FMKB2 isolated from soil samples in Palakkad were identified as *Bacillus subtilis*, while FWKB1 isolated from a water sample in Alappuzha was identified as *Exiguobacterium profundum*. *In vitro* enzyme assays revealed that FMKB1 and FMKB2 produced 53.2 U/mL and 66.8 U/mL of keratinase enzyme, respectively within 24h of incubation at 37°C. A direct keratinase assay with raw feathers exhibited 72.8 U/mL enzyme production by a combination of FMKB1 and FMKB2, completely degrading raw feathers within 45 days at 37°C. Characterisation of exo-enzyme production revealed the absence of lipase, gelatinase, and amylase enzymes. Haemolysis, Congo red binding, biofilm formation and the production of AHL signalling molecule were not detected in the bacterial isolates tested. These isolates can be promoted as environmental probiotics in aquaculture farms for waste management and protein recycling.

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Introduction

Inland aquaculture is surging ahead as a promising bio-industry in India and has emerged as a viable alternative to the depleting marine catches (Katiha, Jena, Pillai, Chakraborty, & Dey, 2005). Though many species, such as the major and minor carps, tilapia, pearl spot, and mullets are suitable for inland finfish aquaculture, pangasius *(Pangasianodon hypophthalmus)* draws global attention due to its hardy nature, remarkable growth rate (Singh & Lakra, 2012) and as a good raw material for making convenient ready to eat and ready to cook products, including fish fillets, fish fingers, fish cutlets, fish balls, fish wafers, fish pickles, smoked fish, canned fish and fish curry in retort pouches (De Silva & Phuong, 2002; Rathod & Pagarkar, 2013). Pangasius is classified as omnivorous and is generally fed with rice bran and pelleted feeds.

In semi-intensive and intensive aquaculture, supplementary feed cost accounts for over 50% of operational costs (Rana, Siriwardena, & Hasan, 2009). The high cost of commercial feeds has prompted farmers to use poultry waste as a nutrient-dense low-cost protein source that has drawn wide interest among aqua farmers to adopt poultry waste-fed pangasius farming as a cost-effective way of aquaculture. However, dumping of poultry waste into aquaculture farms is not advisable since it adversely affects the culture environment and thereby the health of the fish species being cultured. This practice can also result in overloading of aquafarms with poultry waste including legs, bones, skin, and non-degradable chicken feathers,

increasing the risk of pollution and disease transmission between farms.

Ninety percent of poultry feather content is keratin, an insoluble protein with extensively cross-linked disulphide bonds that remain intact in nature for a long period of time. Because of their high keratin content, feathers are often regarded as biological plastics. Usually, keratin cannot be degraded by normal proteolytic enzymes like pepsin, trypsin and papain (Gupta & Ramnani, 2006). Only a special group of metalloprotease enzymes called keratinases can effectively degrade complex keratin into simpler molecules. Keratinase is naturally produced by various microorganisms, including fungi, actinomycetes, and bacteria (Riffel & Brandelli, 2006).

Considering the above facts, there is a need to isolate and characterize microorganisms with good keratinolytic potential from the aquatic environment to address the problem of feather disposal. Till date, many studies have attempted to screen and characterize keratinolytic microorganisms from soils in poultry waste dumped areas (Sekar et al., 2016; Sinha, Shrivastava, & Mathur, 2017; Ashraf, Asad, & Kiran, 2018). The present study intends to isolate keratinolytic bacteria from the gut of chicken waste-fed *P. hypophthalmus,* as well as from the water and soil of those culture ponds, to use such isolates as environmental probiotics to improve the water quality of polluted aquaculture farms.

Materials and Methods

Ten poultry waste-fed *P. hypophthalmus* farms from the Alappuzha and Palakkad districts of Kerala were selected for this study during 2019-20. Geographical points of sampling sites were marked as Azheekkal with latitude (9°44'41.9"N), longitude (76°18'03.9"E) for three Ponds. Two ponds in Harippad were selected with latitude (9°16'39.5"N), longitude (76°24'26.3"E) and latitude (9°17'38.2"N), longitude (76°24'49.1"E), respectively. Three ponds in Palakkad were marked as latitude (10°43'11.8"N), longitude (76°46'56.7"E) and two ponds were with Latitude (10°44'15.5"N), Longitude (76°51'52.4"E). Mud, water and fish samples from aquafarms were collected and brought to the laboratory under iced condition within 4h of sampling and screened for keratinolytic bacteria.

