

Internal distribution code:

- (A) [-] Publication in OJ
- (B) [-] To Chairmen and Members
- (C) [-] To Chairmen
- (D) [X] No distribution

**Datasheet for the decision
of 25 January 2023**

Case Number: T 1238/21 - 3.3.04

Application Number: 08846486.2

Publication Number: 2215110

IPC: C07K14/43

Language of the proceedings: EN

Title of invention:

Alpha-amylase variants with altered properties

Applicant:

Danisco US Inc.

Headword:

Alpha-amylase variants/DANISCO

Relevant legal provisions:

EPC Art. 123(2), 54, 56

Keyword:

Main request - allowable



Beschwerdekammern
Boards of Appeal
Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1238/21 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 25 January 2023

Appellant: Danisco US Inc.
(Applicant) 925 Page Mill Road
Palo Alto, California 94304 (US)

Representative: Mewburn Ellis LLP
Aurora Building
Counterslip
Bristol BS1 6BX (GB)

Decision under appeal: **Decision of the Examining Division of the
European Patent Office posted on 8 February 2021
refusing European patent application No.
08846486.2 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairwoman M. Pregetter
Members: B. Rutz
R. Romandini

Summary of Facts and Submissions

I. The appeal of the applicant ("appellant") lies from the decision of the examining division refusing European patent application No. 08 846 486.2 entitled "*Alpha-amylase variants with altered properties*".

II. In the decision under appeal, the examining division held that the subject-matter of claim 1 of the main request and auxiliary requests 1 to 3 extended beyond the application as filed (Article 123(2) EPC).

III. The following documents are cited in this decision:

D1 WO2004/091544

D2 Uniprot database entry Q12N92

D3 M.S. Kim et al., "*Changes in Optimum pH and Thermostability of α -amylase from Bacillus licheniformis by Site-directed Mutagenesis of His 235 and Asp 328*", Bulletin of the Korean Chemical Society, 15(10), 1994, 832-5

D4 WO2008/153925

IV. With the statement setting out the grounds of appeal, the appellant submitted sets of claims of:

- a main request identical to auxiliary request 3 dealt with in the decision under appeal and originally filed on 18 June 2018
- auxiliary requests 1 and 2

Claim 1 of the main request reads (annotation in square brackets added by the board):

"1. An alpha-amylase variant comprising an amino acid sequence at least 95% identical to that of a parent AmyS-like alpha-amylase, wherein the parent AmyS-like alpha-amylase comprises SEQ ID NO: 2, and having a substitution at an amino acid position corresponding to position 242 of a reference alpha-amylase, wherein the reference alpha-amylase used for numbering the amino acid residues is SEQ ID NO: 2, [feature a)] wherein the substitution is S242A, S242D, S242E, S242M or S242Q, [feature b)] and wherein the variant has improved thermostability as compared to the parent AmyS-like alpha-amylase, and wherein the variant has detectable alpha-amylase activity, wherein thermostability is determined for the parent and variant under the same experimental conditions." [feature c)]

Independent claim 6 relates to an isolated polynucleotide comprising a coding sequence that encodes an amino acid sequence of the alpha-amylase variant of any of claims 1-4; any of SEQ ID NOs: 3 or 4. SEQ ID NO: 3 or 4 represent the amino acid sequences of full-length alpha-amylase from *G. stearothermophilus*, comprising an S242A or S242Q mutation, respectively (see Figure 1 of the application).

Independent claim 7 relates to a vector comprising the isolated polynucleotide of claim 6.

Independent claim 8 relates to an isolated host cell comprising the isolated polynucleotide of claim 6 or the vector of claim 7.

Independent claim 9 relates to a composition comprising
a) an alpha-amylase variant of any of claims 1 to 5 and
b) at least one additional enzyme.

Independent claim 10 relates to a detergent composition comprising the composition of claim 9.

Independent claim 13 relates to a method of treating a starch slurry comprising at least one alpha-amylase variant of any of claims 1 to 5.

Independent claim 16 relates to a method of producing a fermentable substrate comprising at least one alpha-amylase variant of any of claims 1 to 5.

Independent claim 17 relates to a method of treating a starch-containing material or a starch comprising at least one alpha-amylase variant of any of claims 1 to 5.

Independent claim 20 relates to a kit comprising at least one alpha-amylase variant of any of claims 1 to 5.

