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61/059,423 6 June 2008 (06.06.2008) US(71) Applicant (for all designated States except US): **DANISCO US INC.** [US/US]; 925 Page Mill Road, Palo Alto, CA 94304 (US).

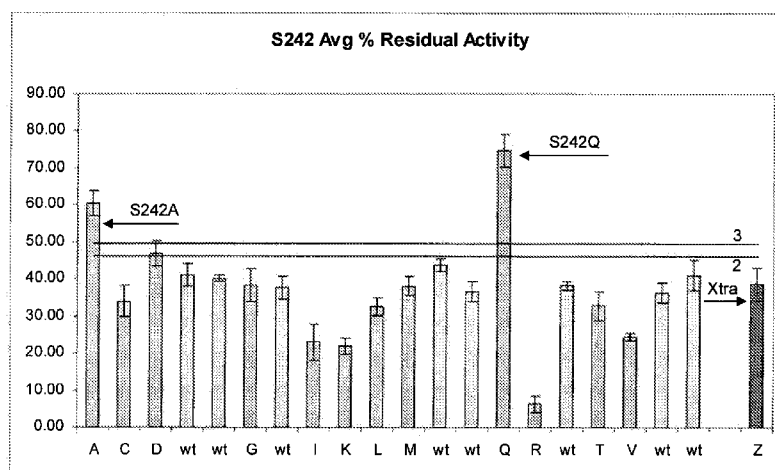
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[Continued on next page]

(54) Title: **GEOBACILLUS STEAROTHERMOPHILUS ALPHA-AMYLASE (AMYS) VARIANTS WITH IMPROVED PROPERTIES****Figure 3**

(57) Abstract: Described are variants of a parent alpha-amylase that exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: specific activity, substrate specificity, substrate binding, substrate cleavage, thermal stability, pH-dependent activity, pH-dependent stability, oxidative stability, calcium dependency, pI, and wash performance. The variants are suitable for starch conversion, ethanol production, laundry washing, dish washing, hard surface cleaning, textile desizing, and/or sweetener production.



OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/046034

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/28 C11D3/386

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, Sequence Search, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 187 576 B1 (SVENDSEN ALLAN [DK]; BORCHERT TORBEN VEDEL [DK]; BISGAARD-FRANTZEN HEN) 13 February 2001 (2001-02-13) abstract; examples 1-2; sequence 3 -----	1-3, 10-18,20
A	HOLM L; ET AL: "RANDOM MUTAGENESIS USED TO PROBE THE STRUCTURE AND FUNCTION OF BACILLUS STEAROTHERMOPHILUS ALPHA-AMYLASE" PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 3, no. 3, 1 January 1990 (1990-01-01) , pages 181-191, XP000087332 ISSN: 0269-2139 the whole document ----- -/--	1-3, 12-14

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

14 December 2009

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18/12/2009

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/046034

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/002472 A2 (DANISCO US INC GENENCOR DIVISI [US]; AEHLE WOLFGANG [US]; ESTELL DAVI) 3 January 2008 (2008-01-03) the whole document	1-3, 10-18,20
A	COHEN N; ABRAMOV S; DROR Y; FREEMAN A: "In vitro enzyme evolution: the screening challenge of isolating the one in a million" TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 19, no. 12, 1 December 2001 (2001-12-01), pages 507-510, XP004321925 ISSN: 0167-7799 the whole document	1-3, 10-18,20
A	NIELSEN J E; BORCHERT T V: "Protein engineering of bacterial alpha-amylases" BIOCHIMICA ET BIOPHYSICA ACTA - PROTEIN STRUCTURE AND MOLECULAR ENZYMOLOGIE, ELSEVIER SCIENCE BV, AMSTERDAM, NL, vol. 1543, no. 2, 29 December 2000 (2000-12-29), pages 253-274, XP004279109 ISSN: 0167-4838 the whole document	1-3, 10-18,20
X,P	WO 2009/061379 A2 (DANISCO US INC GENENCOR DIVISI [US]; YOON MEE-YOUNG [US]) 14 May 2009 (2009-05-14) abstract page 32, line 3 - line 6	1-3, 10-18,20
X,P	WO 2009/061381 A2 (DANISCO US INC GENENCOR DIVISI [US]; SHAW ANDREW [US]; RAMER SANDRA [U]) 14 May 2009 (2009-05-14) abstract page 37, line 19 - line 22	1-3, 10-18,20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/046034

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-3, 10-18, 20(all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3, 10-18, 20(all partially)

A variant polypeptide having α -amylase activity and at least one altered characteristic that improves enzyme performance, the variant polypeptide comprising:

an amino acid sequence having at least 60% amino acid sequence identity to a parental α -amylase polypeptide selected from AmyS (SEQ ID NO: 1) or a truncated variant of AmyS (SEQ ID NO: 2), and

having at least the following mutation at an amino acid residue corresponding to that of the parental α -amylase polypeptide as determined by aligning the variant polypeptides with the parental polypeptide, wherein the mutation changes the amino acid residue from that of the parental polypeptides:

a substitution that introduces a positively charged amino acid residue at position D19;
and uses thereof.