Five grams each of mud, fish gut and 5 mL water samples were enriched separately in 45 mL minimal basal salt media which contained sodium chloride (0.05%), potassium dihydrogen phosphate (0.04%), dipotassium hydrogen phosphate (0.03%) and feather powder (1%) (Govinden & Puchooa, 2012). The samples were incubated for 48h at room temperature with 120 rpm.

Caseinolytic activity assay, a standard method used for determining the protease activity of bacterial strains (Cheng et al., 1995), was used to screen the isolates for protease production. The enriched samples were serially diluted in sterile phosphate-buffered saline (PBS), and 0.5 mL from each dilution was spread plated onto sterile skim milk agar (Peptone-5g, skim milk powder-100g, agar-15g and distilled water-1000 mL; pH 7.2). The plates were incubated at 37°C for 24h. Colonies with clear zone formation were considered positive for non-specific proteolytic property and were isolated and purified over trypticase soy agar (TSA) for further analysis.

Protease producers were streaked on feather meal agar medium (NaCl-0.5g/L, KH_2PO_4- 0.4g/L, K_2HPO_4-0.3g/L, agar-15g/L, feather powder-10g/L) and incubated at 37°C for one week to confirm the keratinolytic activity. The formation of clear zones around the bacterial colony indicated keratinolytic activity (Sekar et al., 2016).

The potential keratinolytic microbial isolates were identified using biochemical tests according to Bergey's Manual of Systematic Bacteriology (Vos et al., 2009) and further confirmed with partial 16S rDNA sequencing. A 50 μL PCR reaction mixture contained 25 μL of 2X master mix (Invitrogen, USA), 2 μL each of forward and reverse primers, 3 μL DNA template and 18 μL of nuclease free water. The forward and reverse primers used for amplification were 27F: 5^{-} -GAGTRTGATCMTYGCTWAC-32^{-} and 1544R: 52^{-} -CGYTAMCTTWTTACGRCT-32^{-} respectively (Phukon, Phukan, Phukan, & Konwar, 2014). Amplification was carried out in thermal cycler (Eppendorf, Germany) with the following cycling conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 52°C for 30 sec, 68°C for 1min for 30 sec and final extension 7 min at 68°C. The PCR products were further purified with gel extraction kit (Bangalore Genei™) and sequenced and compared with sequences in the GenBank database using the BLAST program.

The potential keratinase enzyme producers were evaluated for keratinase production following a standard protocol (Cai, Lou, & Zheng, 2008). The substrate for enzyme assay, *i.e.*, DMSO solubilized

feather keratin, was prepared by the method described by Wawrzkiewicz, Łobarzewski, and Wolski (1987). One-millilitre of appropriately diluted crude enzyme was reacted with 1 mL keratin solution in a water bath at 50°C for 10 min, after which an equal volume of 20% trichloracetic acid (TCA) solution was added to stop the reaction. The solution was then centrifuged at 7000 rpm for 15 min and OD of supernatant was measured at 280 nm. Enzyme activity was calculated as U= 4* n *(A 280/0.01*10), and one unit of keratinase activity was defined as increase in the corrected absorbance (A280) by 0.01 units per mL per minute under the conditions described above.

Actively growing individual bacterial cultures *viz.,* FMKB1, FMKB2 and a combination of FMKB1 and FMKB2 in trypticase soy broth (TSB) (BD & Difco, USA) were adjusted to a concentration of 1 McFarland's standard and further inoculated to 250 mL TSB flask at 5% level. Sterile feather with an average weight of 10 ± 2 mg was added as the substrate for keratinase production in these flasks. Flask with feather but without bacterial inoculation was maintained as control. All flasks were incubated at 37°C for 1 month and were observed for keratinolytic activity (Xu, Zhong, Tang, Yang, & Huang, 2009).

Congo red binding assay with Congo red as indicator was performed to assess the pathogenic potential of bacterial isolates (Qadri et al., 1988). Trypticase soy agar (TSA) supplemented with 0.8 g/L of Congo red dye with incubation for 48h at 37°C was used for this assay. Haemolytic activities of bacterial isolates were studied by streaking over 5% sheep blood agar (Himedia, India), followed by incubation for 48h at 37°C. Exo-enzyme profiling was performed for lipase, amylase, gelatinase and production. Cross-streaking assay with *Chromobacterium violaceum* CVO26 (ATCC) was performed on Luria Bertani (LB) (BD & Difco, USA) plates to confirm the quorum sensing potential of the isolates (Yang et al., 2013). Biofilm formation on polystyrene surface was assessed in 96 well plates according to Coffey and Anderson (2014).

