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Short Communication

Degradation of feathers by bacterial consortium and its application in seed germination

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Abstract

Feathers primarily composed of keratin are largely produced as a waste by-product at poultry plants. Keratin is an insoluble protein macromolecule with very high stability and low degradation rate. Use of keratinolytic bacteria have found a promising effect in shortening the degradation time. In present study consortium of three organisms was used for feather degradation and the feather degraded product was used to check effect on seed germination. It was seen that the consortium was able to degrade 92.6% of feathers within 72 hours. The degraded product proved to be rich source of nitrogen. The application of feather degraded product in seed germination of Gram seeds had astounding results. The effect was measured in terms of root-shoot length, number of secondary roots, root shoot ratio, etc. The root: shoot ratio was increased in control group (2.7) compared with test (1.9) indicating nutrient deficiency. Thus, the feather degraded product could be used to increase seed germination rate.

Keywords: Keratinase, poultry waste, feather degradation, cup method, seed germination.

Introduction

Large quantities of feather waste is generated as a by-product of commercial poultry industry. Feathers account for 5-7% of the total weight of mature chicken[1]. Feathers are almost are very difficult to biodegrade, and no method are known to apply them on an industrial scale. They contain beta keratin constituting 91% of feather protein. Keratin makes feather recalcitrant to most common proteases like trypsin, pepsins, papain. This slows down the degradation process naturally. A great problem of the agricultural industry is managing the enormous amount of waste generated by poultry processing enterprises[2]. Feathers can be converted to feed-stuffs, fertilizers, glues, etc. by mechanical or chemical treatment. Amino acids and peptides can also be produced from feathers. Presently commercial production of feather meal is carried out by treating feathers at elevated temperature and high pressure[3]. Some essential amino acids are lost by this method and also the process uses high energy[4].

Keratin degradation by using microbial technology has varied beneficial applications[5]. The enzymes capable of degrading keratin known as keratinolytic enzymes[6] are extracellular enzyme used for the biodegradation of keratin. Keratinases are reported from many microorganisms such as *Bacillus licheniformis, Pseudomonas*, *Chryseo bacterium, Micro bacterium sp., Actinomycetes* and fungi. Feather degradation by keratinases offers an alternative method for efficient bioconversion, nutritional enhancement and environmental friendliness.

The region selected for study was Baramati region, well known for the number of poultry farms. The major problem in these farms as well as the chicken shops basically is the disposal of feathers, which else cause a nuisance. The conventional method to get rid from these feathers is either burning them or dumping them. Burning losses the entire valuable nitrogen source and dumping them in soil requires a long time period for decomposition.

Keratinolytic enzymes are fetching striking applications in biodegradation. They are used in the manufacturing of amino acids and peptides. Due to numerous potential uses of keratinase, this study was undertaken for use of highly active keratinase to degrade feathers and shorten the decomposition period. Further the use of the nitrogen rich end product was checked for seed germination activity.

Materials and methods

Feather degradation: Feathers are unique source of carbon, nitrogen, sulfur and energy. The selected strains *Sphingomonas paucimobilis, Bacillus brevis* and *Aeromonas hydrophila* were grown in 100ml feather meal broth. The organisms were grown for up to 3 days at 37°C under agitation in an orbital shaker at 180rpm.

Protein estimation by the Folin-Lowry method: Feathers were degraded biologically for 3 days. After 3 days the protein content was detected by Folin-Lowry method. Different

concentrations (20-180µg/ml) of standard protein solution were taken. 5ml of alkaline Cu solution was added, mixed, and allowed to stand for R.T for 10 mins. 0.5ml of Folin reagent was added. The mixture was allowed to stand for 30 min at room temperature. O.D. was read at 750nm against blank. Test sample was treated in the same way as for standard concentrations.

Use of feather degraded product in seed germination: Seed germination cup method: The time it takes for a seed to begin to grow is called germination starting time (GST). The aim of this experiment was to see whether the germination starting time and the rate of germination was reduced by the use of feather degraded product.

Plastic cups were taken and were covered with tissue paper from inner side. Crumpled up tissue papers were placed in the middle of a plastic cup. Gram seeds were selected for the study. Short germination time and ease of handling made Gram seeds, seed of choice. Surface sterilization was done by 0.1% $HgCl_2$. Two seeds were placed in between the wall space of cup and the tissue paper. The tissue papers were sprinkled by water and feather degraded product for control and test respectively. Germination was checked each day. Parameters such as radical-plumule length, width of primary tap root, number of secondary roots etc. were noted.

Results and discussion

Feather degradation by use of consortium: The feather degradation results for individual strains were studied as well as that for consortium was observed. It was found that the feather degradation was more that is 93.6% and the time required was 72 hrs as compared to individual activity of each strain where degradation was seen after 4 to 5 days. Thus consortium of all strains together degrade keratin quickly than individual strain.

Seed germination: Seed germination cup experiment: Unique technique to observe the effect of feather digests on seed germination was done using cup method. The growth of root could be seen directly due to the transparent nature of the cup and the difference in the control and test seed germination was easily noted. The effect was calculated in terms of root length and other parameters. The results were significant in that the length as well as width of the root were more as compared to control.

The root: shoot ratio is one measure to help assess the overall health of the plants. The root-shoot ration will change when plants encounter environmental stresses and nutrition deficiency.

In the current experiment the root:shoot ratio was increased in control group indicating nutrient deficiency. The length of the roots was more as compared to its mass and the length of plumule. The test group showed less root:shoot ratio as compared to control group in relation to the supplements provided by the feather digest.