2-16. claims: 1-3, 10-18, 20(all partially)

Same as invention 1, but wherein the substitution introduces a positively charged amino acid residue at positions N28, E29, Q86, Q89, Q97, N224, N271, N281, D306, D318, Q319, Q358, D393, Q443, and D458, respectively.

17-120. claims: 1, 4, 10-18, 20(all partially)

Same as invention 1, but wherein the substitution introduces the amino acid residue 74A, 115L, 124K, 124R, 132A, 132C, 135A, 145A, 146A, 148A, 148N, 159A, 159C, 159D, 159E, 159F, 159G, 159H, 159K, 159L, 159N, 159R, 159S, 159T, 159V, 169A, 169L, 169M, 169Y, 179A, 181A, 181C, 181D, 181E, 181L, 181P, 181Q, 181V, 181Y, 242A, 242D, 242E, 242Q, 261L, 271A, 271V, 278A, 278H, 278K, 278N, 278R, 281A, 281L, 281M, 302D, 302M, 304D, 304E, 304M, 321A, 321H, 321Q, 321R, 333Q, 378D, 378N, 378R, 382D, 398A, 418A, 418M, 418N, 420A, 421R, 432A, 432D, 432L, 432M, 432N, 432Q, 432R, 432Y, 437D, 437G, 437H, 437L, 437M, 437Y, 446A, 446Y, 454A, 464Q, 464Y, 474A, 474E, 474K, 474L, 474M, 474N, 474P, 474Q, 474R, 474S, and 474V, respectively

121-361. claims: 1, 5, 10-18, 20(all partially)

Same as invention 1, but wherein the substitution introduces the amino acid residue 6I, 6N, 6Q, 6T, 6V, 14T, 16F, 25A, 25C, 25G, 25Q, 27M, 36Q, 36S, 39G, 39V, 50I, 50L, 50M, 50N, 50Q, 52S, 53T, 67N, 67S, 80D, 80I, 90E, 133P, 133V, 137M, 137S, 141E, 141I, 141L, 141M, 141Q, 141R, 141S, 141V, 150E,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

151I, 152G, 155S, 155Y, 168W, 173T, 188P, 193F, 193K, 193L, 193Y, 213L, 213M, 213V, 217Q, 220P, 220Q, 220R, 220S, 220V, 221I, 221S, 249E, 250F, 250I, 250M, 252L, 253Y, 254E, 254F, 254T, 254V, 255F, 255K, 255W, 257L, 257M, 257S, 257V, 258D, 258G, 258H, 258K, 258Q, 258T, 258V, 268F, 274W, 283M, 283N, 283V, 285E, 285Q, 293G, 293K, 294W, 301F, 301I, 301P, 301R, 301T, 301W, 309D, 309V, 312H, 312S, 312V, 312Y, 313G, 313H, 313I, 313L, 313S, 313V, 318T, 338A, 338C, 338G, 338M, 338T, 339K, 339T, 339V, 340A, 340M, 340Q, 340T, 343C, 343I, 343P, 343R, 343Y, 345I, 345Q, 369I, 369T, 370G, 375T, 385T, 386K, 394L, 394V, 400A, 400N, 400V, 402H, 402I, 402T, 402V, 402W, 403A, 403E, 403G, 403Q, 403R, 403T, 403V, 404C, 404E, 404G, 404I, 404V, 419A, 419C, 419M, 419T, 422E, 422G, 433A, 433H, 433I, 433K, 433L, 433M, 433V, 433Y, 442A, 442G, 442N, 442R, 442S, 442T, 442V, 442W, 442Y, 445G, 445I, 445N, 445T, 445V, 445W, 447I, 447N, 447Q, 447W, 447Y, 448C, 448F, 448G, 448H, 448I, 448N, 448Y, 450C, 450H, 450M, 450N, 450R, 450S, 450T, 450W, 455G, 455I, 455P, 455V, 463A, 463M, 463S, 463T, 463V, 463W, 465G, 465I, 465K, 465N, 465T, 465V, 469D, 469W, 469Y, 471I, 471V, 473G, 473Y, 476A, 476G, 476L, 476M, 476N, and 476T, respectively.

