CLAIMS

1. A trehalose phosphorylase comprising an amino acid sequence, wherein the amino acid sequence of the trehalose phosphorylase is at least 80 % identical to SEQ ID NO: 1, wherein the amino acid sequence of the trehalose phosphorylase comprises an amino acid substitution at one or more amino acid positions selected from the group consisting of

an amino acid substitution at position 383 of SEQ ID NO: 1 with the substitution being 383V, 383G, 383M, 383A, 383N, 383C, 383Q, 383S or 383T, preferably 383A, 383G, 383V, 383C, 383S or 383T;

an amino acid substitution at position 712 of SEQ ID NO: 1 with the substitution being 712M;

an amino acid substitution at position 225 of SEQ ID NO: 1 with the substitution being 225V, 225L, 225M, or 225I, preferably 225L, 225M or 225V;

an amino acid substitution at position 304 of SEQ ID NO: 1 with the substitution being 304I or 304L;

an amino acid substitution at position 323 of SEQ ID NO: 1 with the substitution being 323I or 323V, preferably 323I;

an amino acid substitution at position 487 of SEQ ID NO: 1 with the substitution being 487A, 487G, 487L, or 487M;

an amino acid substitution at position 550 of SEQ ID NO: 1 with the substitution being 550I;

an amino acid substitution at position 556 of SEQ ID NO: 1 with the substitution being 556T;

an amino acid substitution at position 705 of SEQ ID NO: 1 with the substitution being 705N;

wherein the trehalose phosphorylase has an increased thermal stability leading to a residual activity after 15 min incubation at 52°C of at least 22 % in comparison to 19 % of trehalose phosphorylase of SEQ ID NO: 1.

2. The trehalose phosphorylase of claim 1, wherein

the amino acid substitution at position 383 of SEQ ID NO: 1 is 383A, 383G, 383V, 383C, 383S or 383T;

the amino acid substitution at position 225 of SEQ ID NO: 1 is 225L, 225M or 225V;

the amino acid substitution at position 323 of SEQ ID NO: 1 is 323I;

wherein the trehalose phosphorylase has at least the characteristic (A), wherein characteristic (A) is thermal stability after incubation at 52°C for 15 minutes defined by a residual activity of from 30% to 100%.

3. The trehalose phosphorylase of any one of the previous claims, wherein the one or more amino acid positions is at amino acid position 383 of SEQ ID NO: 1.

4. The trehalose phosphorylase of any one of the previous claims, wherein the amino acid sequence of the trehalose phosphorylase comprises an amino acid substitution at one or more further amino acid positions, wherein the one or more further amino acid positions are independently and individually selected from the group consisting of amino acid positions 10, 114, 118, 192, 197, 220, 225, 304, 306, 318, 323, 339, 349, 357, 383, 459, 476, 481, 484, 487, 488, 506, 511, 526, 530, 532, 533, 537, 550, 556, 564, 590, 649, 667, 703, 705, and 712 of SEQ ID NO: 1.

5. The trehalose phosphorylase of any one of the previous claims, wherein the trehalose phosphorylase comprises an amino acid substitution at two or more amino acid positions, wherein the two or more amino acid positions are selected from the group consisting of 10 and 304, 10 and 323, 10 and 383, 10 and 487, 10 and 550, 10 and 556, 10 and 705, 10 and 712, 114 and 225, 114 and 304, 114 and 323, 114 and 383, 114 and 487, 114 and 550, 114 and 556, 114 and 705, 114 and 712, 118 and 225, 118 and 304, 118 and 323, 118 and 383, 118 and 487, 118 and 550, 118 and 556, L118 and 705, 118 and 712, 220 and 225, 220 and 304, 220 and 383, 220 and 487, 220 and 550, 220 and 556, 220 and 705, 220 and 712, 225 and 304, 225 and 323, 225and 349, 225 and 357, 225 and 383, 225 and 487, 225 and 506, 225 and 550, 225 and 556, 225 and 564, 225 and 590, 225 and 649, 225 and 705, 225 and 712, 304 and 323, 304 and 349, 304 and 357, 304 and 383, 304 and 487, 304 and 506, 304 and 550, 304 and 556, 304 and 564, 304 and 590, 304 and 649, 304 and 705, 304 and 712, 323 and 349, 323 and 357, 323 and 383, 323 and 487, 323 and 506, 323 and 550, 323 and 556, 323 and 564, 323 and 590, 323 and 649,

