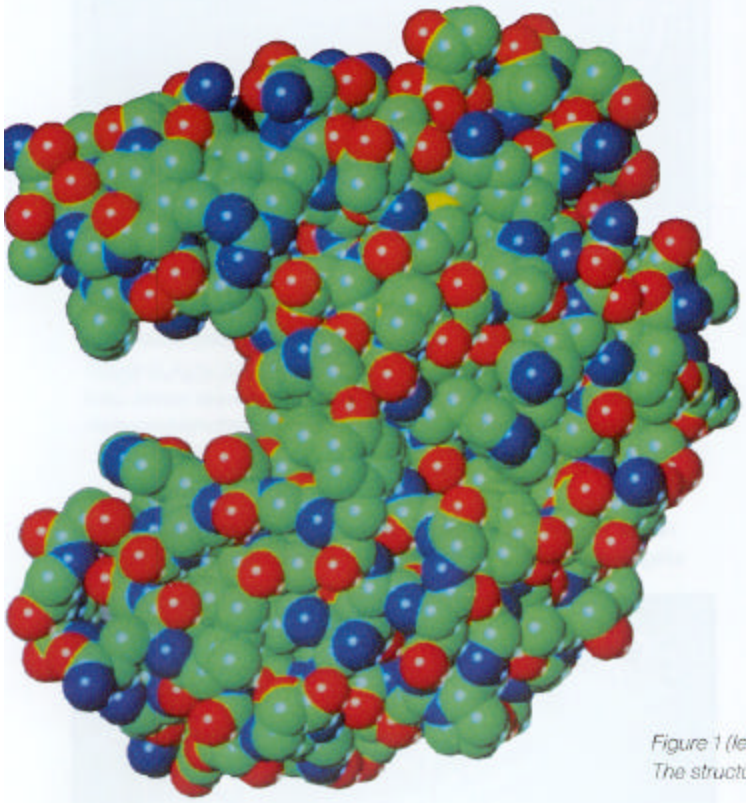
From Microbes to Pork



Enzyme technology has progressed dramatically and is used in many industrial processes. Now, its potential in animal feeds is being realised. Finnfeeds International enzymologist, Dr Andrew Morgan, explains their development and why feed enzymes are making dramatic strides in pig diets.

Figure 1 (left): The 3-dimensional structure of an endo-1,4-ß-xylanase from T.longibrachiatum. The structure was solved by the Cultor Technology Centre and the University of Joensuu.

What are Enzymes?

Enzymes are biological catalysts produced by all living organisms. They facilitate the biochemical reactions that enable microbes, plants and animals to function. Playing a crucial role in digestion, enzymes help to break down food components into simple molecules for absorption and assimilation. Unlike some inorganic catalysts, they have a very high degree of substrate specificity, acting on only one substrate or on a few closely related substances.

Enzymes are classified according to the type of reaction they perform (IUBMB, 1992). Many industrial enzymes are classified as hydrolases. These catalyse different hydrolytic reactions in which water reacts with the target substrate. Such enzymes include those that hydrolyse glycosidic bonds (eg xylanases), peptide bonds (proteases) and ester linkages (eg lipases).

Enzyme specificity is well illustrated by the action of the pancreatic proteases, trypsin and chymotrypsin. Whereas trypsin will split peptide bonds on the carboxyl side of lysine and arginine residues only, the action of chymotrypsin is limited to the carboxyl side of tyrosine, tryptophan and phenlyalanine and to large hydrophobic residues such as methionine. By comparison, bacterial subtilisins - proteases produced by selected strains of *Bacillus subtilis* and related species - are far less discriminating about the nature of the side chains adjacent to the peptide bond to be cleaved (Stryer, 1988).

Most of the enzymes that have been characterised are proteins, which consist of chains of amino acids. Enzyme proteins generally contain more than 100 amino acid residues giving them a molecular mass of more than 10 kilodaltons and a diameter of more than 25 Å. The amino acid chain folds into a three-dimensional structure in such a way that amino acid residues that directly participate in substrate binding and the reaction catalysed come together to form the *active site*.

These key amino acid residues are often widely separated in the linear amino acid sequence. Figure 1 shows a three-dimensional model of a fungal xylanase. A feature of most enzymes is the presence of an active site cleft or crevice, which this xylanase clearly shows. The amino acid residues for binding the xylan substrate and the catalytic residues that participate in the hydrolysis of the β-(1,4) xylan linkages have been tentatively identified (Törrönen *et al*, 1994).