V. The appellant's arguments submitted in writing may be summarised as follows.

Main request

Added subject-matter (Article 123(2) EPC)

Basis for "feature a)" was found e.g. on page 4, line 27 to page 5, line 2. The sequence of SEQ ID NO: 2

related to a "parent" *Geobacillus stearothermophilus* alpha-amylase (see page 37, lines 7 to 8 and page 38, lines 5 to 6 of the application as filed).

Basis for "feature b)" was the list of position 242 substitutions (S242A, S242D, S242E, S242F, S242G, S242H, S242L, S242M, S242N, S242Q and S242T) found throughout the application as filed (e.g. on page 4, lines 10 to 12; page 7, lines 20 to 22; page 75, lines 11 to 12 and page 76, lines 25 to 27). Although five of the disclosed substitutions were selected from this list of eleven, this was a selection of preferred options from a single list. Example 3 described the screening of thermal stability of the variants with position 242 substitutions. S242A and S242Q were shown to have the highest thermal stability, but S242D, S242E and S242M were also shown to retain more residual activity than the wild-type AmyS amylase when averaged across four experiments, as described from page 81, line 13 to page 82, line 1 (including Table 3-1).

With regard to "feature c)", the application as filed disclosed thermostability as a property that could be altered in the disclosed amylase variants (see page 2, lines 8 and 18; page 22, lines 7 to 8 and page 36, lines 14 to 15). The application clearly and unambiguously disclosed improved thermostability to be particularly desirable (see Figures 3, 5 and 6 and Examples 3 and 4).

Basis for improved thermostability relative to the parent amylase, determined under the same experimental conditions, was found on page 22, lines 7 to 13 of the application as filed.

In conclusion, the application as filed provided clear pointers towards features b) and c). Moreover, features b) and c) were substantively interrelated with each other, meaning that the skilled person would directly and unambiguously derive their combination from the application as filed.

Basis for the other claims could be found in the application as filed as follows:

claim 2: page 3, line 19 to page 4, line 9

claim 3: page 10, line 24; Figures 3, 5 and 6; and Examples 3 and 6

claim 4: page 2, lines 6 to 18

claim 5: page 4, lines 17 to 18

claim 6: page 4, lines 19 to 21

claim 7: page 4, lines 19 to 20

claim 8: page 4, lines 19 to 26

claim 9: page 5, lines 3 to 5

claim 10: page 62, line 8

claim 11: page 5, line 11; page 6, lines 3 to 4

claim 12: page 5, line 29 to page 6, line 4

claim 13: page 6, lines 3 to 13

claim 14: page 74, line 27 to page 75, line 3

claim 15: page 6, line 28 to page 7, line 4

claim 16: page 8, lines 3 to 19

claim 17: page 9, lines 18 to 23

claim 18: page 8, lines 24 to page 9, line 2; claim 45

claim 19: page 9, lines 3 to 5

claim 20: page 76, line 28 to page 77, line 8; claim 49

VI. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request filed with the statement of grounds of appeal or, alternatively, based on auxiliary request 1 or 2. Oral proceedings were requested "*if the Board*

of Appeal intends to reject the appeal as inadmissible".

Reasons for the Decision

Main request

Added subject-matter (Article 123(2) EPC) - claim 1

1. In addition to the passage referred to by the appellant as basis for "feature a)" (i.e. page 4, line 27 to page 5, line 2 of the application as filed), the board considers claim 1 as filed to be of relevance. The requirement of "*further comprising one or more of*" in claim 1 as filed includes under point "*n) modification of one or more amino acid residues corresponding to ... S242 ... where SEQ ID NO: 1 or 2 are the reference alpha-amylase*". The board therefore interprets this claim to also disclose alpha-amylases which contain only a substitution in position S242 and no further modifications.

2. In addition, dependent claim 2 as filed discloses "*S242A, S242D, S242E, ... S242M, ..., S242Q*"; dependent claim 3 as filed discloses "*detectable alpha-amylase activity and is altered, relative to the parent AmyS-like alpha-amylase, in any one of [sic] more: ... (e) thermal stability*"; and dependent claim 4 as filed discloses "*wherein the parent AmyS-like alpha-amylase comprises any of SEQ ID NOs: 1, 2*". Claims 2, 3 and 4 are all dependent on claim 1. The passage on page 22, lines 7 to 11 of the application as filed discloses that thermostability is determined "*under the same experimental conditions*".

