Submission

A1096
Xylanase from Bacillus licheniformis as a Processing Aid (Enzyme)

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Peter Hvass
Senior Science Manager, Regulatory Affairs
Novozymes A/S, Krogshoejvej 36
2880 Bagsvaerd Denmark
[redacted]
Web: www.novozymes.com

Background

Novozymes submits this document as a response to the call for submissions on Application A1096 published by FSANZ 25 July 2014.

Novozymes respectfully request FSANZ to reconsider its view with respect to the enzyme protein subject of this application being considered as 'novel protein', as it e.g. is stated in the Executive summary on page 4 in the consultation document:

"The enzyme protein of this preparation differs by one amino acid to that found in nature. Because no evidence has been provided or located that the enzyme protein is identical to that found in nature, the enzyme protein would be considered novel in relation to the definition in Standard 1.5.2 – Food Produced using Gene Technology."

Novozymes request

Novozymes requests that the enzyme object of A1096, a xylanase (Xyl264) from *Bacillus licheniformis*, is not considered 'novel protein'. This is justifiable because the change in protein sequence is negligible compared to the variation of native xylanases even in a reduced subset of xylanases within the *Bacillus licheniformis* species and because there is evidence that the specific change introduced in Xyl264 occurs in nature.

Scientific support

In support of Novozymes' proposal above, the following conclusions were reached based on scientific assessments on the natural sequence variation in xylanase enzymes, including those already mentioned in the FSANZ positive list:

1. Known xylanases, incl. those in the FSANZ positive list, are not identical in sequence – on the contrary, a high level of variation is found in nature
2. Also, within the same xylanase family as Xyl264, the GH8 family, the overall level of sequence variation is significantly high, and the sequence of Xyl264 is well within the natural variation of xylanases in general and also within the GH8 family

3. In addition to the evidence included in A1096 that the specific change in Xyl264 occurs in nature, several GH8 xylanases were found to display the same sequence at the region that was modified in Xyl264
4. Even comparing to the narrow group of wild-type GH8 xylanases from found in strains within the *B. licheniformis* species, the sequence variation of Xyl264 is negligible compared to the variation found in nature

Details on above 1 and 2 are provided below, while evidence for 3 and 4 is provided in a separate, confidential document.

To assess the variation of known xylanases found in nature, incl. those in the FSANZ positive list, public and Novozymes proprietary databases were searched for amino acid sequence information for enzymes with confirmed xylanase activity. The relevant species included in the FSANZ positive list are those that are mentioned as production strains in case of non-GMM or as donor in case of GMM strains.

The origins of xylanase entries in the current FSANZ positive list are:

Table to clause 17

Enzyme	Source
Hemicellulase endo-1,4-β-xylanase EC 3.2.1.8	*Aspergillus niger* *Aspergillus oryzae* *Aspergillus oryzae*, containing the gene for Endo-1,4-β-xylanase isolated from *Aspergillus aculeatus* *Aspergillus oryzae*, containing the gene for Endo-1,4-β-xylanase isolated from *Thermomyces lanuginosus* *Bacillus amyloliquefaciens* *Bacillus subtilis* *Humicola insolens* *Trichoderma reesei*

Fig. 1. Xylanases in current FSANZ positive list

The resulting amino acid sequences were assessed with respect to homology and pictured as a 'Phylogenetic tree' (Fig.2) or a 'Dot plot' (Fig. 3) view as shown below.

