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Datasheet for the decision of 2 May 2023

Case Number: T 0946/20 - 3.3.04

Application Number: 08846670.1

Publication Number: 2215202

IPC: C11D3/386, C12N9/28

Language of the proceedings: EN

Title of invention:
Variants of bacillus sp. TS-23 alpha-amylase with altered properties

Patent Proprietor:
Danisco US Inc.

Opponent:
Novozymes A/S

Headword:
Bacillus amylase variants/DANISCO

Relevant legal provisions:
RPBA 2020 Art. 13(2)
EPC Art. 56, 123(2)

Keyword:

Amendment after summons - exceptional circumstances (no)
Inventive step - main request, auxiliary request 1 (no)
Amendments - auxiliary requests 2 and 3: extension beyond the content of the application as filed (yes)

Decisions cited:

T 0824/05, T 1742/12, T 0405/14, T 1705/18, T 0424/21

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Case Number: T 0946/20 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 2 May 2023

Appellant: (Patent Proprietor)	Danisco US Inc. 925 Page Mill Road Palo Alto, CA 94304 (US)
Representative:	Mewburn Ellis LLP Aurora Building Counterslip Bristol BS1 6BX (GB)
Respondent: (Opponent)	Novozymes A/S Krogshøjvej 36 2880 Bagsværd (DK)
Representative:	Potter Clarkson The Belgrave Centre Talbot Street Nottingham NG1 5GG (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 6 February 2020 concerning maintenance of the European Patent No. 2215202 in amended form.**

Composition of the Board:

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

Summary of Facts and Submissions

I. The appeal by the patent proprietor (appellant) lies from the opposition division's decision to maintain European Patent No. EP 2 215 202 based on auxiliary request 24.

II. The patent had been opposed on the grounds of Article 100(a) EPC, in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC), and of Article 100(b) and (c) EPC.

III. In the decision under appeal, the opposition division decided with regard to the main request and auxiliary requests 1 to 7 that claim 1 thereof did not comply with Article 123(2) EPC.

IV. Claim 1 of auxiliary requests 8 to 15 was found not to comply with Articles 84 and 123(2) EPC.

V. Claims 1 and 2 of auxiliary requests 16 to 23 were found not to comply with Article 123(2) EPC and claim 2 of these requests was found not to comply with Article 84 EPC.

VI. With its statement of grounds of appeal, the appellant re-filed sets of claims of a main request and of auxiliary requests 1 to 7 (identical to the respective requests on which the decision under appeal was based) and sets of claims of auxiliary requests 8 to 23 in which, compared to the respective requests dealt with in the decision under appeal, claim 2 had been amended.

VII. The opponent (respondent) replied to the appeal and filed document D17.

VIII. With its letter of 2 July 2021, the appellant responded to the opponent's reply and filed a new main request and auxiliary requests 1 to 3, which correspond to previously filed auxiliary requests 16, 19, 21 and 23, respectively, except for the deletion of the previous claim 2, as well as auxiliary request 4, which is identical to previously filed auxiliary request 24 and was held allowable by the opposition division.

IX. Claim 1 of the main request reads as follows.

"1. A variant of a parent *Bacillus* sp. TS-23 alpha-amylase, wherein the variant is encoded by a sequence that has at least 98% identity to SEQ ID NO: 4, and wherein said variant comprises a) and b), as present in SEQ ID NO: 5, and optionally any one or more of c) to k):
a) a truncation of the C terminus;
b) R180 and/or S181 deleted;
c) M201L;
d) Q87 to E, R;
e) N225 to E, R;
f) N272 to E or R;
g) N282 to E or R;
h) T182 delete;
i) G183 delete;
j) Q98R, M201L, S243Q, R309A, Q320R, Q359E, and K444E; or
k) S243Q, A, E,D,
wherein said variant exhibits alpha-amylase activity."

Claim 1 of auxiliary request 1 differs from claim 1 of the main request in that "said variant has improved thermostability relative to the parent *Bacillus* sp. TS-23 alpha-amylase".

Claim 1 of auxiliary request 2 reads as follows:
"1. A variant of a parent *Bacillus* sp. TS-23 alpha-amylase, wherein the variant is encoded by a sequence that has at least 98% identity to SEQ ID NO: 4, and wherein said variant comprises a) and b), as present in SEQ ID NO: 5, and c):
a) a truncation of the C terminus;
b) R180 and/or S181 deleted;
c) S243Q,
wherein said variant exhibits alpha-amylase activity."