3. The examining division considered the selection of the five mutations from a list of eleven mutations in combination with the functional feature "*improved thermostability*" to add subject-matter (see annex to the summons to the second oral proceedings, dated 12 February 2020). The improvement in thermostability of these five variants shown in Examples 1, 3 and 20 was noted by the examining division but found not to be generalisable. A general link between the mutants and improved thermostability was not recognised.

4. The board does not agree and finds that the skilled person would have considered that the list on page 4, lines 10 to 12 (see also claim 2 as filed) represented a pool of preferred substitutions in position 242 which are tested in the application for "*properties of interest*" (see page 2, lines 6 to 18). Altered thermostability is one of those properties (see page 2, lines 16 to 18). As disclosed on page 22, lines 7 to 13, "*thermostable*" in the context of the application means "*more thermostable than a reference enzyme*". The examples provide results for thermostability of nineteen S242 substitutions compared to the wild-type (in duplicate) and a positive control (see Table 20-1 on pages 117 to 118). The residual activity for the A, D, E, M and Q substitutions in position 242 is higher (above 40%) than for the other substitutions or for the wild-type controls (34% and 32.3%, respectively, see Table 20-1, last column). Those five variants are improved with regard to thermostability and thus represent preferred variants in the application. The argument by the examining division that the teaching from the examples could not be generalised is not convincing because neither the application nor common general knowledge gives reasons to doubt that the effect of those mutations would also arise in the

context of the slightly modified amylases ("*at least 95% identical to that of a parent AmyS-like alpha-amylase*") as claimed.

5. The subject-matter of the claim does not extend beyond the content of the application as filed.

Claims 2 to 20

6. The board is satisfied with the basis for the further amendments of claims 2 to 20 provided in the statement of grounds of appeal, pages 3 to 4 (see above point V.).

Novelty (Article 54 EPC)

7. Document D4 does not enjoy priority for the subject-matter of alpha-amylases with a substitution at position 242. It is therefore not comprised in the state of the art under Article 54(3) EPC.
8. The subject-matter of claim 1 of auxiliary request 4 (current auxiliary request 2) was recognised by the examining division as being novel. The board considers that the same applies to 95% identical variants as claimed in claim 1 because the amylases disclosed in the state of the art (e.g. documents D1 and D3) do not contain substitutions at position 242 as required in claim 1.
9. The same applies to the remaining independent claims, which all comprise either an alpha-amylase according to claim 1 or a polynucleotide encoding this alpha-amylase.
10. The claimed subject-matter is novel (Article 54 EPC).

Inventive step (Article 56 EPC)

11. Document D1 can be considered the closest prior art because it discloses alpha-amylases, e.g. from *B. stearothermophilus* (see SEQ ID NO: 88), and alpha-amylase variants with improved thermostability. The difference of the subject-matter of claim 1 resides in the substitutions at position 242 (KFSFFPDWL in wild-type; KFSF[ADEMQ]PDWL in the variants). The technical effect linked to these substitutions is that thermostable proteins are obtained. Since an improvement over the alpha-amylase variants disclosed in document D1 has not been shown, the objective technical problem can be formulated as the provision of further thermostable alpha-amylases. As shown in Tables 3-1 and 20-1, the claimed mutants of *B. stearothermophilus* have improved thermostability compared to the wild-type protein. Neither the closest prior art nor other documents in the state of the art (e.g. documents D2 or D3) point to position 242 as being of any relevance for the thermostability of an alpha-amylase. Additionally, not all substitutions at this position, as shown in the examples (Table 20-1), give rise to increased thermostability. These substitutions are therefore not obvious over the disclosure of the state of the art.
12. The same applies to the remaining independent claims, which all comprise either an alpha-amylase according to claim 1 or a polynucleotide encoding this alpha-amylase.
13. The claimed subject-matter involves an inventive step (Article 56 EPC).

Oral proceedings (Article 116 EPC)

14. As the board finds the main request allowable, the request for oral proceedings is moot.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the examining division with the order to grant a patent with the claims of the main request filed on 18 June 2018 as auxiliary request 3 and a description possibly to be adapted.

The Registrar:

The Chairwoman:



I. Aperribay

M. Pregetter

Decision electronically authenticated