

Industrial Production of *Trichoderma reesei* Enzymes by Liquid State Fermentation and their Early Application in Animal Feeds in P.R. China

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Introduction

The Ascomycota fungi fulfil a central role in most land-based ecosystems. They are important decomposers, breaking down organic materials, such as dead leaves and animals, and helping the detritivores (animals that feed on decomposing material) to obtain their nutrients.

Ascomycetes, along with other fungi, can break down large molecules such as cellulose or lignin, and thus have important roles in nutrient cycling such as the carbon cycle.

The genus *Trichoderma* comprises a group of filamentous ascomycetes that are widely used in industrial applications because of their ability to produce extracellular lignocellulose-degrading hydrolases in large amounts. Enzymes secreted by *Trichoderma* have received widespread industrial interest, leading to commercial applications in the textile industry, the food and feed industries, and the pulp and paper industry. *Trichoderma reesei* has the capacity to secrete enzymes in high yields and this property can be exploited when using *Trichoderma reesei* as an industrial host for homologous and heterologous enzyme production [1].

Trichoderma reesei's potential as a rapid and efficient biomass degrader was first recognized in the 1950s when it was isolated from United States Army textiles during World War

II. The microbe secreted cellulases that were degrading cotton-based tents and clothing of service members stationed on the Solomon Islands. In the 1970s, at the time of the first global oil crisis, research interest in *Trichoderma reesei* gained popularity as it was explored as part of the solution to the world's growing dependence on fossil

fuels. This early lineage was used as a starting point for both academic research with the goal of understanding secretion and regulation of expression of the complex mixture of enzymes required for cellulosic biomass decay as well as for its development as a host for industrial enzyme production. In 2001, at the onset of the second major oil crisis, research programs focused in microbial cellulases to produce ethanol from biomass led to another surge in the study of *Trichoderma reesei*. In addition to *Trichoderma reesei*'s role in bio-ethanol production, it is used to produce industrial enzymes with a broad range of applications supporting the bio-based economy. To date there are around 243 commercially available enzyme products manufactured by fermentation of microorganisms; 30 of these are made using *Trichoderma reesei* as a host, 21 of which are recombinant products sold for use in food, feed, and technical applications including textiles and pulp and paper [2].

Enzymes were globally first used commercially in animal feeds in Finland in the 1980s when a β -glucanase was added to a barley-based feed for poultry, and it has been the poultry industry that has seen the biggest use of feed enzymes. The swine industry is following this trend, and to a lesser extent the ruminant sector. The size of the animal feed production industry means that there is huge potential for enzymes, and the current penetration rate of approximately 10 % of all monogastric feeds shows that there is room for growth. However, in the European Union the level of use is already very high; with the majority of poultry feed already containing enzyme supplements [3]. The largest component of animal feeds is in the form of cereal, and depending the local availability this can be either a viscous cereal (wheat, barley, rye, or triticale) or non-viscous cereal (maize or sorghum). Although animals produce their own endogenous enzymes, these are not sufficient to digest all the

feed. Swine are unable to digest approximately one-fifth of their daily ration.

In viscous diets that contain large proportion of wheat, barley, rye, or triticale the main antinutritive factor is the high fiber content of the diet. The fiber present consists mainly of nonstarch polysaccharides – arabinoxylan (pentosan) in wheat and rye, and β -glucan in barley and oats. The nonstarch polysaccharides are antinutritive because in the soluble form they can increase the viscosity of the digesta in the animals' small intestine and thus reduce the degree and rate of nutrient digestion and decrease the animals' performance. Enzymes targeted against these antinutritional factors formed the initial basis of commercial products, with xylanases aimed at the arabinoxylan backbone of wheat-based diets and β -glucanases at the β -glucan backbone of barley [4]. In a study on the effect of β -glucanase and

xylanase addition on the viscosity of digesta on chicken in rye-feeding, it was found that enzyme addition decreased the viscosity of the digesta in anterior part of the intestines by 55 % and in the posterior part by 52 % [5, 6].

Enzymes degrade also the cell walls of cereals thus liberating nutrients for the animals. In a study on *Trichoderma reesei* xylanase and cellulase on wheat microstructure the scientists found that enhanced cell wall polysaccharide extraction due to xylanase was accompanied by increased extraction of protein. Cellulase appeared to enhance further the efficacy in the extraction of protein and the release of protein from the cereal aleurone layer. The scientists concluded that the xylanase and cellulase are capable of cell wall degradation and liberation of entrapped nutrients, especially protein [7].