323 and 705, 323 and 712, 349 and 383, 349 and 487, 349 and 550, 349 and 556, 349 and 705, 349 and 712, 357 and 383, 357 and 487, 357 and 550, 357 and 556, 357 and 705, 357 and 712, 383 and 487, 383 and 506, 383 and 550, 383 and 556, 383 and 564, 383 and 590, 383 and 649, 383 and 705, 383 and 712, 487 and 506, 487 and 550, 487 and 556, 487 and 564, 487 and 590, 487 and 649, 487 and 705, 487 and 712, 506 and 550, 506 and 556, 506 and 705, 506 and 712, 550 and 556, 550 and 564, 550 and 590, 550 and 649, 550 and 705, 550 and 712, 556 and 564, 556 and 590, 556 and 649, 556 and 705, 556 and 712, 564 and 705, 564 and 712, 590 and 705, 590 and 712, 649 and 712, 649 and 705, and 705 and 712.

6. The trehalose phosphorylase of any one of the previous claims, wherein the trehalose phosphorylase comprises an amino acid substitution at two or more amino acid positions, wherein the two or more amino acid positions are selected from the group consisting of10 and 712, 114 and 304, 114 and 383, 114 and 712, 118 and 304, 118 and 383, 118 and 556, 118 and 712, 225 and 304, 225 and 383, 225 and 487, 225 and 550, 225 and 556, 225 and 590, 304 and 323, 304 and 357, 304 and 383, 304 and 487, 304 and 556, 304 and 564, 304 and 590, 304 and 712, 323 and 357, 323 and 487, 323 and 556, 323 and 564, 323 and 590, 323 and 649, 349 and 383, 357 and 383, 357 and 705, 357 and 712, 383 and 487, 383 and 550, 383 and 556, 383 and 564, 383 and 590, 383 and 649, 383 and 712, 487 and 564, 487 and 590, 487 and 649, 550 and 590, 556 and 564, 556 and 590, 556 and 649, 564 and 712, 590 and 712, and 649 and 712.

7. The trehalose phosphorylase of any one of the previous claims, wherein the trehalose phosphorylase comprises an amino acid substitution at two or more amino acid positions, wherein the two or more amino acid positions are selected from the group consisting of 118 and 383, 118 and 556, 225 and 304, 225 and 383, 225 and 487, 225 and 550, 225 and 556, 225 and 590, 304 and 323, 304 and 383, 304 and 487, 304 and 556, 304 and 564, 304 and 590, 323 and 357, 323 and 383, 323 and 487, 323 and 556, 323 and 564, 323 and 590, 323 and 649, 349 and 383, 357 and 383, 357 and 487, 383 and 487, 383 and 550, 383 and 556, 383 and 564, 383 and 590, 383 and 649, 383 and 712, 487 and 564, 487 and 590, 487 and 649, 550 and 590, 556 and 564, 556 and 590, 556 and 649, 564 and 712, 590 and 712, 649 and 712, and 705 and 712.

8. The trehalose phosphorylase of any of the previous claims, wherein the amino acid sequence of the trehalose phosphorylase comprises one or more substitutions, wherein the one or more substitution is/are selected from the group consisting of

an amino acid substitution at position 10 of SEQ ID NO: 1 with the substitution being V10R, V10H or V10K, preferably V10R;

an amino acid substitution at position 114 of SEQ ID NO: 1 with the substitution being L114A, L114G, L114I, L114M, L114P or L114V, preferably L114I;

an amino acid substitution at position 118 of SEQ ID NO: 1 with the substitution being I118A, I118G, I118L, I118M, I118P or I118V, preferably I118V;

an amino acid substitution at position 192 of SEQ ID NO: 1 with the substitution being S192A, S192G, S192I, S192L, S192M, S192P or S192V, preferably S192V;

an amino acid substitution at position 197 of SEQ ID NO: 1 with the substitution being S197A, S197G, S197I, S197L, S197M, S197P or S197V, preferably S197G;

an amino acid substitution at position 220 of SEQ ID NO: 1 with the substitution being Y220F or Y220W, preferably Y220F;

an amino acid substitution at position 306 of SEQ ID NO: 1 with the substitution being D306R, D306H or D306K, preferably D306H;