Claim 1 of auxiliary request 3 differs from claim 1 of auxiliary request 2 in that "said variant has improved thermostability relative to the parent *Bacillus* sp. TS-23 alpha-amylase".

X. The board summoned the parties to oral proceedings and informed them of its preliminary opinion in a communication pursuant to Article 15(1) RPBA.

XI. Oral proceedings before the board took place on 2 May 2023 in the form of a videoconference as requested by both attending parties.

XII. At the end of the oral proceedings, the Chairwoman announced the board's decision.

XIII. The following documents are cited in this decision:

D1 H.-F. Lo et al., "*Deletion analysis of the C-terminal region of the α-amylase of Bacillus sp. strain TS-23*", Archives of Microbiology 178(2), 2002, 115-123.

D2 WO 96/23873

D3 R.-J. Shiau et al.,"*Improving the Thermostability of Raw-Starch-Digesting Amylase from a Cytophaga sp. by Site-Directed Mutagenesis*", Applied and Environmental Microbiology 69(4), 2003, 2383-2385.

D4 WO 99/19467

D5 WO 00/60060

D17 Declaration of C. Andersen, 12 October 2020, 5 pages

XIV. The appellant's arguments insofar as relevant to the decision may be summarised as follows.

Admittance of new facts and arguments (Article 13(2) RPBA)

The passage on pages 118 to 119 of document D1 concerning stability and expression of the truncated amylase was relevant to inventive step. Indeed, it confirmed that the skilled person would not have started from the truncated amylase when aiming to improve thermostability of the enzyme.

Main request and auxiliary request 1 - claim 1 Inventive step (Article 56 EPC)

The truncated alpha-amylase disclosed in document D1 could not be considered the closest prior art because it was not the most promising starting point. Its selection required knowledge of the invention. The skilled person would have concluded from document D1 that the truncated alpha-amylase was not the most suitable enzyme for the purpose of industrial

application. It was apparent from Figure 6 of this document that the truncated form showed a 10 to 15% decrease in activity at higher temperatures compared to the full-length enzyme. The skilled person when aiming at providing a more thermostable enzyme would have started with the full-length enzyme. Documents D2 to D5, which disclosed additional mutations, did not suggest combining these with a truncated variant either.

Even when considering the truncated alpha-amylase variant as closest prior art, the subject-matter of claim 1 of the main request was inventive for essentially the same reasons as given by the opposition division in connection with the maintained claims, i.e. then auxiliary request 24:
"*When faced with the objective technical problem of improving the thermostability of the C-terminally truncated TS-23 alpha-amylase of document D1, the skilled person would start by adding the C-terminal 98 amino acids of the wild-type TS-23 alpha amylase because it is shown in Figure 6 of document D1 that this improves thermostability at temperatures above 50°C. The skilled person would then further introduce variations which have been shown to improve thermostability in other alpha-amylases, such as deletion of R180 and S181 (see documents D2 and D3). The skilled person would thus not arrive at the claimed alpha-amylase.*" (point 8.3.7 of the decision under appeal).

The above reasoning of the opposition division applied equally to claim 1 of the main request.

Data supporting the inventiveness of the claimed variant were shown in Table 13-2 of the patent, from

which it was clear that, compared to the truncated TS23 alpha-amylase ("Base") of the prior art, the additional deletion of R180 and S181 ("Ace") of the claimed invention resulted in a surprisingly higher level of thermostability.

Claim 1 of auxiliary request 1 specified the increased thermostability in claim 1.

Auxiliary requests 2 and 3 - claim 1
Amendments (Article 123(2) EPC)

Claim 1 of auxiliary requests 2 and 3 defined the variant as having the C terminal truncation, the R180/ S181 deletion and the S243Q mutation. This was disclosed throughout the application as filed, e.g. on page 3, lines 8 to 21 and page 4, lines 1 to 3, page 108, lines 17 to 22 and in figures 21, 23 and 25. In the application as filed, the truncated TS23 alpha-amylase was referred to as "Base" (see page 107, line 1) and Base ΔR180S181 was referred to as "Ace" (see page 107, line 9). Both the Ace and the Base variants were combined with the mutation S243Q (see Example 13, page 108, lines 17 to 22 and Table 13-2). A further indication of the preferred nature of the S243Q mutation was the fact that it appeared twice in the list of optional variations on page 3, namely under points j) and k) (lines 20 and 21) and g) and h) (lines 31 and 32). Finally, the S243Q mutation was mentioned in Figures 20 to 25 and their corresponding figure legends (see page 9).