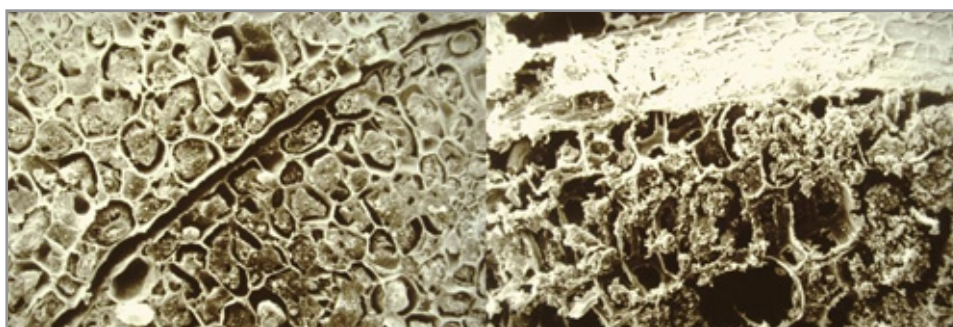


Figure 1: Scanning electron micrograph of cereal grain cell walls before and after xylanase enzyme treatment

The outer cell-layers of the wheat grain contain substantial amounts of protein, in many cases the limiting nutrient in diets in China, thus enzyme effect on the cell walls can have proportionally higher effect on animal nutrition in feed enzyme addition. Rice husks contain on the other hand substantial amounts of oils, high in energy, giving higher digestible energy levels in the diets with feed enzyme addition. Feed enzymes allow also usage of local potential feed ingredients in the animal's diets. Table 1.

shows the effect of xylanase and β -glucanase on the high wheat pollard diet in pigs. The feeding trial was carried out in Yueyian, Hunan province by Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing. Wheat pollard, is a mix of bran, cereal endosperm, and some flour, resulting in a higher energy and protein content with lower final fiber content with enzyme addition [5].

Table 1: Effect of xylanase and β -glucanase feed enzymes on growing pig's daily weight gain and feed conversion rate on high wheat pollard diet, comparison to control feed and commercial feed [5].

Feed Type	Daily Weight Gain (g)	Change	Feed Conversion Rate	Change
Commercial Feed	622	-11,9 %	3,01	-5,6 %
40 % Wheat Pollard	706	Control	2,85	Control
40 % Wheat Pollard + Enzyme	763	+ 8,1 %	2,72	+ 4,6

Finland was the initiator of commercial animal feed use of enzymes in the 80s. VTT Technical Research Centre of Finland has had extensive research on *Trichoderma*-technology. Based on the research there was established three private company fermentation plants for *Trichoderma* enzyme production. Extracellular enzyme production in industrial scale by *Trichoderma reesei* fungi is by the use of an aerobic submerged culture in a stirred-tank reactor. The fermentation process involves ea. culture maintenance, preparation of seed material, inoculum fermentation, main fermentation, analysis and recovery of the enzyme protein. Yield improvement involves both the strain and process development and optimization research. Application re-

search involves matrix analyses, polymer estimations, and animal feeding trials. Private companies and their technology centres conducted extensive research in the application of enzyme technology in the feed industry in Finland. The author worked in the 1980s and 1990s as a manager of the Animal Feed Biotechnology Department of a Technology Centre in Finland.

At that time in China there were some forty factories producing enzymes commercially. The application areas were food, pharmaceutical, textile, leather, feed, and detergent industries. The organisms used were among others; *Aspergillus niger*, *Monascus serrubescens*, *Bacillus megaterium*, and *Bacillus subtilis* [8].

The *Trichoderma reesei* –technology was non-existent, and the feed enzyme technology was mainly based on solid state fermentation of fungi on target matrix platform eq. rice bran. There were also innovative solutions at that time, like preparations extracted from cattle rumen fluids to be used as cellulose hydrolysing feed additives for poultry, these preparations increased chicken growth by 10 % and feed conversion rate by 2 % [9].

In May 1991 China State Science and Technology Commission invited the author to visit Chinese universities and research centers and evaluate the technological level of research, manufacture and application of feed enzymes in China as well as lecturing the present status of feed enzymes application in Finland. The visit focal points were, China State Science and Technology Commission HQ in Beijing, Nanjing Agricultural University and Yanxheng Science and Technology Exchange Center in Jiangsu Province, Zhejiang Agricultural University, Analytical Testing Center of Zhejiang Province in Hangzhou, and Sichuan Academy of Food and Fermentation Industries in Chengdu, as well as the local Provincial Science and Technology Commissions in the three provinces. Official Hang Sanba from China State Science and Technology Commission contributed to the organizing of the mission and later international cooperation between Finland and P.R.China.

The State Science and Technology Commission was transformed in 1998 into the Ministry of Science and Technology (MOST) of the People's Republic of China, which now is the central government ministry which coordinates science and technology activities in the country. In 2018 MOST absorbed the functions of the State Administration of Foreign Experts Affairs.

In early 1990s there was prominent increase of scientific cooperation between China and Finland in enzyme application studies in animal feeds. Liquid state fermented *Trichoderma reesei*-enzymes were tested in several animal trials in China, in chicken [10, 11], in swine [5] and in ruminants [12]. Joint Sino-Finnish projects were conducted; Feasibility Study – Application of Biotechnology in Feed Industry in China, 1992 in 41 pages, China Animal Feed Sector Study 1997 in 77 pages (in English and in Chinese). These projects were funded by the Ministry of Trade and Industry of Finland. The results were reported in conferences i.e. in Beijing 1996, Zhaoqing 1997, and in Hangzhou 1998.

Among the pioneers in enzyme technology application in animal feed industry were professor Wang Jing in Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing

and professor Xu Zirong, director of Feed Science Institute of the Zhejiang Agricultural University, Hangzhou.

The Chinese feed industry took opportunity of the use of *Trichoderma reesei* enzymes produced in Finland.

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