Results and Discussion

The present study aimed to isolate and characterize keratinolytic bacteria from poultry waste-fed aqua farms in Kerala, India. A total of 116 proteolytic bacteria were isolated from soil, water and fish gut samples from Palakkad and Alappuzha districts of Kerala during initial screening for keratinase producers (Table 1). Of these, 6.9% of the isolates showed keratinolytic activity in feather meal agar. The prevalence of proteolytic bacteria was high (47.4%) among soil microbes, while in water and fish gut, the prevalence was 36.2% and 16.4%, respectively. Close association of keratinolytic bacteria with keratin-containing substrates such as feathers, hairs, hooves, horns etc has been reported previously by many studies (Williams, Richter, Mackenzie, & Shih, 1990; Riffel & Brandelli, 2002, 2006; Joshi, Tejashwini, Revati, Sridevi, & Roma, 2007; Govarthanan, Kumar, Prakash, & Manoharan, 2010; Agrahari & Wadhwa, 2010; Rajesh, Rajasekar, Mathan, & Anandaraj, 2016; Sinha et al., 2017). Significant proteolytic activity among keratinolytic bacterial population makes the proteolytic assay as an efficient tool in initial screening (Cheng et al., 1995; Sekar et al., 2016; Wibowo et al., 2017; Ashraf et al., 2018). The present study also justifies the same and their proteolytic activity (zone formation) in 24h was found to be more than their keratinolytic activity. This might be due to the production of other broad-spectrum proteases as reported in *B. licheniformes* (Wakil, Dada, & Onilude, 2011).

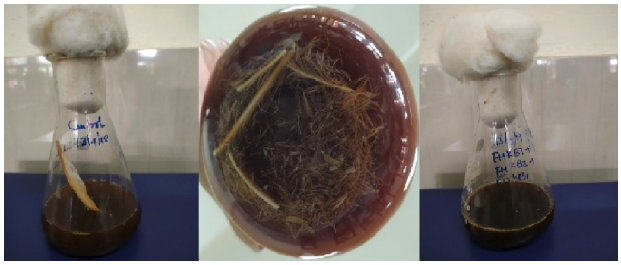

Fig. 1. a). Control raw feather b). Partially degraded raw feather by FMKB1 and FMKB2 within one month c). Completely dissolved raw feather by FMKB1 and FMKB2 within 45 days

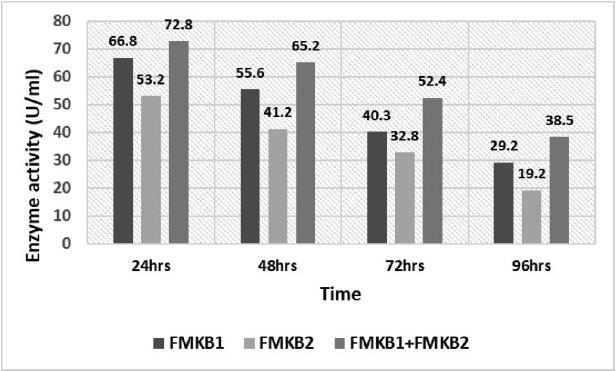

Fig. 2. The keratinase enzyme production (U/mL) by FMKB1, FMKB2 and combination of FMKB1 & FMKB2 with time

Table 1. Proteolytic and keratinolytic isolates obtained from different aqua farms