In addition to high substrate specificity, enzymes are extremely powerful catalysts, accelerating reactions by at least a million-fold whilst not being consumed or irreversibly altered during the reaction. Once the reaction is completed, and the products of the reaction released, an enzyme molecule goes on to catalyse further reaction events until its activity is inhibited, down-regulated or the protein structure is denatured or degraded. As with other proteins, enzymes are eventually decomposed as part of the nitrogen cycle.

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What Are They Used For?

Man has made use of enzymes, often unknowingly, throughout history. For example, Homer describes the process of cheese making in which milk is stirred with a twig from a fig tree. The fig twig produces a protease, ficin, which makes the milk coagulate. Another traditional method has been the coagulation of milk with an extract, rennet containing chymosin, from the calf stomach. Modern microbial enzyme technology has replaced the need for this source of chymosin.

Today, enzymes are used in a diverse range of applications including detergents, starch processing, textiles, leather manufacture, pulp and paper, waste management, biomass conversion, food processing and in animal feed (Cabral *et al*, 1993). Each process demands enzymes with different characteristics. For example, xylanases for pulp and paper applications need to function at high temperatures (70+ °C) and at alkaline pH levels, whereas enzymes for use in animal feeds need to operate at moderate temperatures (around 40 °C) and over the range of acidic to neutral pH conditions found in the upper gastrointestinal tract of the pig and chicken.

One of the advantages of using enzymes is that they are natural biodegradable proteins offering an environmentally-friendly alternative to chemical processes. In the case of feed enzyme applications, once their task is completed, these enzymes are digested along with other residual proteins in the lower digestive tract and do not leave any residues in animal produce.

How Are Enzymes Produced?

Enzymes for use in food and feed or other industrial applications are mainly produced by fermentation using harmless bacteria and fungi, which represent a diverse resource for the isolation and production of industrial enzymes. Several microbes, originally isolated from the environment, have been selected for their ability:

- to produce specific enzymes
- to secrete enzymes efficiently into the medium in which they are grown
- to grow well in defined media and in large scale fermentation
- and for their amenability to classical and molecular strain improvement procedures.

Table 1 lists some examples.

Table 1: Examples of microbial strains used in the production of certain industrial enzymes.

Producing Micro-organism	Enzyme Product
Fungi	
Trichoderma longibrachiatum	Cellulase, β-Glucanase, Xylanase
Aspergillus niger	α-Amylase, α-Galactosidase, Pectinase, Phytase, Protease
Humicola insolens	Cellulase, β- Glucanase, Xylanase
Rhizomucor miehei	Lipase, Protease
Yeasts	
Kluyveromyces marxianus	Invertase, Lactase
Saccharomyces cerevisiae	Invertase
Bacteria	
Bacillus subtilis (& related strains)	α-Amylase, β-Amylase. Protease
Streptomyces murinus	Xylose Isomerase/Glucose Isomerase

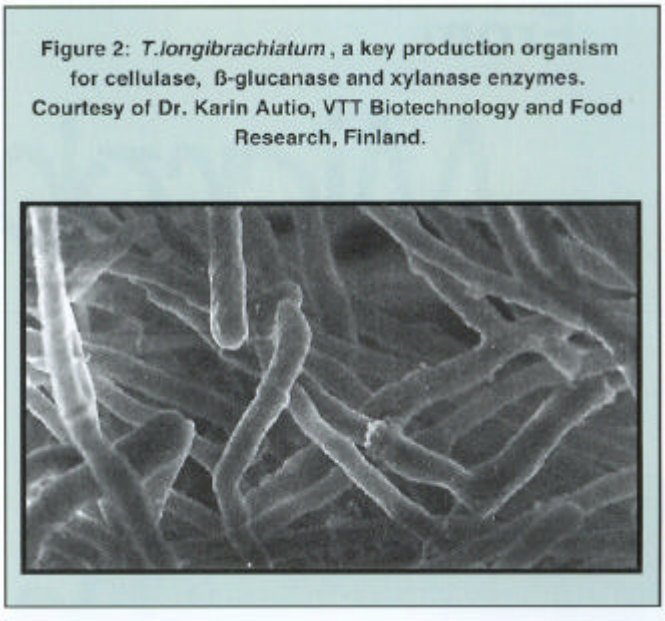
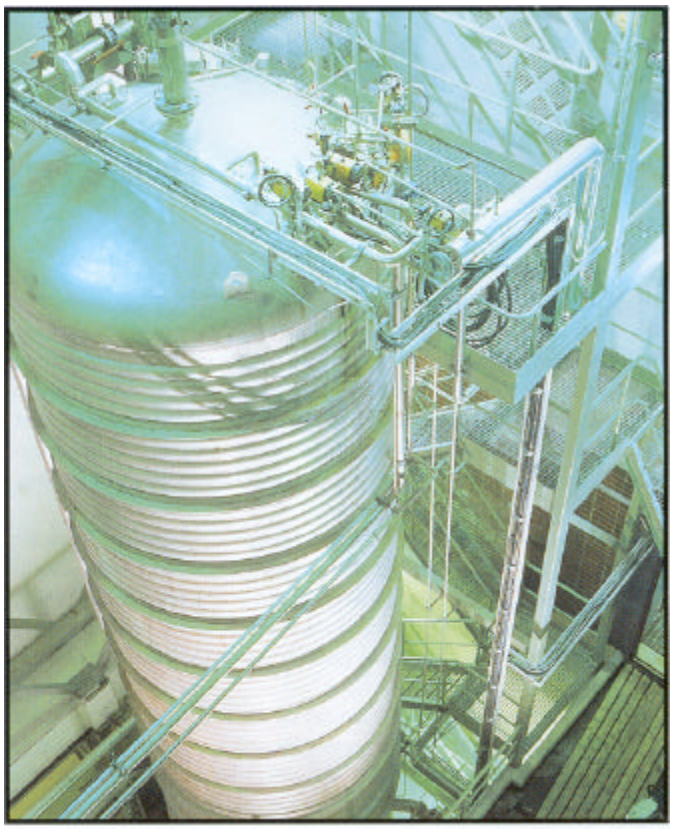