The preferred selection of the S243Q mutation and its combination with the remaining features in claim 1 was therefore disclosed in the application as filed.

XV. The respondent's arguments insofar as relevant to the decision may be summarised as follows.

Admittance of new facts and arguments (Article 13(2) RPBA)

The passage on pages 118 to 119 of document D1 concerning stability and expression of the truncated amylase had not been cited before in the proceedings. It constituted an amendment to the case and should not be admitted.

Main request and auxiliary request 1 - claim 1 Inventive step (Article 56 EPC)

Document D1 disclosed the *Bacillus* TS-23 alpha amylase truncated at the C-terminus, teaching that up to 98 amino acids could be deleted from the C-terminal end of the enzyme without significant effect on starch hydrolytic activity or thermal stability (see Abstract, last 8 lines), i.e. a variant of the TS23 alpha amylase which was encoded by a sequence having at least 98% identity to SEQ ID NO:4 and having feature (a) of claim 1. Document D1 recognised the importance of alpha amylases in various industrial processes (see page 115, right hand column, first full paragraph, last sentence), and stated that the *Bacillus* TS-23 alpha-amylase had properties that made it useful for industrial applications.

The objective technical problem could be formulated as improving the thermostability of the alpha-amylase (see the patent, page 2, lines 6, 8 and 55 and page 49, Example 6). The solution to that problem was provided by feature (b) of claim 1 of the Main Request, namely the deletion of R180 and/or S181.

The solution was obvious from:

- document D2 which disclosed that the "double deletion" increased thermal stability in various alpha-amylases
- document D3 which disclosed 20-fold enhancement of stability achieved by the double deletion (of the corresponding amino acid residues R178 and G179) in an alpha-amylase from *Cytophaga* sp. (see Abstract) and showed that the loop area within domain B of alpha-amylase played a crucial role in improving thermostability (page 2383, left-hand column, paragraph 2)
- documents D4 (page 11, Table 2) and D5 (page 18, Table 2) which also disclosed the double deletion

Given the general disclosure in documents D2 to D5 that the double deletion was beneficial in a range of different amylases and the very high similarity between the sequences in the region of the deletion, it would have been obvious to the skilled person to carry out the deletion of R180 and S181 in order to improve the stability of the truncated *Bacillus* TS-23 alpha-amylase.

Auxiliary requests 2 and 3 - claim 1
Amendments (Article 123(2) EPC)

Claim 1 of these requests had been amended to specify that the variant additionally contained the S243Q mutation, which was previously presented as an optional feature in that claim. The amendment contravened Article 123(2) EPC because two further selections were needed to arrive at the particular mutation specified in the claim:

- the group of S243 mutations specified in part (k) of claim 1 as filed
- the particular S243Q mutation

Those two selections - in addition to those needed to arrive at the remainder of the claim wording - meant that the resulting subject matter was not clearly and unambiguously derivable from the application as filed.

XVI. The appellant requested that the decision under appeal be set aside and the patent maintained on the basis of the set of claims of the main request, or, alternatively, of the set of claims of one of auxiliary requests 1 to 3 (all filed with the letter of 2 July 2021).

The respondent requested that the appeal be dismissed and the decision upheld and that document D17 be admitted into the proceedings.

Reasons for the Decision

Admittance of requests (Article 13(1)) RPBA)

1. The main request and auxiliary requests 1 to 3 were filed in response to the reply to the appeal. Since the opponent withdrew its request not to admit these claim requests, the board considered it more expedient to discuss them on their merits. In view of the outcome (see below) it is not necessary for the board to provide reasons for their admittance.

Admittance of document D17 (Article 12(4) and (6) RPBA)

2. Document D17 was filed with the reply to the appeal. The document was not required to reach a conclusion on

inventive step. Hence, it was not necessary to decide on its admittance.

Admittance of new facts and arguments (Article 13(2) RPBA)

3. During oral proceedings, the appellant cited a passage on pages 118 and 119 of document D1 as a basis for putting forth an argument about the solubility and expression of the disclosed truncated amylase in the context of inventive step. This passage and the corresponding line of argument were brought forward for the first time. The board decided not to admit the new fact, namely that document D1 included a passage relating to solubility and expression of the truncated amylase, and the new argument, namely that this information about solubility and expression would deter the skilled person from considering the truncated amylase, because there were no exceptional circumstances which had been justified by cogent reasons (Article 13(2) RPBA).