Sl No.	Pond site	Sample	No. of proteolytic isolates	No. of keratinolytic isolates
1	Azheekkal [Latitude (9°44'41.9"N), Longitude (76°18'03.9"E)]	Fish	4	0
		Soil	7	0
		Water	9	0
2	Harippadu [Latitude (9°16'39.5"N), Longitude (76° 24'26.3"E)]	Fish	5	0
		Soil	1	0
		Water	3	0
3	Harippadu [Latitude (9° 17'38.2"N), Longitude (76° 24'49.1"E)]	Fish	4	0
		Soil	4	1
		Water	1	0
4	Azheekkal [Latitude (9°44 '41.9"N), Longitude (76°18'03.9"E)]	Fish	2	0
		Soil	5	0
		Water	3	0
5	Azheekkal [Latitude (9°44'41.9"N), Longitude (76°18'03.9"E)]	Fish	4	0
		Soil	6	0
		Water	6	1
6	Palakkad [Latitude (10°43'11.8"N), Longitude (76°46'56.7"E)]	Fish	0	0
		Soil	8	1
		Water	4	0
7	Palakkad [Latitude (10°44'15.5"N), Longitude (76° 51'52.4"E)]	Fish	0	0
		Soil	8	1
		Water	4	1
8	Palakkad [(Latitude (10°43'11.8"N), Longitude (76°46'56.7"E)]	Fish	0	0
		Soil	6	2
		Water	5	1
9	Palakkad [Latitude (10°43'11.8"N), Longitude (76°46'56.7"E)]	Fish	0	0
		Soil	6	0
		Water	3	0
10	Palakkad [Latitude (10°44'15.5"N), Longitude (76° 51'52.4"E)]	Fish	0	0
		Soil	4	0
		Water	4	0

Potential keratinase producers (FMKB1 and FMKB2) isolated from mud of pangasius farm in Palakkad district of Kerala were identified by bio-typing and 16S rDNA sequencing as *Bacillus subtilis* (GenBank accession numbers MN340035.1 and MN340032.1, respectively). The keratinolytic bacterium isolated from water of pangasius farm in Alappuzha district of Kerala, *i.e.,* FWKB1 was identified as *Exiguobacterium profundum* (GenBank accession no. MN340033.1). In general, all three isolates showed an increasing trend in zone formation with incubation time up to 72h. *E. profundum* did not show any growth and zone formation after 72h of incubation. Keratinase production among various *Bacillus* species indigenous to poultry has been reported previously (Brandelli, 2008; Sivakumar, Shankar, Vijayabaskar, & Ramasubramanian, 2012; Lakshmi, Chitturi, & Lakshmi, 2013; Femi-Ol, Akinbobola, & Oluwaniyi, 2015), and the most potential among them was *B. licheniformis* (Xu et al., 2009; Wakil et al., 2011; Iruolaje, Ogbeba, Tula, Ijebor, & Dogo, 2015). Sekar et al. (2016) reported the highest keratinolytic activity with a maximum zone production of 20 mm diameter by *B. subtilis* within 72h. According to this study, *Bacillus subtilis* strains produced clear zones ranging from 12±2 mm up to 43±3 mm within seven days and confirm the results of many earlier studies (Kim, Lim, & Suh, 2001; Cortezi, Contiero, de Lima, Lovaglio, & Monti, 2008; Macedo, Beys da Silva, & Termignoni, 2008; Hassan, Haroun, Amara, & Serour, 2013; He et al., 2018).

Assessment of direct degradation of feather by the keratinolytic bacterial isolates showed that the raw feather was partially degraded within two weeks and was completely hydrolyzed within 45 days using a combination of FMKB1 and FMKB2 (Fig. 1). The sterile raw feather in control flask remained intact without any degradation during the entire study period. Direct degradation of feather keratin in broth cultures is preferred over plate screening method as it is less expensive, providing easy-to-interpret visual results (Sivakumar et al., 2012; Tamilkani, Karnan, Kanimozhi, & Panneerselvam, 2017; Ashraf et al., 2018).

The keratinase enzyme assay revealed that the most active keratinolytic isolates FMKB1 and FMKB2 exhibited 53.2 U/mL and 66.8 U/mL of enzyme production, respectively within 24h of incubation at 37°C. The combination of FMKB1 and FMKB2 exhibited a maximum value of 72.8 U/mL keratinase production, and this synergistic action resulted in efficient and fast degradation compared to individual bacteria. After 24h the keratinase enzyme production appeared to reduce in successive hours of incubation over 96h (Fig. 2), which could be due to the unstable nature of the enzyme in non-ambient conditions. Keratinase enzyme production in *Bacillus* species is reported to be strictly temperature dependent and varied from 96 to 187.5 U/mL under optimum conditions (Riffel & Brandelli, 2006; Cortezi et al., 2008; Chhimpa, Yadav, & John, 2016). Therefore, further studies are necessary to determine the production of keratinase under different time-temperature combinations.