an amino acid substitution at position 318 of SEQ ID NO: 1 with the substitution being P318R, P318H or P318K, preferably P318H;

an amino acid substitution at position 339 of SEQ ID NO: 1 with the substitution being L339A, L339G, L339I, L339M, L339P or L339V, preferably L339I;

an amino acid substitution at position 349 of SEQ ID NO: 1 with the substitution being F349W or F349Y, preferably F349Y;

an amino acid substitution at position 357 of SEQ ID NO: 1 with the substitution being G357A, G357I, G357L, G357M, G357P or G357V, preferably G357A;

an amino acid substitution at position 459 of SEQ ID NO: 1 with the substitution being A459N, A459C, A459Q or A459S, A459T, preferably A459S;

an amino acid substitution at position 476 of SEQ ID NO: 1 with the substitution being Q476A, Q476G, Q476I, Q476L, Q476M, Q476P or Q476V, preferably Q476G;

an amino acid substitution at position 481 of SEQ ID NO: 1 with the substitution being E481A, E481G, E481I, E481L, E481M, E481P or E481V, preferably E481I;

an amino acid substitution at position 484 of SEQ ID NO: 1 with the substitution being A484N, A484C, A484Q, A484S or A484T, preferably A484S;

an amino acid substitution at position 488 of SEQ ID NO: 1 with the substitution being K488A, K488G, K488I, K488L, K488M, K488P or K488V, preferably K488A;

an amino acid substitution at position 506 of SEQ ID NO: 1 with the substitution being A506N, A506C, A506Q, A506S or A506T, preferably A506S;

an amino acid substitution at position A511 of SEQ ID NO: 1 with the substitution being A511N, A511C, A511Q, A511S or A511T, preferably A511S;

an amino acid substitution at position 526 of SEQ ID NO: 1 with the substitution being R526D or R526E, preferably R526E;

an amino acid substitution at position 530 of SEQ ID NO: 1 with the substitution being E530A, E530G, E530I, E530L, E530M, E530P, E530V, preferably E530V;

an amino acid substitution at position 532 of SEQ ID NO: 1 with the substitution being G532R, G532H or G532K, preferably G532R;

an amino acid substitution at position 533 of SEQ ID NO: 1 with the substitution being D533A, D533G, D533I, D533L, D533M, D533P or D533V, preferably D533G;

an amino acid substitution at position 537 of SEQ ID NO: 1 with the substitution being D537A, D537G, D537I, D537L, D537M, D537P or D537V, preferably D537M;

an amino acid substitution at position 564 of SEQ ID NO: 1 with the substitution being T564D or T564E, preferably T564E;

an amino acid substitution at position 590 of SEQ ID NO: 1 with the substitution being D590N, D590C, D590Q, D590S, D590T, D590A, D590G, D590I, D590L, D590M, D590P or D590V, preferably D590N, D590G or D590A, and more preferably D590N;

an amino acid substitution at position 649 of SEQ ID NO: 1 with the substitution being A649D or A649E, preferably A649E;

an amino acid substitution at position 667 of SEQ ID NO: 1 with the substitution being R667D, R667E, R667R, R667H or R667K, preferably R667E or R667K, more preferably R667E;

an amino acid substitution at position 703 of SEQ ID NO: 1 with the substitution being A703D or A703E, preferably A703E.

9. The trehalose phosphorylase of any one of the previous claims, wherein the amino acid sequence of the trehalose phosphorylase comprises

(a) an amino acid substitution at the three amino acid positions 383, 556, and 590 of SEQ ID NO: 1;

(b) an amino acid substitution at the four amino acid positions 712, 383, 114 and 118 of SEQ ID NO: 1; and/or 383, 487, 556, and 590 of SEQ ID NO: 1; and/or 383, 225, 556, and 590 of SEQ ID NO: 1;

(c) an amino acid substitution at the five amino acid positions 712, 383, 114, I118 and 304 of SEQ ID NO: 1; and/or 712, 383, 114, 118 and 357 of SEQ ID NO: 1; and/or 383, 225, 304, 556, and 590 of SEQ ID NO: 1; and/or 383, 225, 487, 556, and 590 of SEQ ID NO: 1;

(d) an amino acid substitution at the six amino acid positions 712, 383, 114, 118, 304 and 357 of SEQ ID NO: 1; and/or 383, 225, 304, 487, 556, and 590 of SEQ ID NO: 1.