Figure 2: *T.longibrachiatum*, a key production organism for cellulase, ß-glucanase and xylanase enzymes. Courtesy of Dr. Karin Autio, VTT Biotechnology and Food Research, Finland.

One of the most important microbes to be used to produce enzymes on an industrial scale is *Trichoderma longibrachiatum* (Figure 2). This is a filamentous fungus that secretes large amounts of enzymes, mainly cellulases (including ß-glucanases) and hemicellulases (eg xylanase) and is well suited for fermentation on an industrial scale (Figure 3).

Figure 3: An industrial fermenter for the production of enzymes. Courtesy of Genencor International Europe Ltd.



Combining advanced genetics and state-of-the-art fermentation technology, it is now possible to obtain very high yields of enzyme proteins from selected strains of *Trichoderma longibrachiatum*. In

recent years, there has been a rapid development of enzymes for animal feed with the recognition that this is an increasingly important market. As a result, enzymes are being developed specifically with optimum characteristics for the feed sector.

Enzyme Applications in Feed

Currently, the most widely used enzyme in the feed industry is a xylanase from *Trichoderma longibrachiatum* (this enzyme is produced by Genencor International Ltd exclusively for feed enzyme products of Finnfeeds International Ltd). Amongst all of those which are industrially produced, this specific xylanase has some unique properties that makes it ideal for use as a feed enzyme (see Table 2). Particularly, the dual action of highly effective viscosity-reduction (Figure 4) and cell wall degradation (Figure 5) makes it ideally suited for use in products for pig diets containing wheat, triticale, rye or by-products of these cereals.

Table 2 Characteristics of T. *longibrachiatum* xylanase and its effects in vivo.

Enzyme Characteristics	In vivo Significance
Broad pH activity profile (pH3.5 to 6.5)	Functions efficiently in both gastric region of digestive tract and in upper small intestine.
High relative activity at 40 degrees Celsius	Functions efficiently in digestive tract
High viscosity-reducing activity. Randomly depolymerises soluble high molecular weight arabinoxylans	Increases nutrient availability by reducing viscosity in vivo and thereby enhances the efficiency of digestion in diets based on wheat, triticale and rye.
Solubilises insoluble arabinoxylans from cell walls of cereal grains.	Increases permeability of cell walls enhancing the ability of endogenous enzymes to penetrate cells and degrade entrapped nutrients.

As part of the development work for the *Porzyme 8300* product, the *T. longibrachiatum xylanase* was tested in piglets in the starter period. In this study, it was found that the xylanase significantly improved ($p < 0.05$) growth and feed conversion in piglets fed a wheat-based diet (Figure 6).

The development and introduction of *Porzyme 9300* for grower/ finisher pigs is perhaps of even greater significance for the pig industry and will be the subject of a second paper (Schulze and Partridge, in preparation).

Finally, the future for the development and production of new enzymes is progressing rapidly. We can expect to see even more effective enzymes extended to an even wider range of cereal and vegetable protein substrates delivering even greater benefit to the customer.