Discussion: Nitrogen-based fertilizers are in demand, which are cheap and readily available. Feathers prove to be rich source of protein in turn of nitrogen. The consortium used in the present study degraded feathers within less time increasing nitrogen availability. Reports have highlighted the prospective role of feather degraded product in promotion of plant growth. It was found to be a rich source of nitrogen[7,8]. The germination of mung bean was positively affected by the degraded metabolites of chicken feathers probably by providing the nitrogen sources. Soil enriched with feather digest showed increased length and numbers of root hairs. This result was analogous with that of Paul *et al.*[9] who found the function of feather digest in increasing the number of root hairs. Promotion of plant growth was revealed by increased weight of shoot and plumule length. Plant growth support was observed by Anwar *et al.*[10] in *Brassica juncea* following inoculation with *B. cereus*. The metabolites required to synthesize the major nutrient and hormones are probably provided by the feather digest. Nitrogen fertilizers or soil amendments produced by the enzymatic conversion of feathers could be beneficial for plants[11-13]. Comparative effectiveness of animal manures on soil chemical properties, yield and root growth of *Amaranthus cruentus* L. were observed[14].

Figure-1: Feather degradation.

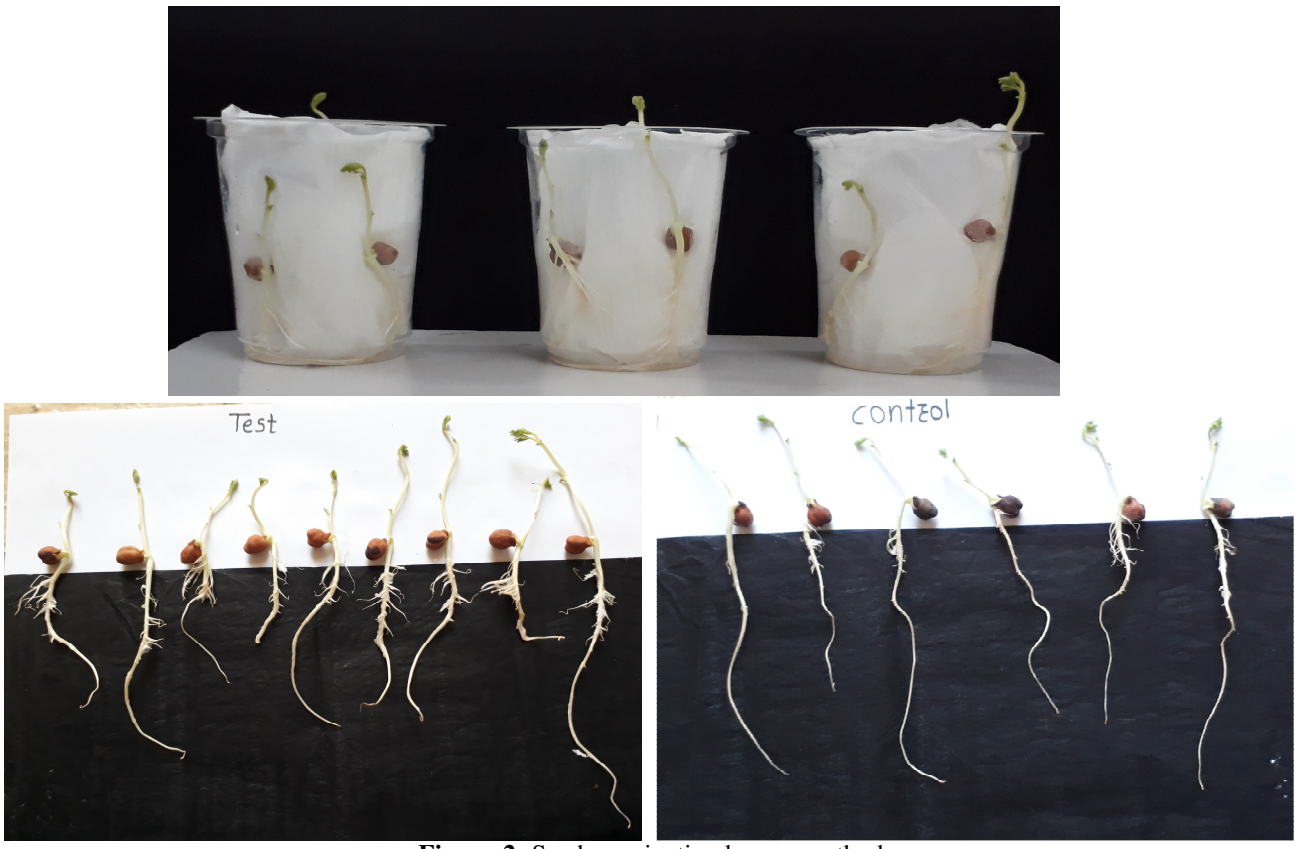

Figure-2: Seed germination by cup method.

Table-1: Seed germination effect in terms of root length and other parameters.

Content	Length of radical (cm)	Length of plumule (cm)	No. of secondary roots	Dry wt. of radical (g)	Dry wt. of plumule (g)	Root/Shoot ratio
Control	10.4±1.74	3.8±0.46	5±1.2	0.027±0.004	0.041±0.005	2.7
Test	10.03±2.18	5.1±1.08	7±1.5	0.031±0.003	0.048±0.004	1.9

Conclusion

Feed additives or soil fertilizers can be prepared by the enzymatic degradation of keratin. The consortium was able to degrade feathers rapidly within 72 hours. There was a remarkable effect of feather degraded product on the seed germination. Absorption of nutrients from soil can be enhanced by the increased root surface area, which is achieved by increase in root hairs. The capability of microbial keratinases to speed up the degradation of feathers could be a practical and environmental friendly method of recycling these organic wastes into nitrogen-rich fertilizers.

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