362-430. claims: 1, 6, 10-18, 20(all partially)

Same as invention 1, but wherein the substitution introduces the amino acid residue 124N, 125A, 125K, 125N, 130A, 130S, 159A, 159D, 159E, 159G, 159H, 159K, 159L, 159N, 159R, 159S, 159T, 166F, 166G, 166H, 166S, 166Y, 169L, 179A, 179P, 180A, 180D, 180H, 180K, 180L, 180N, 180T, 180V, 180Y, 181A, 181D, 181E, 181G, 181P, 181R, 181S, 181V, 187A, 187C, 187K, 187N, 187P, 187Q, 187R, 187S, 242H, 242N, 278H, 278K, 278N, 278R, 281M, 302D, 304M, 304Y, 321H, 321Q, 321R, 333Q, 432Q, 437Y, 446A, 474Q, and 474S, respectively

431-663. claims: 1, 7, 10-18, 20(all partially)

Same as invention 1, but wherein the substitution introduces the amino acid residue 6A, 6D, 6E, 6H, 6I, 6K, 6L, 6M, 6N, 6P, 6Q, 6R, 6S, 6T, 6V, 6W, 6Y, 13K, 14F, 14T, 14Y, 15A, 15D, 15E, 015G, 15H, 15K, 15N, 15P, 15Q, 15R, 15S, 15T, 15W, 16A, 16E, 16G, 16H, 16K, 16N, 16P, 16Q, 16R, 16T, 25C, 39D, 39E, 39N, 39Q, 81Y, 121P, 139D, 139H, 139R, 139Y, 177A, 188D, 191H, 191K, 192A, 192D, 192G, 192N, 192P, 192Q, 192S, 192T, 192V, 192Y, 196A, 196C, 196D, 196E, 196F, 196H, 196I, 196K, 196P, 196R, 196S, 196T, 196V, 201A, 201E, 201G, 201H, 201M, 202H, 216E, 216G, 216H, 216M, 216Q, 216R, 216S, 216T, 216Y, 221A, 221D, 221F, 221I, 221L, 221M, 221N, 221R, 221S, 221V, 221Y, 237G, 240G, 240N, 240P, 240Q, 240R, 240T, 246R, 250A, 250D, 250E, 250F, 250G, 250I, 250K, 250L, 250M, 250N, 250Q, 250R, 250S, 250W, 252K, 268A, 268D, 268E, 268G, 268H, 268K, 268N, 268P, 268Q, 268R, 268S, 274A, 274D, 274G, 274I, 274K, 274L, 274N, 274Q, 274R, 274S, 274T, 275K, 285Q, 285Y, 293K, 293R, 318A, 318F, 318G, 318I, 318K, 318L, 318M, 318R,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

318S, 318T, 318V, 318Y, 319C, 319D, 319H, 319I, 319K, 319R, 319Y, 320K, 320R, 320T, 338A, 338G, 338I, 338M, 338P, 338S, 338V, 339G, 339P, 340A, 340D, 340E, 340H, 340K, 340N, 340Q, 345E, 363D, 363E, 363M, 363N, 363Q, 363S, 366Q, 370A, 370D, 370E, 370H, 370K, 370N, 370Q, 370S, 375A, 375D, 375E, 375K, 375N, 375Q, 375R, 375S, 419A, 419I, 419M, 419P, 419S, 419V, 448Y, 452N, 452Q, 452R, 452S, 471R, and 471Y, respectively.

664-673. claims: 1, 8, 10-20(all partially)

Same as invention 1, but wherein the substitution introduces the amino acid residue I181A, I181P, I181C, I181E, I181Y, S242A, S242E, G132A, N193Y, and E188P, respectively.

674. claims: 9, 16-18, 20(all partially)

A variant α -amylase polypeptide, comprising an amino acid sequence derived from a parental α -amylase polypeptide, and having a combination of three or more mutations at positions selected from the group consisting of 5, 6, 13, 14, 15, 16, 18, 20, 25, 27, 29, 36, 39, 50, 52, 53, 54, 67, 71, 73, 75, 77, 80, 81, 83, 85, 90, 92, 107, 111, 113, 114, 120, 121, 126, 128, 131, 133, 137, 138, 139, 141, 143, 147, 149, 150, 151, 152, 155, 160, 165, 168, 172, 173, 177, 188, 191, 192, 193, 196, 200, 201, 202, 213, 216, 217, 220, 221, 227, 232, 235, 237, 238, 240, 246, 249, 250, 252, 253, 254, 255, 257, 258, 268, 272, 274, 275, 279, 283, 285, 293, 294, 297, 300, 301, 306, 309, 312, 313, 317, 318, 319, 320, 338, 339, 340, 343, 345, 363, 366, 369, 370, 375, 379, 381, 385, 386, 391, 392, 393, 394, 400, 402, 403, 404, 406, 407, 410, 413, 414, 416, 419, 422, 427, 433, 436, 439, 442, 445, 447, 448, 450, 452, 455, 463, 465, 469, 471, 473, and 476, wherein the polypeptide has α -amylase activity, and where each of the at least three or more mutations introduces an amino acid residue that differs from that in the parental polypeptide; and uses thereof.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/046034

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6187576	B1	13-02-2001	NONE
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