FROM FEATHER TO FEED

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Background, Purpose and Hypothesis

Feathers are produced in large amounts as a byproduct at poultry processing plants, reaching millions of tons annually. Since feathers are almost pure keratin protein consisting of amino acids, feather wastes represent a potential alternative to expensive dietary ingredients for animal feedstuffs.

Keratin, the insoluble protein of feathers, is known for its high stability. However, feathers do not accumulate in nature, suggesting the existence of natural microbial decomposers of feathers. The development of a biotechnological approach to chicken feathers hydrolysis is the *main goal* of this project.

The *hypothesis* is that if keratin-degrading microorganisms do exist in nature, then it will be possible to isolate them and use them for hydrolysis of chicken feathers.

Procedure

Part 1: Isolation of feather degrading microorganisms

Strong selective conditions were created, using an enrichment medium containing chicken feathers as the only source of carbon and nitrogen, in order to isolate feather-degrading microorganisms from soil.

1) A few soil samples were collected at a local chicken farm and then mixed together.

2) Feathers were washed 3 times with water and sterilized by boiling for 30 minutes.

3) An enrichment medium was prepared consisting of cut, chopped and ground chicken feathers suspended in a 0.1% phosphate buffer (pH 7.0) solution.

4) 1g of the soil mix was added to a first enrichment flask (500ml capacity) containing 50ml of enrichment medium.

5) The first enrichment flask was incubated for 7 days at ~30ºC. Aeration of the culture was achieved by shaking the flask every hour for ten-fifteen minutes.

6) 1ml of broth was taken from the first enrichment flask, re-inoculated into 50ml of fresh enrichment medium, and cultivated under the same conditions until the turbidity became noticeable due to bacterial growth. The same procedure was repeated a third time.

7) The final broth was filtered through filter paper to remove solid particles and dispersed into plastic test tubes. The preparation was preserved in a freezer (around -10ºC) in the presence of 15% glycerol.

Part 2: Development of Conditions for Feather Hydrolysis

Microorganisms obtained at the end of the enrichment procedure were used for chicken feather hydrolysis. Hydrolysis is the reaction with water where the breakdown of long polymeric molecules into monomers occurs. The chicken feather (keratin) is the long polymeric molecule that is broken down into amino acids and short peptides. This reaction is catalyzed by the enzyme keratinase produced by the isolated microorganisms.

Turbidity is a measure of cloudiness of a suspension. Any decrease of turbidity of the feather suspension is an indicator of feather hydrolysis. Feather hydrolysis was evaluated by a visual comparison of turbidity of experimental samples with the standard ones (Table 1). The scale of turbidity was created using 5 test tubes to prepare feather suspensions with concentrations of 5%, 2.5%, 1.25%, 0.50% and 0% in 0.1% phosphate buffer (pH 7.0).

Table 1. Turbidity Standards

Feather concentration (%)	5.00	2.50	1.25	0.50	0.00
Turbidity	100	50	25	10	0

I. Influence of bacterial amount on feathers hydrolysis

Six test tubes each containing 10 ml of 5% feathers suspension in 0.1% phosphate buffer (pH 7.0) were mixed with 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the culture broth. Incubation at ~30^{o}C continued until the turbidity in the test tube with 1.0ml of culture broth was very low. Results are presented in Table 2.

Table 2. Feather hydrolysis versus amount of culture

Cultural broth (ml)	0	0.2	0.4	0.6	0.8	1.0
Turbidity	100	90	80	30	10	5

The results demonstrated that feather hydrolysis increased with an increase of bacterial amount.

The numbers represent an average of three test tubes that underwent the exact same treatment.

II. Effect of temperature on feathers hydrolysis

Three test tubes containing 10 ml of 5% suspension in 0.1% phosphate buffer (pH 7.0) were mixed with 1.0 ml of the culture and incubated at ~30ºC (on top of fridge), ~20ºC (on living room floor), and ~5ºC (cold room in basement). Incubation continued until the suspension incubated at 30ºC had almost no turbidity. Results are in Table 3.

Table 3. Feather hydrolysis versus temperature of incubation

Temperature (^{0}C)	**Turbidity**
~30	5
~20	50
~5	90

The most effective hydrolysis of feathers was observed at 30°C.

The numbers represent an average of three test tubes that underwent the exact same treatment.

III. Dependence of feather hydrolysis upon the amount of feathers

4 test tubes each containing 10 ml of 20%, 15%, 10% and 5% feather suspensions in 0.1 % phosphate buffer (pH 7.0) were prepared. 1ml of microorganism was added to each suspension. The test tubes were incubated at ~30°C until the test tube containing 5% suspension had almost no turbidity. For comparison with the turbidity standards (Table 1), experimental tubes containing 10-, 15-, and 20% of feather suspension were evaluated

after 2-, 3- and 4-times dilution in phosphate buffer. Results are presented in Table 4.

Table 4. Feather hydrolysis versus feather concentration

Feather concentration (%)	Turbidity
20	100
15	80
10	50
5	5

The results showed that the most effective hydrolysis occurs with 5% feather suspension.

The numbers represent an average of three test tubes that underwent the exact same treatment.

IV. Preparation of dry chicken feathers hydrolysate.

10ml of 5% feather suspension containing 1ml of culture was incubated at ~30°C for seven days. The hydrolysate was filtered through filter paper to remove undigested particles and brought to boiling. Simmering continued until all of the liquid was evaporated and a dry powder was left. The control, with no microorganisms, underwent the same treatment. The results demonstrated that almost all feathers were hydrolyzed as a result of microbial treatment. The yield of the process was found to be 86.5%. Results are presented in Table 5.

Table 5. Preparation of dried feather hydrolysate

Sample	Control	Experiment
Weight (g)	0.52	0.45

The numbers represent an average of three test tubes that underwent the exact same treatment.

Results, Observations and Conclusions

This report describes isolation of a soil microbial consortium capable of chicken feather degradation and the use of obtained microorganisms for feather hydrolysis.

The theoretical background for the selection of feather-degrading microorganisms was: **(i)** keratinase secreting microorganism are able to digest feathers; and **(ii)** products of the hydrolysis, amino acids and small peptide, are taken up by these microorganisms and used as a source of carbon and nitrogen.

The following results were achieved:

1) Feather degrading microorganisms were isolated. The microbes were able to grow in the presence of feathers as a source of carbon and nitrogen.

2) When chicken feathers were treated with the isolated microorganisms, almost complete hydrolysis of the feathers occurred.

3) The optimal conditions for feather hydrolysis are: **a)** an incubation time of ~7 days; **b)** a temperature of ~30^0C; **c)** concentration of feather suspension- 5% and **d)** 1 ml of culture per 10 ml of feather suspension.

4) Both liquid and dried preparations of chicken feathers hydrolysates were obtained.

The results from this work would be very useful for industrial chicken farms. Usually feathers are burnt or buried in landfills The ability to turn waste feathers into feed would reduce feed costs, and since this process would reduce the amount of pollutants going into the atmosphere and save space in landfills it could be beneficial to the environment.

Safety considerations

1) Feathers were sterilized by boiling for 30 minutes. This treatment kills all microbial contaminants. **2)** Due to the strong selective conditions used, only feather degrading microbes were able to grow and reproduce. Non-growing microbes were supplanted by the growing ones and, therefore, eliminated. **3)** The final product, chicken feather hydrolysate, was boiled and dried at end of process, which was another sterilization step in order to kill any bacterial contaminant.

All of these measures eliminate the possibility of **any** microbial contaminants from feathers being passed on to the animals consuming the hydrolysate.

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Appendix: Bibliography

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