Main request - claim 1
Inventive step (Article 56 EPC)

4. In the decision under appeal, the C-terminally truncated alpha-amylase disclosed in document D1 was considered an appropriate starting point for analysing inventive step.

5. The appellant agreed to this in writing (see letter of the appellant dated 2 July 2021, page 3). However, during the oral proceedings, the appellant stated that the truncated amylase was not an appropriate starting point as its selection involved hindsight. The board disagrees for two reasons.

6. Firstly, according to Article 56 EPC, an invention involves an inventive step if it is not obvious having regard to the whole state of the art, in practice the cited prior art, and not only the closest prior art as identified by one of the parties. If the claimed invention is obvious to a person skilled in the art in view of at least one of the disclosures which form part of the state of the art, it is not inventive (see e.g. T 1705/18, point 1.1 of the reasons and T 424/21, point 43 of the reasons).

7. Thus, a disclosure forming part of the state of the art cannot be disregarded simply because another disclosure is considered by one of the parties to be "closer" to the claimed invention, or because, allegedly, the skilled person would not have started from it or considered it (see also T 405/14, points 18 and 19 of the reasons). Otherwise such an approach could lead to the paradoxical situation of a claimed invention becoming inventive because new "closer" prior art had been found (see also T 1742/12, point 6.5 of the reasons and T 824/05, point 6.2 of the reasons). Or, put differently, a claimed invention cannot be rendered inventive by the fact that an allegedly "better" starting point has been identified in the state of the art. In this regard it also does not make any difference whether the different starting points are part of the same disclosure, e.g. different embodiments within the same document, or are present in different disclosures, e.g. different documents. The only requirement is that the disclosure is state of the art within the meaning of Article 54(2) EPC.

8. Secondly, even if the board were to subscribe to the theory that only disclosures which the person skilled in the art would consider a suitable starting point

could serve as closest prior art for the purpose of the problem-solution approach, the board would still be of the view that in the present case the skilled person would not have dismissed the truncated alpha-amylase as not suitable for the purpose of the claimed invention. Indeed, even though Figure 6 of document D1 shows that its activity at certain temperatures is slightly below that of the full-length enzyme, the authors of document D1 state that "*the thermal stability of Bacillus sp. strain TS-23 α-amylase is not related to its C-terminal region*" (see page 121, left-hand column).

9. The board therefore agrees with the decision under appeal that the truncated alpha-amylase disclosed in document D1 is an appropriate starting point for analysing inventive step.

10. It is undisputed that embodiments of the claimed subject-matter differ from the truncated alpha-amylase disclosed in document D1 only in the deletion of amino acids R180 and/or S181. It is also undisputed that the objective technical problem is the provision of alpha-amylases with improved thermostability and that the problem has been solved.

11. The decision under appeal in relation to obviousness concluded from Figure 6 of document D1 that the skilled person would not combine the C-terminal truncated form of the alpha-amylase disclosed therein with a deletion of amino acids R180 and S181 (see point 8.3.7 of the decision).

12. The board disagrees for the following reason. The abstract of D1 states that "[up] *to 98 amino acids from the C-terminal end of the α-amylase could be deleted without significant effect on the raw-starch hydrolytic*

activity or thermal stability" (see Abstract, last 8 lines). The authors furthermore state on page 121, first full paragraph, with regard to Figure 6 that "[b]*oth enzymes remained stable at temperatures below 50 °C and showed similar inactivation profiles with a 60% residual activity at 90 °C, suggesting that the thermal stability of Bacillus sp. strain TS-23 α-amylase is not related to its C-terminal region*". The skilled person would have concluded from these passages that the difference between wild-type and truncated variant was within the margin of error and thus not significant.

13. The board also does not agree with the decision under appeal that "*when faced with the objective problem of improving the thermostability of the C-terminally truncated TS-23 alpha-amylase of document D1, the skilled person would start by adding the C-terminal 98 amino acids of the wild-type TS-23 alpha amylase*" (see point 8.3.7). This conclusion would be contrary to the clear teaching of document D1 that the C-terminal region was not required for thermal stability (see above).

