

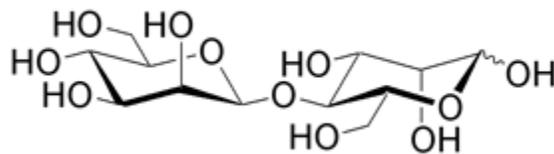


## Technology Summary: $\beta$ -Mannanase

### Opportunity Statement

Animal fodder is mainly comprised of agricultural and forestry plant residues including processed grass and grain products and by-products, fats, proteins, salts, calcium-containing compounds, zinc-containing compounds, acids and enzymes. The plant residue added in the animal fodder contains mannan, a hemicelluloses, which is considered to be undesirable in the animal feed due to its hydrophilic nature. Mannan easily absorbs water in the mono-gastric animal alimentary canal, enhances the viscosity of the alimentary canal contents, resists the gastrointestinal motility, and affects animal digestion and absorption of nutrients.

In order to degrade the mannan, an enzyme must be added to the animal feed.  $\beta$ -Mannanases are widely used in animal fodders for this purpose.  $\beta$ -Mannanases isolated from *Aspergillus niger*, also referred to as acidic  $\beta$ -Mannanases, are a kind of endohydrolases for hydrolyzing  $\beta$ -1,4,-D-mannan glycosidic bonds. Acidic  $\beta$ -Mannanases are particularly useful due to their stability at low pH as compared to other reported alkaline or neutral enzymes.



*Chemical Structure of mannan*

### **Problem**

The rate of production of  $\beta$ -Mannanases directly from *Aspergillus niger* is very low and the optimal temperature range does not match the internal temperature of most animals.

***Therefore, there is a need for a solution which addresses the limitations of current technologies and provides a cost-effective, efficient, safe and eco-friendly answer to this problem.***

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### 360ip's Partner Solution

The technology developed by 360ip's partner relates to a recombinant enzyme with improved properties and yield over conventional products in the market.

The process includes the following steps:

1. Obtaining  $\beta$ -Mannanase of *Aspergillus niger* from NCBI database; analyzing its sequence and optimizing its gene sequence according to preference of yeast *Pichia pastoris* codon.
2. Amplification of the optimized gene.
3. Connecting the gene to pGEM-T vector and inserting it into DH5 $\alpha$ -competent cells. The positive clones (white colonies) are obtained by blue-white spot screening.
4. Extraction of pGEM-man by alkaline lysis from the positive clones.
5. Cutting of pGEM-man, pGAPZ $\alpha$ , and pPICZ $\alpha$  with Xho I and Xba I.
6. Insertion of acidic  $\beta$ -Mannanase gene between restriction enzyme sites of Xho I and Xba I in plasmids pGAPZ $\alpha$  and pPICZ $\alpha$ . The inserted gene is located downstream of GAP promoter.
7. Electrotransformation of competent cells of yeast *Pichia pastoris* X-33 with the recombinant plasmid containing  $\beta$ -Mannanase gene (pGAPZ $\alpha$ -man and pPICZ $\alpha$ -man).
8. Identification and screening of stable recombinant strain.
9. Expression of the interest protein and its analysis.

The key advantages associated with this technology are as follows:

- The  $\beta$ -Mannanase of this invention has higher thermal stability as compared with  $\beta$ -Mannanases obtained from *Aspergillus sulphureus* and *Bacillus subtilis*. In addition, its optimal temperature range is closer to the rumen of most animals. (see Annex A)
- The  $\beta$ -Mannanase functions at an optimum pH of 5.0, and has good stability at pH: 3.0-7.0. It easily exerts its function in the stomach (pH of about 3.0) and intestinal tract (pH of about 6.0) of pigs & poultry.
- The technology achieves a yield of more than 75%.

### **Patents**

360ip's partner has filed one patent application on this invention.

***360ip is seeking interested parties for the licensing, further development and commercialization of this technology-based solution.***

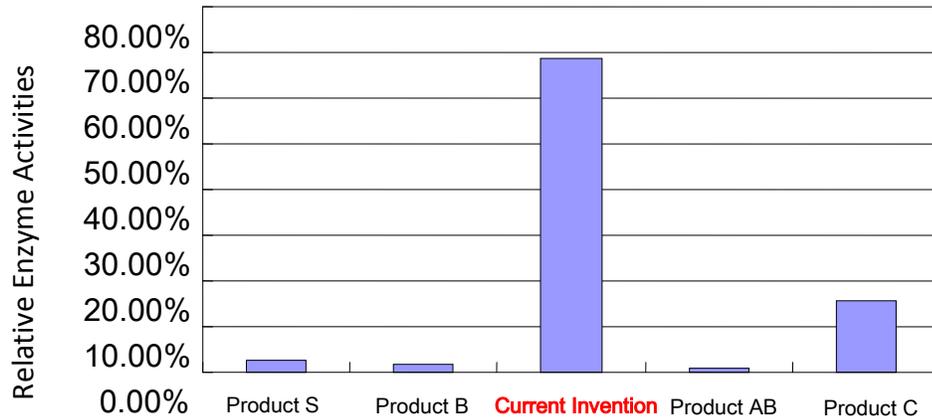
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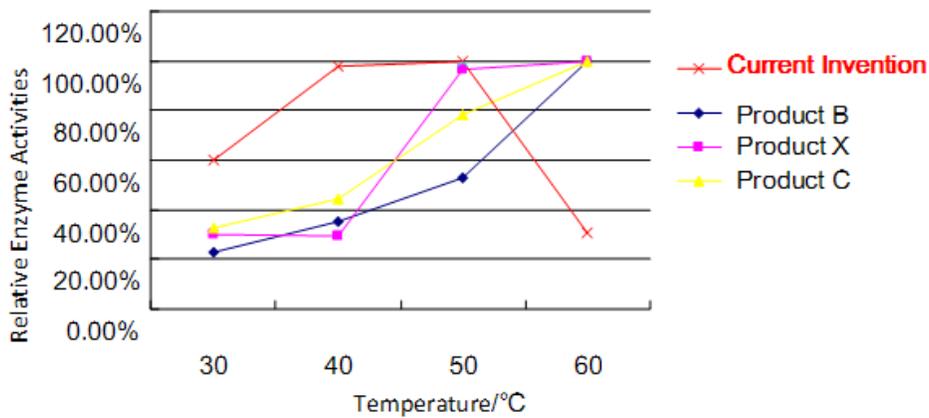
## Annex A (Performance comparison with other industrial enzymes in the market)

### 1. Thermal Resistance Measurements



Enzyme activity measured when samples cooled to room temperature after processing at 95 °C for 5 min. Results shows that the partner's enzyme is most resistant to high-temperature processing.

### 2. Enzyme Activities measurement at different temperatures



Compared to other enzymes, the partner's enzyme has the advantage that its optimal temperature range (30-40 °C) is closer to the internal temperature of most animals.