Pig Bristles – An Underestimated Biomass Resource

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Abstract - The potential of novel technology to convert biological residual resources into new alternative high value products are to be considered in this ongoing project. Contemporary industrial meat processing produces large quantities of residual by-products such as pig bristles, rich in the protein keratin. The aim of this study is to develop a gentle method of processing with increased concentrations of intact amino acids, digestibility and nutritional value of the final product. Efficient and gentle bioconversion of keratin will be conferred by the enzymatic action of microbial strains. Therefore, suitable screening methods to find potential keratin degrader microorganisms are a prerequisite for the success of the project. The scope of this paper is a presentation of an enrichment method and keratinase screening assays, designed and performed for this purpose [1].

Key words: keratin, microbial degradation

I. INTRODUCTION

Due to the large amounts produced by the meat industry, as well as the high protein content and potential nutritional value, feathers and pig bristles are particularly interesting as a biomass resource. This study is focussing on pig bristles. A slaughtered pig delivers about 0.9 kg bristles, which in Denmark alone totals approximately 16,000 tons annually. The slaughterhouses collect both bristles and hooves (nails) during the dehairing process.

Basically, the raw material is a low-value product. However, the amino acid composition in the protein fraction has a high nutritional potential but is hampered by a low bioavailability. This is the core area of study in the research project *Keratin2Protein*, *http://www1.bio.ku.dk/projects/keratin2protein/*. In pig bristles, the content of the sulphur-containing amino acid cysteine is high. Nevertheless, this is also the main challenge as digestibility of pig bristles is low due to its highly stable structure.

Pig bristles consist primarily of keratin (90% or more), an insoluble protein packed with cross-linked fibres by disulfide bonds. Keratin is known for its high mechanical stability, insolubility and recalcitrance to degradation by common proteolytic enzymes such as trypsin, pepsin and papain [2].

The traditional technology for degradation of raw pig bristles is based on long-term heating, alkaline hydrolysis or treatment with high pressure and heat (6 bars, 150°C for at least 20 min.). Unfortunately, the process also destroys components with high biological value, primarily amino acids [3]. The form and the duration of the process determine whether you choose increasing digestibility or reduced amino acid content. The digestibility of bristle meal is approximately 30% [4].

The potential of novel technology for efficient conversion of low value bio-residues from pig bristles into high value nutritionally beneficial bio-products will be investigated. Action of microbes will be the innovative biotechnological tool to devise new technological methods for efficient bioconversion of keratin. Optimal enzyme production by fungal and bacterial solutions from nature will aid to improve resource efficiency when producing eco friendly, high protein content products with improved bioavailability and increased nutritional and economic value. A costly waste problem will be turned into a profitable product, thereby making food production more

competitive, sustainable and environmentally friendly.

II. MATERIALS AND METHODS

Keratin waste material collection and preparation

Raw bristles and hooves were collected from a Danish slaughterhouse. Bristles and hooves were removed from the pig at the slaughterhouse immediately after sticking. Before milling, the pig bristles were soaked in warm water with hand soap for 1 hour. Then they were rinsed three times with lukewarm water. The precipitate was separated, and finally the bristles were dried at 60°C overnight. Preparing the bristles meal was carried out by grinding the bristles with the Planetary Ball Mill PM 100 [5] in a 125 ml grinding jar with 7x20mm milling balls, made of stainless steel (revolution speed 500 rpm for 20 minutes).

Growth conditions: Microcosms

The microcosm incubation was performed in glass bottles containing a minimum liquid media supplemented with pigs' bristles and hooves as the sole nutrient source. The microcosm's medium consisted of: 10 g/l of milled bristles and hooves, 2 mM potassium phosphate buffer at pH 7.5, 1 mM $MgSO_4$ and 10 ml/l of a trace element solution containing 27 mM $CaCl_2$, 4 mM Fe(III)Citrate, 1.3 mM $MnSO_4$, 0.7 mM $ZnCl_2$, 0.16 mM $CuSO_4$, 0.17 mM $CoCl_2$, 0.10 mM Na_2MoO_4 and 0.26 mM $Na_2B_4O_7$ [6]. The medium was homogenized and sterilized by autoclaving for 15 min at 115°C.

Protease assay: Azocasein

Azocasein hydrolysis procedure was performed as described by [3]. Absorbance was measured with an ELISA spectrophotometer at 415 nm. The negative controls were treated with Tris-HCl buffer instead of sample. The result consisted on average of three replicas. The pH measurements were performed after each assay.

Agars

Agars were prepared using the minimum liquid medium mentioned before with 1% substrate and 1% agar. The solution was autoclaved for 15 min at 115°C. The agar was allowed to set in square petri dishes (120 mmx120 mm). Subsequently, isolates were inoculated using a Boekel 140500 Microplate Replicator with 96 Pins, 1.5 mm L x 1.5 mm W Pin Size, 2.54 cm Pin Length.