Assessment of probiotic potential of these identified cultures of *B. subtilis* and *E. profundum* were also carried out and was revealed that the isolates lacked amylase, lipase and gelatinase enzymes. Haemolysis, Congo red binding, biofilm formation and AHL signalling molecule production were not detected among the isolates. All these findings suggest the absence of pathogenic potential in these isolates. Ramnani and Gupta (2007) suggested that the cell free keratinase is incapable of feather degradation and continuous supply of live bacterial cells is very crucial for complete feather degradation. The results of this study suggest that FMKB1, FMKB2 and FWKB1 are good sources of keratinase and can be potentially useful to combat the problem of feathers in poultry-waste fed aquafarms. The degradation of poultry feathers by microbial keratinases might also offer an alternative technique for bioconversion of keratin.

Conventional methods of feather degradation such as acid/alkali hydrolysis and steam pressure cooking destroy the heat sensitive essential amino acids such as lysine, methionine, and tryptophan and generate non-nutritive amino acids apart from consuming energy. This study identified FMKB1 and FMKB2 strains of *B. subtilis* as effective keratinase producers capable of degrading poultry feathers within 45 days at 37°C. In aquafarms fed with poultry waste, these isolates could be used as environmental probiotics to improve the water quality in an environmentally friendly approach. Future studies should focus on optimizing keratinase production and evaluating the field efficacy of these isolates in aquaculture system.

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References

Agrahari, S., & Wadhwa, N. (2010). Degradation of chicken feather a poultry waste product by keratinolytic bacteria isolated from dumping site at Ghazipur poultry processing plant. *International Journal of Poultry Science*, *9*(5), 482-489. https://doi.org/10.3923/ijps.2010.482.489.

Ashraf, M. M., Asad, W., & Kiran, T. (2018). Isolation of keratinolytic bacterial strains from poultry farm soil samples; production, optimization and thermal characterization of the keratinase enzyme(s). *Journal of Chemical Society of Pakistan*, *40*(5), 953-960.

Brandelli, A. (2008). Bacterial keratinases: useful enzymes for bioprocessing agroindustrial wastes and beyond. *Food and Bioprocess Technology*, *1*(2), 105-116. https://doi.org/10.1007/s11947-007-0025-y.

Cai, C. G., Lou, B. G., & Zheng, X. D. (2008). Keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis*. *Journal of Zhejiang University Science B*, *9*(1), 60-67. https://doi.org/10.1631/jzus.B061620.

Cheng, S. W., Hu, H. M., Shen, S. W., Takagi, H., Asano, M., & Tsai, Y. C. (1995). Production and characterization of keratinase of a feather-degrading *Bacillus licheniformis* PWD-1. *Bioscience, Biotechnology, and Biochemistry*, *59*(12), 2239-2243. https://doi.org/10.1271/bbb.59.2239.

Chhimpa, S., Yadav, C. S., & John, P. J. (2016). Isolation and identification of keratin degrading (keratinolytic) bacteria from poultry feather dumping sites. *Journal of Biodiversity and Environmental Sciences*, *8*(6), 109-119.

Coffey, B. M., & Anderson, G. G. (2014). Biofilm formation in the 96-well microtiter plate. In A. Filloux, & J. L. Ramos (Eds.), *Pseudomonas Methods and Protocols* (pp. 631-641). Humana, New York.

Cortezi, M., Contiero, J., de Lima, C. J. B., Lovaglio, R. B., & Monti, R. (2008). Characterization of a feather degrading by *Bacillus amyloliquefaciens* protease: a new strain. *World Journal of Agricultural Sciences*, *4*(5), 648-656.

De Silva, S. S., & Phuong, N. T. (2011). Striped catfish farming in the Mekong Delta, Vietnam: a tumultuous path to a global success. *Reviews in Aquaculture*, *3*(2), 45-73. https://doi.org/10.1111/j.1753-5131.2011.01046.x.

Femi-Ola, T. O., Akinbobola, O. S., & Oluwaniyi, T. T. (2015). Isolation and characterization of feather degrading bacteria from poultry soil. *Agriculture and Biology Journal of North America*, *6*(5), 146-154. https://doi.org/10.5251/abjna.2015.6.5.146.154.

Govarthanan, M., Kumar, T. S., Prakash, S. A., & Manoharan, P. K. (2010). Keratinolytic bacteria isolated from chick faecal waste. *Environmental Science*, *5*(4), 223-225.

Govinden, G., & Puchooa, D. (2012). Isolation and characterization of feather degrading bacteria from Mauritian soil. *African Journal of Biotechnology*, *11*(71), 13591-13600. https://doi.org/10.5897/AJB12.1683.

