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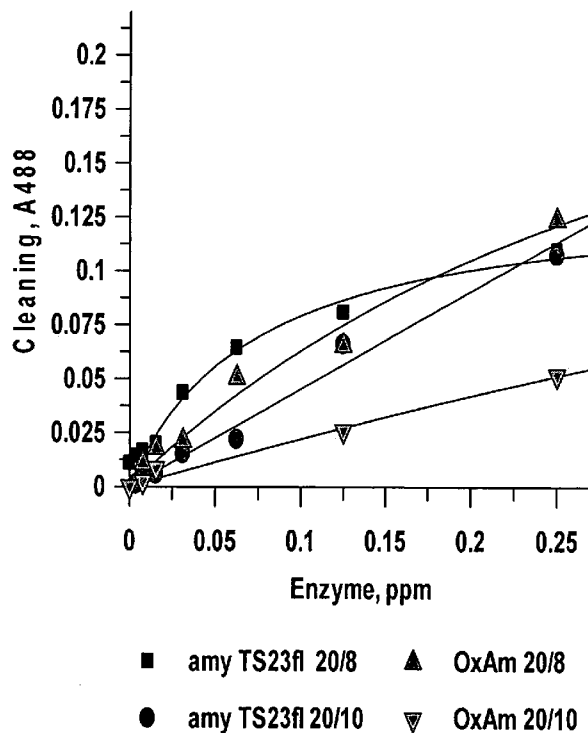
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(54) Title: VARIANTS OF BACILLUS sup. TS-23 ALPHA-AMYLASE WITH ALTERED PROPERTIES



(57) Abstract: Variants of Bacillus sp. TS-23 strain alpha-amylases exhibit improved enzymatic performance, including increased thermostability, reduced calcium dependence, increased washing / cleaning performance, and baking ability. Compositions comprising these variants are useful in methods of starch processing, starch liquefaction, fermentation, starch saccharification, cleaning, laundry, textile desizing, baking, and biofilm removal.



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AMENDED CLAIMS

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1. A variant of a parent *Bacillus* sp. TS-23 alpha-amylase, wherein the variant has 90% identity to SEQ ID NO: 4, and wherein said variant comprises at least two of a), b), or c), as present in SEQ ID NO: 1, and optionally any one or more of d) 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.

2. The variant of claim 1, wherein variant has an amino acid sequence which has at least about 90% sequence identity to SEQ ID NO: 4, and comprises a Δ RS delete at positions 180-181 of SEQ ID NO: 4 and M201L of SEQ ID NO: 4, and at least one of the following:

- a) Q87 to E, R;
- b) N225 to E, R;
- c) N272 to E or R;
- d) N282 to E or R;
- e) T182 delete;
- f) G183 delete;
- g) Q98R, M201L, S243Q, R309A, Q320R, Q359E, and K444E; or
- h) S243Q, A, E, D.

3. The variant of claim 1, wherein said variant has at least about 95% sequence identity to SEQ ID NO: 4.

4. The variant of claim 3, wherein said variant has at least about 98% sequence identity to SEQ ID NO: 4.

5. The variant of claim 1, wherein said variant has the following characteristics:

- a) requires less than 60 ppm calcium ions for enzymatic activity;
- b) improved oxidative stability relative to the parent *Bacillus* sp. TS-23 alpha-amylase; and/or
- c) improved thermostability relative to the parent *Bacillus* sp. TS-23 alpha-amylase.

6. The variant of claim 1, wherein the truncation of the C terminus is 1 amino acid to 100 amino acids.

7. An isolated nucleic acid encoding a variant of a parent *Bacillus* sp. TS-23 alpha-amylase, wherein said variant comprises at least a), b), or c) and optionally any one or more of d) 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;

k) S243Q, A, E, D;

corresponding to SEQ ID NO: 1 and having at least about 90% sequence identity to SEQ ID NO: 1, and wherein said variant exhibits alpha-amylase activity.