*Finnfeeds International Ltd has recently produced a booklet, *Feed Enzymes - a working guide* - which provides a simple guide to the development of enzymes, how they work and their role in animal feeds. Free copies are available from Finnfeeds International Ltd, PO Box 777, Marlborough, Wilts, SN8 1XN.

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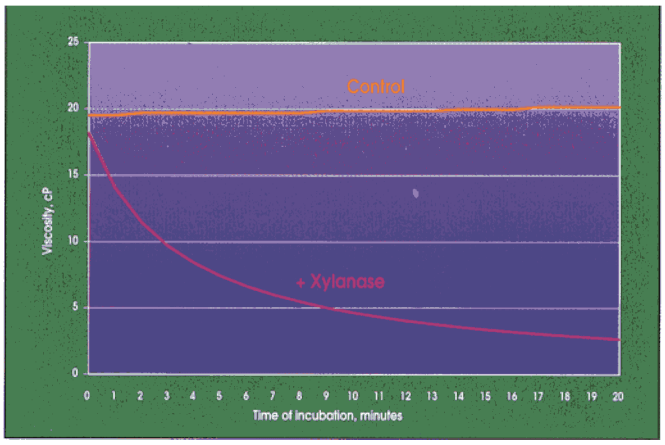

Figure 4: The viscosity-reducing effects of *T. longibrachiatum* xylanase on soluble wheat arabinoxylan. A 1.2 % solution of soluble wheat arabinoxylan (Megazyme Pty) was made in a buffer at pH 6.5, 40 °C and the effect of the xylanase monitored using a Brookfield spinning disk viscometer.

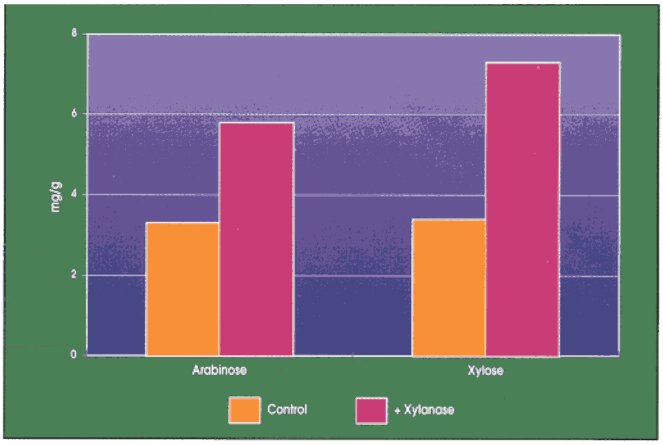

Figure 5: The effects of *T. longibrachiatum* xylanase on the solubilisation of wheat cell wall arabinoxylans following in vitro digestion simulation. Supernatants were analysed by Dionex HPAEC following acid hydrolysis. The effect of enzyme was significant ($p < 0.005$).

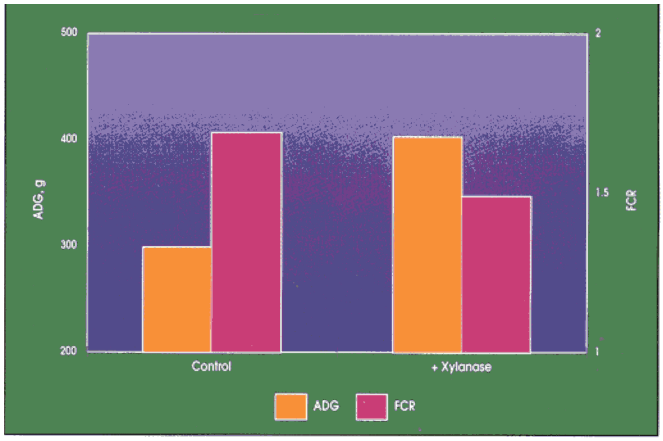

Figure 6: Piglets were weaned at around 24 days. Average daily gain (ADG) and feed intake were measured at 64 days of age. The diet contained 70% wheat / 22% soybean meal with and without 0.1% of a premix containing *T. longibrachiatum* xylanase. The effects of xylanase on ADG and FCR were significant ($p < 0.05$). Courtesy of Dr. Hagen Schulze (FFI) and Dr. Pinder Gill (MLC).

Keywords: Porzyme tp100, Porzyme 8300, Porzyme 8100, Porzyme 9100, Porzyme 9300, Pig, Piglet, Wheat, Barley, Rye, Triticale, Swine, Xylanase, Beta-glucanase, Amylase, Protease