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(71) Ansøger: Novozymes A/S, Krogshøjvej 36, 2880 Bagsværd, Danmark

(54) Benævnelse: Subtilase variants

(57) Sammendrag:

The present invention relates to novel subtilase variants exhibiting alterations relative to the parent subtilase in one or more properties including: Wash performance, thermal stability, storage stability or catalytic activity. The variants of the invention are suitable for use in e.g. cleaning or detergent compositions, such as laundry detergent compositions and dishwash compositions, including automatic dishwash compositions.

CLAIMS

1. A subtilase variant comprising one or more of the following alterations:

X11{A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V}, deletion, insertion;

X30{A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V}, deletion, insertion;

X31{R,N,D,Q,E,H,K,M,P,W,Y}, deletion, insertion;

X32{A,R,D,C,E,G,H,I,L,K,M,F,P,T,W,Y,V}, insertion;

X34{A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V}, deletion;

X65{A,R,C,G,H,I,L,K,M,F,T,W,Y,V}, deletion, insertion;

X66{A,R,C,H,I,L,K,M,F,T,W,Y,V}, deletion, insertion;

X67{A,R,N,Q,G,H,I,K,M,F,P,S,T,W,Y,V};

X68{R,N,D,Q,E,G,H,I,L,K,F,P,S,T,W,Y,V}, deletion, insertion;

X69{A,R,N,D,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V}, deletion, insertion;

X70G;

X71T;

X77N;

X83G, insertion;

X84V;

X85{A,R,N,D,Q,E,G,H,I,L,M,F,S,T,W,Y,V};

X90{A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V}, deletion, insertion;

X95{R,K,F,W,Y,V}, deletion;

X107I

X110G, insertion;

X121V, insertion;

X122 insertion;

X123N;

X125S;

X150{A,R,N,D,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V}, deletion, insertion;

X152{A,R,N,D,Q,E,H,K,F,P,W,Y,V};

X153{R,N,D,Q,E,G,H,I,L,K,M,F,T,W,Y,V};

X154{A,R,G,H,I,L,M,F,W,Y,V};

X164{A,R,H,T,W};
X165{R,L,F,W,Y};
X166{W,Y}, insertion;
X175{R,N,D,Q,E,G,H,K,M,F,P,T,W,Y,V};
X177{R,N,D,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V}, deletion, insertion;
X178{A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V}, deletion, insertion;
X180{A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V}, deletion, insertion;
X199{R,L,K,F,W,Y,V}, deletion, insertion;
X200{A,R,I,L,K,M,F,W,Y,V}, deletion, insertion;
X201{A,R,H,I,L,K,M,F,P,T,W,Y,V}, deletion, insertion;
X202{A,R,C,G,H,I,L,K,M,F,T,W,Y,V};
X207{A,R,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V};
X220T, insertion;
X223A, insertion;
X225P, insertion;
X226H, insertion;
X227V, insertion;
X228A, insertion;
X229G, insertion;
X230 insertion;
X231A, insertion;
X253{A,R,N,D,Q,E,G,H,I,L,M,F,S,T,W,Y,V}, deletion, insertion;
X264{A,R,N,Q,G,H,I,L,M,F,S,T,W,Y,V}, deletion, insertion;
X266{A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V}, deletion, insertion; wherein

(a) the alteration(s) are independently, as specified,

(i) a substitution of the amino acid that occupies the position with a different amino acid,

(ii) a deletion of the amino acid which occupies the position, or

(iii) an insertion of at least one additional amino acid downstream of the amino acid that occupies the position,

(b) the variant has protease activity, and

(c) each position corresponds to a position of the amino acid sequence of subtilisin BPN', shown in Figure 1.

2. The variant according to claim 1, wherein the parent subtilase belongs to the sub-group I-S1.

3. The variant according to claim 2, wherein the parent subtilase is selected from the group consisting of BSS168, BSSDY and BLSCAR, or functional variants thereof having retained the characteristics of sub-group I-S1.

4. The variant according to claim 1, wherein the parent subtilase belongs to the sub-group I-S2.

5. The variant according to claim 4, wherein the parent subtilase is selected from the group consisting of BLS147, BLSAVI (Savinase®), BAPB92, TVTHER and BYSYAB, or functional variants thereof having retained the characteristics of sub-group I-S2.

6. The variant according to claim 5, wherein the parent subtilase is BLSAVI (Savinase®).

7. The variant according to any of claims 4-6, wherein the variant further comprises at least one modification in the positions 27, 36, 56, 76, 87, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 120, 123, 159, 167, 170, 206, 218, 222, 224, 232, 235, 236, 245, 248, 252 or 274 (BASBPN numbering).

8. The variant according to claim 7, wherein the variant further comprises the modification S101G+S103A+V104I+G159D+A232V+Q236H+Q245R+N248D+N252K.

9. An isolated DNA sequence encoding a subtilase variant as defined in any of claims 1-8.

10. An expression vector comprising the isolated DNA sequence of claim 9.

11. A microbial host cell transformed with the expression vector of claim 10.

12. A microbial host cell according to claim 11, which is a bacterium, preferably a Bacillus, especially a *B. lentus*.

13. A microbial host cell according to claim 11, which is a fungus or yeast, preferably a filamentous fungus, especially an Aspergillus.

14. A method for producing a subtilase variant as defined in any of claims 1-8, wherein a host as defined in any of claims 11-13 is cultured under conditions conducive to the expression and secretion of the variant, and the variant is recovered.

15. A cleaning or detergent composition, preferably a laundry or dish wash composition, comprising the variant as defined in any of claims 1-8.

16. A composition according to claim 15, which additionally comprises a cellulase, a lipase, a cutinase, an oxidoreductase, another protease, an amylase or a mixture thereof.

17. Use of a variant as defined in any of claims 1-8 in a laundry and/or a dish wash detergent.

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No: 1         10         20         30         40         50
a)  AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASM
b)  AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI*STHPDLNIRGGASF

No:           60         70         80         90         100
a)  VPSETNPFQDNNSHGTHVAGTVAALNNSIGVLGVAPSASLYAVKVLGADG
b)  VPGEPST*QDGNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASG

No:           110        120        130        140        150
a)  SGQYSWIINGIEWAIANNMDVINMSLGGPSGSAALKAAVDKAVASGVVVV
b)  SGSVSSIAQGLEWAGNNGMHVANLSLGSPSPSATLEQAVNSATSRGVLVV

No:           160        170        180        190        200
a)  AAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFSSVGPELDVMA
b)  AASGNSG*AGS***ISYPARYANAMAVGATDQNNNRASFSQYGAGLDIVA

No:           210        220        230        240        250
a)  PGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWTNTQVRSSL
b)  PGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPSWSNVQIRNHL

No:           260        270  275
a)  ENTTTKLGDSFYYGKGLINVQAAAQ
b)  KNTATSLGSTNLYGSGLVNAEAATR
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