14. Rather, the skilled person aiming to solve the objective technical problem would introduce variations which were shown to improve thermostability in other alpha-amylases (see documents D2 to D5) into the truncated alpha-amylase disclosed in document D1.

15. Document D2, for example, discloses alpha-amylases from various *Bacillus* strains (see page 4, lines 22 to 27 and page 12, lines 1 to 13) and finds that deletion of amino acid residues R181 and G182 increases thermal stability in several alpha-amylases (see Example 4 A and B, pages 75 to 77). It is undisputed that this

deletion corresponds to the deletion of R180 and S181 in claim 1 and that the alpha-amylase disclosed in document D1 and the alpha-amylases in document D2 are highly conserved in the respective region.

16. The board has not been presented with evidence of particular difficulties when introducing the corresponding R180 and S181 deletions into the truncated alpha-amylase disclosed in document D1 or that the skilled person would have had no reasonable expectation of success in obtaining a more thermostable enzyme when doing so. It was therefore obvious for the skilled person to arrive at the claimed subject-matter.

17. The subject-matter of claim 1 lacks an inventive step.

Auxiliary request 1 - claim 1
Inventive step (Article 56 EPC)

18. The feature "said variant has improved thermostability relative to the parent *Bacillus* sp. TS-23 alpha-amylase" does not further distinguish the claimed subject-matter from the state of the art. The same considerations with regard to inventive step apply as for the main request.

19. The subject-matter of claim 1 lacks an inventive step.

Auxiliary requests 2 and 3 - claim 1
Amendments (Article 123(2) EPC)

20. Claim 1 of auxiliary requests 2 and 3 combines "[a] variant of a parent *Bacillus* sp. TS-23 alpha-amylase, wherein the variant is encoded by a sequence that has at least 98% identity to SEQ ID NO: 4" with further

features, namely that the "variant comprises a) and b), as present in SEQ ID NO: 5, and c):
a) a truncation of the C terminus;
b) R180 and/or S181 deleted;
c) S243Q,
wherein said variant exhibits alpha-amylase activity."

21. It is undisputed that the C-terminal truncation is already present in the translated product of SEQ ID NO: 4. Feature b), the deletion of R180 and/or S181, however, is only disclosed in the application as filed in relation to sequences other than the variants encoded by SEQ ID NO: 4. On page 3, lines 9 to 12 and lines 22 to 24 and in claims 1 and 2 as filed, the reference is to an amino acid variant which has 90% identity to SEQ ID NO: 4 which is a nucleic acid (see Fig. 4), i.e. the reference is contradictory and unclear. On page 4, lines 13 to 28 and in claim 7 as filed, the reference is to a variant which has at least 90% identity to SEQ ID NO: 1, i.e. the full-length alpha-amylase (see Fig. 1).

22. Feature b) "R180 and/or S181 deleted" is therefore not disclosed in combination with "a variant [which] is encoded by a sequence that has at least 98% identity to SEQ ID NO: 4".

23. The appellant argued that it was apparent from the application as filed as a whole that the S234Q mutation was preferred and that the combination of this mutation with the remaining features of the claim was therefore directly and unambiguously disclosed. The appellant referred in this regard in particular to the lists of features on pages 3 and 4 as filed in which the mutation S243Q appeared twice (see page 3, lines 8 to 21, and page 4, lines 1 to 10) and to the Examples,

e.g. the "Base" and "Ace" variants in combination with the S234Q mutation (see Table 13-2; page 108, lines 19 to 22, and figures 21, 23 and 25).

24. The board does not agree because the mutation "S243Q" is not disclosed in combination with "a variant [which] is encoded by a sequence that has at least 98% identity to SEQ ID NO: 4" (see point 21. above).

25. Furthermore, the variants exemplified in the application as filed which contain the S234Q mutation either have no RS deletion (AmyTS23-7mut, see Example 9) or have a double deletion of R180 and S181 ("Base" and "Ace", see Example 13). By contrast, the combination of S234Q with R189 and/or S181, i.e. including single deletions, is not disclosed as an exemplary embodiment.

26. In conclusion, the application as filed does not disclose the combination of features
b) R180 and/or S181 deleted, and
c) S243Q
in the context of a "*variant [which] is encoded by a sequence that has at least 98% identity to SEQ ID NO: 4*".

27. The amendment of claim 1 of auxiliary requests 2 and 3 extends beyond the content of the application as filed (Article 123(2) EPC).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

I. Aperribay M. Pregetter

Decision electronically authenticated