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Darmstadt 2018-03-07

Statement: Call for Submissions: A1153 – Endo xylanase from T. reesei as a Processing Aid

AB Enzymes is providing clarification and additional data regarding "Labelling Requirements for food produced using gene technology" Section 2.3.1.1 from the Call for Submissions, dated February 15, 2018.

We respectfully request that FSANZ reviews the information provided in this response for the call for submissions, with respect to the fact that the truncated xylanase can be found in nature and thus fulfills Subsection 1.5.2—4(5) for labelling of foods using gene technology, such that labelling on final foods is not required.

Please find attached a confidential version (confidential section marked in red) and a non-confidential version (confidential sections redacted). The information deemed confidential is company specific data and is proprietary in nature.

AB Enzymes GmbH

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Marja Paloheimo 27 February, 2018

Nonomuraea flexuosa [redacted] xylanase in nature and recombinant xylanase in enzyme products of AB Enzymes

1. Amino acid sequence of [redacted] xylanase in AB Enzymes´ enzyme products vs. the native [redacted]

The [redacted] xylanase in AB Enzymes´ enzyme products originates from [redacted] xylanase of *Nonomuraea flexuosa* [redacted]

The full-length mature [redacted] xylanase consists of [redacted] amino acids and contains an enzymatically active core, a carbohydrate binding domain (CBM) and a linker region combining these two separately folding units (Fig. 1). The expression cassette [redacted] transformed to *T. reesei* in construction of the [redacted] production strain RF5427 encodes an active [redacted] xylanase [redacted] No amino acid modifications are encoded by the expression construction and thus the amino acid sequence [redacted] is identical to that of the wild-type [redacted]

[redacted]

Fig. 1. The amino acid sequence of *Nonomuraea flexuosa* [redacted] xylanase. The mature amino acid sequence starts from [redacted] (the N-terminal peptide DTTITQ, determined from the purified [redacted] protein is underlined). The active site glutamic acids are marked with open boxes. The location of the linker and CBM are indicated by a dotted line and markings below the amino acid sequence. [redacted]

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[redacted]

2. Different forms of xylanases detected and purified from culture supernatants of *N. flexuosa* and recombinant *T. reesei*

The full-length native [redacted] xylanase has been purified from the culture medium of the *N. flexuosa* gene donor strain [redacted] Also, recombinant [redacted] xylanases have been purified from the *T. reesei* strains producing the full-length mature xylanase [redacted] In addition to the full-length recombinant [redacted] the *T. reesei* culture media were shown to contain shorter, active xylanase polypeptides. [redacted]

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Similar degradation products from the native [redacted] to those characterised from the *T. reesei* culture media are also present in the culture medium of the *N. flexuosa* used as a gene donor. This has been shown by Western blot analysis (Fig. 3[redacted]

A. **B.**

[redacted]

Fig. 3[redacted]

3. Carbohydrate degrading enzymes with multi domain structure – sensitivity of linker to proteases

It is well known that many enzymes, such as cellulases and xylanases having a multi domain structure and wherein the domains are linked by a linker region are often particularly susceptible to protease cleavage, even by the own native host´s proteases. The linker is believed to be sensitive to proteases due to its flexibility. Also, in [redacted] the linker has been shown to be sticking out from the molecular structure of the core [redacted] which makes it an easy target for proteases. The multi domain enzymes, when developed as industrial products, thus often require careful engineering of the joining sequences (core – linker, linker – CBM) to improve stability, in addition to a need to use a low protease host and optimization of cultivation conditions (Vehmaanperä *et al*., 2014; Paloheimo *et al.*, 2016).

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4. Conclusion

The recombinant [redacted] xylanase produced by *T. reesei* [redacted] is identical to that of the native [redacted] in both sequence and structure. [redacted] However, it is well known that the linker regions of different multi modular glycoside hydrolases are easily degraded by proteases, even with the native proteases. It has also been shown that [redacted] degradation products of different molecular masses [redacted] can be detected from the culture media of the *N. flexuosa* gene donor strain as well as from the *T. reesei* strain producing the recombinant [redacted]

References

[redacted]

[redacted]

[redacted]

Paloheimo, M., Mäkinen, S., Punt, P., Juntunen, K., Puranen, T. and Vehmaanperä, J. 2016. Methods for controlling protease production. Priority 20.02.2015 FI 20155112.

Vehmaanperä, J., Puranen, T., Valtakari, L., Kallio, J., Alapuranen, M. and Paloheimo, M. 2014. Improved cellulases. EP 1 874 927 B1. Priority 29.04.2005 FI 20055202 and US 110526.

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