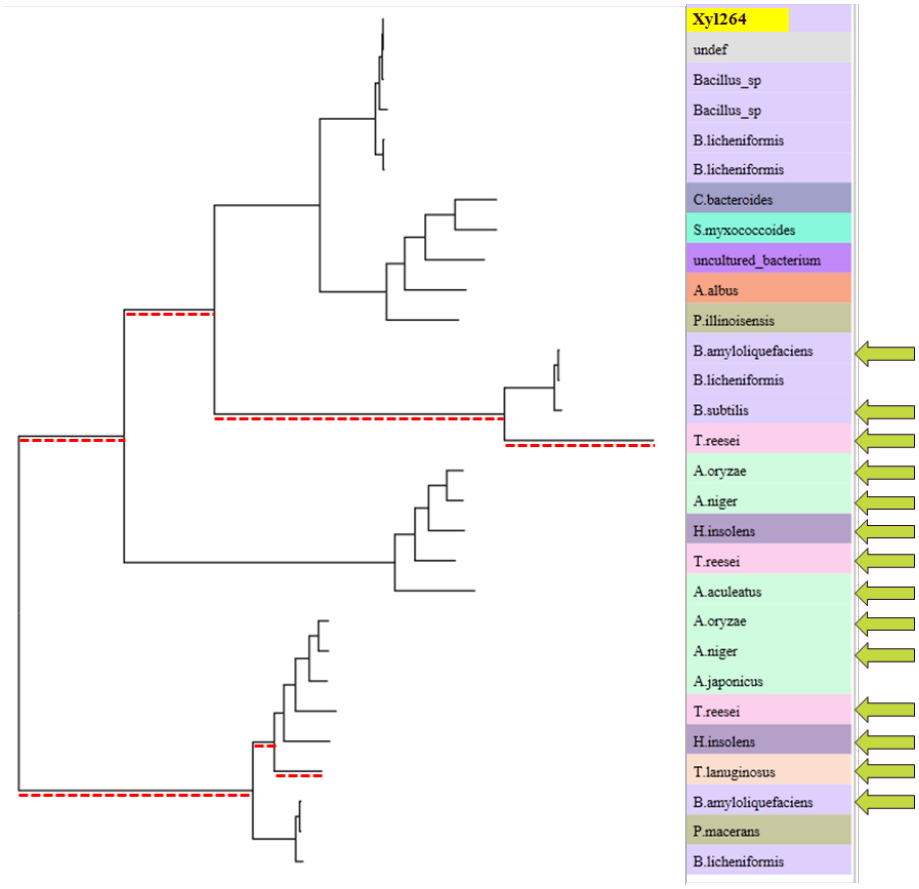

Fig. 2. Phylogenetic tree of xylanases found in nature, including those in the current FSANZ positive list (indicated with green arrow to the right). The distance between two sequences is indicated by adding up the length of all the horizontal lines between the sequences, *e.g.*, the total length of red, dotted line represents the distance between *T. reesei* and *T. lanuginosus* xylanases.

As shown in the phylogenetic tree in Fig. 2, there is high level of variation between known xylanases found in nature. It is also seen that the sequences are somewhat clustered into groups of more closely related sequences, the so-called families. Xyl264 belongs to one such family, designated as the GH8 xylanase family.

Another way of representing the sequence variation between the xylanases is a 'Dot plot' – a data analysis tool to visualizing similarities and differences in two-dimensional plots. This is shown in Fig. 3 below, where members of the GH8 xylanase family are indicated with a green circle. The radius of the dots may include more than one enzyme if there is only small sequence variations. In fact, Xyl264 and the native enzyme are represented in a single dot (Xyl264). Importantly, the dot is shown to be well within the sequence variation between xylanases in general and also within GH8 xylanases.

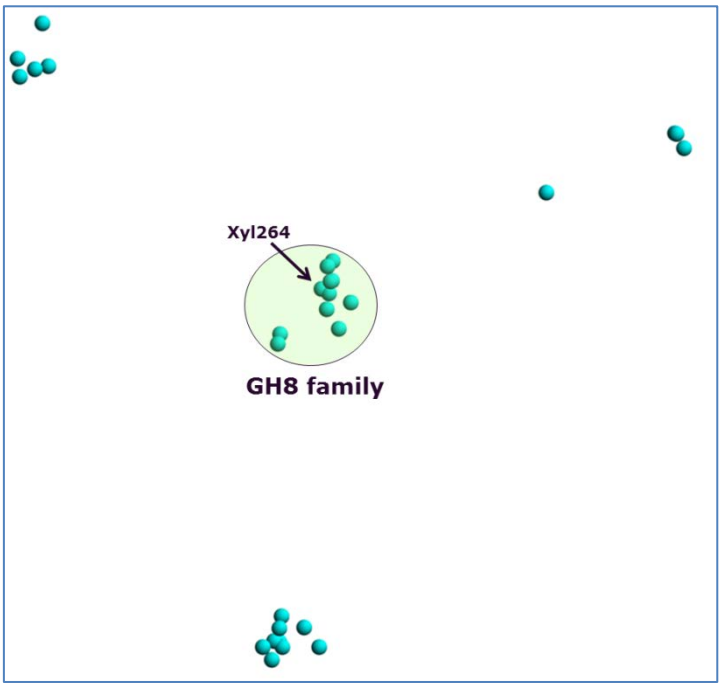

Fig. 3. Dot plot representation of sequence variation of xylanases found in nature, including those in the current FSANZ positive list.

Furthermore, in an assessment of the natural sequence variation in the GH8 family of xylanases, a high degree of variation among GH8 xylanases (down to 40 % sequence identity) was found. The level of variation found in this limited set of sequences defines the frame for variation for GH8 xylanases that can be found in nature. This is visualized in Fig. 4 below.

Fig. 4. Sequence alignment of GH8 xylanases.

The below mentioned information is requested to be treated as confidential commercial information (CCI), justified by the fact that sequence information for Xyl264 would provide an unambiguous possibility for competitors to link a certain xylanase to a specific commercial product and make it extremely easy to copy it or benchmark it against their products. The following CCI is therefore placed in a separate, confidential document:

- Sequence alignment of GH8 xylanases at the region modified in Xyl264, showing that the specific change introduced in Xyl264 is also found in wild-types
- The detailed amino acid alignment of Xyl264 to 3 wild-type GH8 xylanases from strains within the *B. licheniformis* species, showing a homology of Xyl264 to the wild-type from which it was

generated of 99.8%, whereas the variation between wild-type GH8 xylanases from strains within the *B. licheniformis* species is down to 91.5% homology.

Based on the documentation provided in this submission, Novozymes finds it evidenced that:

- The change in protein sequence of Xyl264 is insignificant compared to the variation of native xylanases from strains within the *Bacillus licheniformis* species
- Similar sequences at the region modified in Xyl264 are found in wild-type GH8 xylanases and
- The specific change introduced in Xyl264 occurs indeed in nature.

Novozymes therefore hopes that this submission is sufficient to justify FSANZ to reconsider and conclude that the enzyme object of A1096 is not considered 'novel protein'.

Please do not hesitate to contact us, if you have any questions or require additional information.

Kind regards,

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Peter Hvass
Regulatory Affairs

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