8. An isolated nucleic acid comprising a nucleic acid sequence that encodes the variant of any of claims 1-6.

9. The isolated nucleic acid of claim 8, wherein said nucleic acid consists of a nucleic acid sequence that encodes the variant.

10. The vector comprising the nucleic acid of any of claims 7 to 8.

11. An isolated host cell comprising the nucleic acid of any of claims 7 to 8

12. An isolated host cell comprising the vector of claim 11, and wherein the host cell is a prokaryote or an eukaryote.

13. The isolated host cell of claim 12, wherein the isolated host cell is a bacterium or a fungus.

14. The isolated host cell of claim 13, wherein the bacterium is a Gram positive bacterium selected from the group consisting of *Bacillus subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. thuringiensis*, *Streptomyces lividans*, and *S. murinus*; or a Gram negative bacterium, wherein said Gram negative bacterium is *Escherichia coli* and a *Pseudomonas sp.*

15. A detergent additive comprising the variant of any of claims 1 to 6.
16. The detergent additive of claim 15, further comprising one or more enzymes from the group consisting of: a cellulase, a protease, an aminopeptidase, an amylase, a carbohydrase, a carboxypeptidase, a catalase, a chitinase, a cutinase, a cyclodextrin glucanotransferase, a deoxyribonuclease, an esterase, an α -galactosidase, a β -galactosidase, a glucoamylase, α -glucosidase, a β -glucosidase, a haloperoxidase, an invertase, a laccase, a lipase, a mannosidase, an oxidase, a pectinolytic enzyme, a peptidoglutaminase, a peroxidase, a phytase, a polyphenoloxidase, a proteolytic enzyme, a ribonuclease, a transglutaminase, a xylanase, a pullulanase, an isoamylase, a carrageenase, or any combination thereof.
17. The detergent additive of claim 16 in the form of a non-dusting granulate, microgranulate, stabilized liquid, or protected enzyme.
18. A detergent composition comprising the detergent additive of claim 16.
19. A detergent composition comprising a surfactant and a variant of any of claims 4 to 10.
20. The detergent composition of claim 19, wherein said detergent composition is a laundry detergent or a dish detergent.
21. The detergent composition of claim 20, further comprising one or more enzymes selected from the group consisting of a cellulase, a protease, an aminopeptidase, an amylase, a carbohydrase, a carboxypeptidase, a catalase, a chitinase, a cutinase, a cyclodextrin glucanotransferase, a deoxyribonuclease, an esterase, an α -galactosidase, a β -galactosidase, a glucoamylase, an α -glucosidase, a β -glucosidase, a haloperoxidase, an invertase, a laccase, a

lipase, a mannosidase, an oxidase, a pectinolytic enzyme, a peptidoglutaminase, a peroxidase, a phytase, a polyphenoloxidase, a proteolytic enzyme, a ribonuclease, a transglutaminase, a xylanase, a pullulanase, an isoamylase, a carrageenase, and any combination thereof.

22. A laundry detergent composition comprising the laundry additive of claim 21, and further comprising one or more of a surfactant, detergent builder, complexing agent, polymer, bleaching system, stabilizer, foam booster, suds suppressor, anti-corrosion agent, soil-suspending agent, anti-soil redeposition agent, dye, bactericide, hydrotone, optical brightener, fabric conditioner, and perfume.

23. A biofilm hydrolyzing composition comprising the variant of any claims 1 to 6 in a solution or gel, and optionally further comprising a cellulase, a hemicellulase, a xylanase, a lipase, a protease, a pectinase, an antimicrobial agent, or any combination thereof.

24. A method of hydrolyzing a biofilm comprising administering the composition of claim 24 for a period sufficient to process said biofilm.

26. A baking composition comprising the variant of any of claims 1 to 6 in a solution or gel.

27. A method of baking, comprising administering the baking composition of claim 26.

STATEMENT UNDER PCT ARTICLE 19(1)

The amendment of claim 15 is made in order to remove an editorial comment. The amendment consisting of the deletion of the editorial comment does not go beyond the disclosure of the international application as filed.