Gupta, R., & Ramnani, P. (2006). Microbial keratinases and their prospective applications: an overview. *Applied Microbiology and Biotechnology*, *70*(1), 21-33. https://doi.org/10.1007/s00253-005-0239-8.

Hassan, M. A., Haroun, B. M., Amara, A. A., & Serour, E. A. (2013). Production and characterization of keratinolytic protease from new wool-degrading *Bacillus* species isolated from Egyptian ecosystem. *BioMed Research International*, *2013*(1), Article 175012. https://doi.org/10.1155/2013/175012.

He, Z., Sun, R., Tang, Z., Bu, T., Wu, Q., Li, C., & Chen, H. (2018). Biodegradation of feather waste keratin by the keratin-degrading strain *Bacillus subtilis* 8. *Journal of Microbiology and Biotechnology*, *28*(2), 314-322. https://doi.org/10.4014/jmb.1708.08077.

Iruolaje, F. O., Ogbeba, J., Tula, M. Y., Ijebor, J. A., & Dogo, B. A. (2016). Isolation and identification of keratinolytic bacteria that exhibit feather-degrading potentials. *Journal of Advances in Biology & Biotechnology*, *5*(2), 1-9. https://doi.org/10.9734/JABB/2016/22082.

Lakshmi, P. J., Chitturi, C. M. K., & Lakshmi, V. V. (2013). Efficient degradation of feather by keratinase producing *Bacillus* sp. *International Journal of Microbiology*, *2013*(1), Article 608321. https://doi.org/10.1155/2013/608321.

Joshi, S. G., Tejashwini, M. M., Revati, N., Sridevi, R., & Roma, D. (2007). Isolation, identification and characterization of a feather degrading bacterium. *International Journal of Poultry Science*, *6*(9), 689-693. https://doi.org/10.3923/ijps.2007.689.693.

Katiha, P. K., Jena, J. K., Pillai, N. G. K., Chakraborty, C., & Dey, M. M. (2005). Inland aquaculture in India: past trend, present status and future prospects. *Aquaculture Economics & Management*, *9*(1-2), 237-264. https://doi.org/10.1080/13657300590961573.

Kim, J. M., Lim, W. J., & Suh, H. J. (2001). Feather-degrading *Bacillus* species from poultry waste. *Process Biochemistry*, *37*(3), 287-291. https://doi.org/10.1016/S0032-9592(01)00206-0.

Macedo, A. J., Beys da Silva, W. O., & Termignoni, C. (2008). Properties of a non collagen-degrading *Bacillus subtilis* keratinase. *Canadian Journal of Microbiology*, *54*(3), 180-188. https://doi.org/10.1139/W07-124.

Phukon, P., Phukan, M. M., Phukan, S., & Konwar, B. K. (2014). Polyhydroxyalkanoate production by indigenously isolated *Pseudomonas aeruginosa* using glycerol by-product of KCDL biodiesel as an inexpensive carbon source. *Annals of Microbiology*, *64*(4), 1567-1574. https://doi.org/10.1007/s13213-014-0800-8.

Qadri, F., Hossain, S. A., Ciznár, I., Haider, K., Ljungh, A., Wadstrom, T., & Sack, D. A. (1988). Congo red binding and salt aggregation as indicators of virulence in *Shigella* species. *Journal of Clinical Microbiology*, *26*(7), 1343-1348. https://doi.org/10.1128/jcm.26.7.1343-1348.1988.

Rajesh, T. P., Rajasekar, S., Mathan, R. K. H., & Anandaraj, B. (2016). Isolation and identification of feather degrading bacteria from feather-dumped soil. *International Journal of Environment and Sustainable Development*, *15*(3), 293-299. https://doi.org/10.1504/IJESD.2016.077393.

Ramnani, P., & Gupta, R. (2007). Keratinases vis-à-vis conventional proteases and feather degradation. *World Journal of Microbiology and Biotechnology*, *23*(11), 1537-1540. https://doi.org/10.1007/s11274-007-9398-3.

Rana, K. J., Siriwardena, S., & Hasan, M. R. (2009). *Impact of rising feed ingredient prices on aquafeeds and aquaculture production* (1st ed.). FAO, Rome.