Experimental design

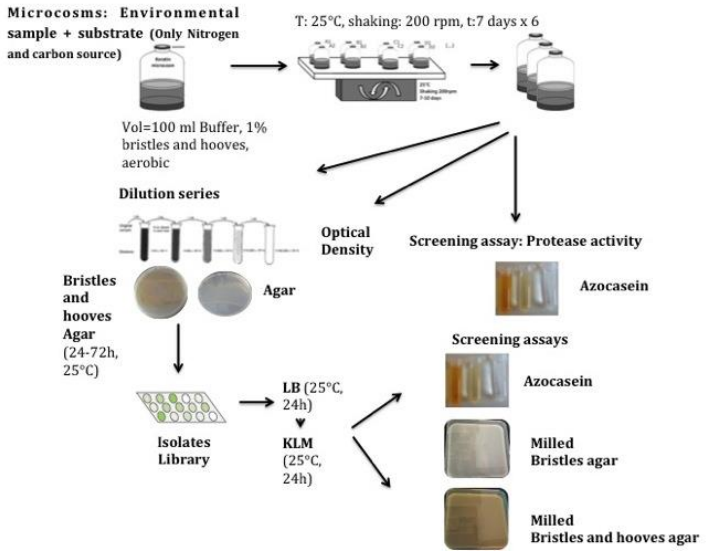


Figure 1. Experimental design of microcosms, isolation of single colonies and screening assays to find keratin degrading microorganisms [1].

III. RESULTS AND DISCUSSION

The use of pig's bristles and hooves as the only source of carbon and nitrogen promotes the growth of keratin degrading microorganisms. After six weeks, an enrichment process involving pig waste plus an environmental sample showed increased biomass and a reduction in microbial diversity (data not shown), representing a microbial community able to degrade keratin. This finding infers that an initial environment can hold thousands of microorganisms with different abilities, but a few of them will be useful for keratin degradation. A proper enrichment step is necessary in order to break down the original flora of bacteria and only the ones able to use keratin will survive.

From the microcosms procedure, it was possible to isolate single microbial strains using bristles and hooves agar, as shown in Figure 2. As a negative control, agar was used to make sure colonies were growing in the keratin. So far these strains are unknown.

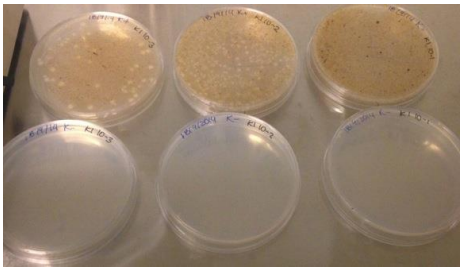
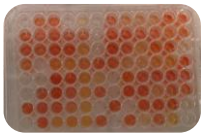
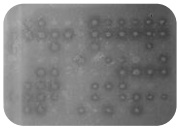


Figure 2. Colonies from the microcosms enrichment process. Up: bristles and hooves agar, down: agar. Dilution factors: 10^{-1}, 10^{-2} and 10^{-3}.

The selected isolates were tested for protease and keratinase activity, and a library was made for overview. Azocasein assay was prepared first to identify broad range proteolytic activity, as shown in Figure 3a, in which the presence of colour indicates activity. Subsequently, agar supplemented with different types of substrate were made. For example, Figure 3b shows how isolates with keratinase ability produce a halo. These results match the Azocasein assay (Figure 3a), indicating that the protease activity definitely is a keratinase activity. Figure 3c does not show halos, which might imply that the isolates are able to degrade certain types of keratin, but not all of them. Certainly, bristles could be an easier substrate to break up compared to hooves, due to less disulfide bonding. This outcome demonstrates that keratin structure is different from substrate, and probably different types of enzymes are needed to disrupt the whole assembly.





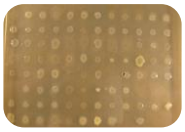


Figure 3a. Azocasein assay on isolates. Figure 3b. Isolates inoculated on milled bristles agar. Figure 3c. Isolates inoculated on milled bristles and hooves agar.

IV. CONCLUSION

The enrichment process using pig's bristles and hooves has successfully increased the biomass growth and reduced the initial microbial diversity of an environmental sample over time. Additionally, it was possible to isolate single strains from this procedure. Moreover, protease and keratinase screening assays have proved the presence of microbial strains able to degrade keratin. Therefore, this is a suitable method to screen for keratin degrading microorganisms. It is planned in the future to compare the difference on the substrates (bristles, hooves as well as bristles and hooves).

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