Rathod, N., & Pagarkar, A. (2013). Biochemical and sensory quality changes of fish cutlets, made from pangasius fish (*Pangasianodon hypophthalmus*), during storage in refrigerated display unit at -15 to-18ºC. *International Journal of Food, Agriculture and Veterinary Sciences*, *3*(1), 1-8.

Riffel, A., & Brandelli, A. (2002). Isolation and characterization of a feather-degrading bacterium from the poultry processing industry. *Journal of Industrial Microbiology and Biotechnology*, *29*(5), 255-258. https://doi.org/10.1038/sj.jim.7000307.

Riffel, A., & Brandelli, A. (2006). Keratinolytic bacteria isolated from feather waste. *Brazilian Journal of Microbiology*, *37*(3), 395-399. https://doi.org/10.1590/S1517-83822006000300036.

Sekar, V., Kannan, M., Ganesan, R., Dheeba, B., Sivakumar, N., & Kannan, K. (2016). Isolation and screening of keratinolytic bacteria from feather dumping soil in and around Cuddalore and Villupuram, Tamil Nadu. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *86*(3), 567-575. https://doi.org/10.1007/s40011-014-0483-8.

Singh, A. K., & Lakra, W. S. (2012). Culture of *Pangasianodon hypophthalmus* into India: impacts and present scenario. *Pakistan Journal of Biological Sciences*, *15*(1), 19-26. http://dx.doi.org/10.3923/pjbs.2012.19.26.

Sinha, S., Shrivastava, V., & Mathur, A. (2017). First Report on screening of keratinolytic bacteria from soil samples of poultry waste dump sites of wide Delhi-NCR region. *European Journal of Pharmaceutical and Medical Research*, *4*(9), 491-494. http://dx.doi.org/10.13140/RG.2.2.28086.19525.

Sivakumar, T., Shankar, T., Vijayabaskar, P., & Ramasubramanian, V. (2012). Optimization for keratinase enzyme production using *Bacillus thuringiensis* TS2. *Academic Journal of Plant Sciences*, *5*(3), 102-109. https://doi.org/10.5829/idosi.ajps.2012.5.3.6279.

Tamilkani, P., Karnan, M., Kanimozhi, K., & Panneerselvam, A. (2017) Screening of Keratinolytic Bacteria Form Keratin Waste Dumped Soil in Thanjavur (Dt), Tamil Nadu, India. *International Journal of Pharmacy & Pharmaceutical Research, 8*(2), 25-32.

Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K. H., & Whitman, W. B. (2009). *Bergey's manual of systematic bacteriology: Volume 3, the firmicutes.* Springer, Berlin.

Wakil, S. M., Dada, M. T., & Onilude, A. A. (2011). Isolation and characterization of keratinase-producing bacteria from poultry waste. *Journal of Pure and Applied Microbiology*, *5*(2), 567-580.

Wawrzkiewicz, K., Łobarzewski, J., & Wolski, T. (1987). Intracellular keratinase of *Trichophyton gallinae*. *Journal of Medical and Veterinary Mycology*, *25*(4), 261-268. https://doi.org/10.1080/02681218780000601.

Wibowo, R. L. M. S. A., Bachruddin, Z., Fitriyanto, N. A., Nakagawa, T., Hayakawa, T., & Pertiwiningrum, A. (2017). Screening and characterization of keratinolytic bacteria from puffer fish skin waste. *Pakistan Journal of Nutrition*, *16*(7), 488-496. https://doi.org/10.3923/pjn.2017.488.496

Williams, C. M., Richter, C. S., Mackenzie, J. M., & Shih, J. C. H. (1990). Isolation, identification, and characterization of a feather-degrading bacterium. *Applied and Environmental Microbiology*, *56*(6), 1509-1515. https://doi.org/10.1128/aem.56.6.1509-1515.1990.

Xu, B., Zhong, Q., Tang, X., Yang, Y., & Huang, Z. (2009). Isolation and characterization of a new keratinolytic bacterium that exhibits significant feather-degrading capability. *African Journal of Biotechnology*, *8*(18), 4590-4596.

Yang, Y., Yao, F., Zhou, M., Zhu, J., Zhang, X., Bao, W., Wu. S., Hardwidge, P. R., & Zhu, G. (2013). F18ab *Escherichia coli* flagella expression is regulated by acyl-homoserine lactone and contributes to bacterial virulence. *Veterinary Microbiology*, *165*(3-4), 378-383. https://doi.org/10.1016/j.vetmic.2013.04.020.