10. The trehalose phosphorylase of any one of the previous claims, wherein the identity of the amino acid sequence of the trehalose phosphorylase with the amino acid sequence of SEQ ID NO: 1 is at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, still more preferably at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, yet more preferably at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, and most preferably at least 96%, or at least 97%, or at least 98%, or at least 99%, at least 99.1%, or at least 99.2%, or at least 99.3%, or at least 99.4%, or at least 99.5%, or at least 99.6%, or at least 99.7%, or at least 99.8%, and in particular at least 99.9%, or 100%.

11. The trehalose phosphorylase of claim 1, wherein the amino acid sequence of the trehalose phosphorylase comprises or consists of any one of amino acid sequences of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, or 190, preferably wherein the amino acid sequence of the trehalose phosphorylase comprises or consists of the amino acid sequence of SEQ ID NO: 14.

12. The trehalose phosphorylase of any one of the previous claims, wherein the amino acid sequence of the trehalose phosphorylase comprises or consists of any one of amino acid sequences of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 87, 88, 89, 91, 93, 94, 95, 97, 98, 101, 103, 104, 105, 107, 109, 110, 111, 113, 114, 115, 117, 119, 121, 123, 125, 128, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 146, 147, 149, 150, 151, 152, 154, 155, 156, 157, 158, 159, or 190, preferably wherein the amino acid sequence of the trehalose phosphorylase comprises or consists of the amino acid sequence of SEQ ID NO: 14.

13. The trehalose phosphorylase of any one of the previous claims, wherein the trehalose phosphorylase is capable of catalyzing conversion of
glucose and alpha-D-glucose-1 phosphate to trehalose and inorganic phosphate
and/or
conversion of trehalose and inorganic phosphate to glucose and alpha-D-glucose-1 phosphate.

14. The trehalose phosphorylase, wherein the trehalose phosphorylase has at least one of characteristics (A), (B), (C), (D) and (E), or any combination thereof, wherein

characteristic (A) is thermal stability after incubation at 52°C for 15 minutes defined by a residual activity of from 30% to 100%;

characteristic (B) is thermal stability after incubation at 52°C for 15 minutes which is characterized by

i) a Tm30-value of at least 52 °C, and/or
ii) a Tm50-value of at least 52 °C;

characteristic (C) is thermal stability characterized by
i) a Tm30-value between 52 °C and 90°C and/or
ii) a Tm50-value between 52 °C and 90°C;

characteristic (D) is thermal stability characterized by
i) a process stability characterized by a half-life at 45 °C of from 3 hours to 9 days or more; or
ii) a process stability characterized by a half-life at 45 °C of from 24 hours to 9 days or more, or
iii) a process stability characterized by a half-life at 45 °C of 4 days to 9 days or more; and

characteristic (E) is relative activity expressed as 100/500-ratio of between 0.65 and 1.0, wherein
the100/500-ratio is defined as the ratio of
[trehalose activity at 100 mM glucose and 100 mM alpha-glucose-1 phosphate]/
[trehalose activity at 500 mM glucose and 100 mM alpha-glucose-1 phosphate],

wherein the trehalose phosphorylase is a trehalose phosphorylase as defined in any one of claims 1 to 12.

15. A trehalose phosphorylase variant, wherein the variant is a polypeptide that comprises an amino acid sequence, wherein the amino acid sequence is SEQ ID NO: 1, 160, 191, 192, or 193, , wherein the variant comprises an amino acid substitution at one or more, two or more, three or more, four or more, five or more, six or more, preferably seven or more, of the following amino acid positions selected from the group consisting of amino acid positions corresponding to positions 383, 712, 225, 304, 323, 487, 550, or 556 of SEQ ID NO: 1, wherein the trehalose phosphorylase variant comprises an amino acid substitution at one or more amino acid positions selected from the group consisting of

an amino acid substitution at position 712 of SEQ ID NO: 1 with the substitution being 712M, and

an amino acid substitution at position 383 of SEQ ID NO: 1 with the substitution being 383V, and

an amino acid substitution at position 225 of SEQ ID NO: 1 with the substitution being 225V, and

an amino acid substitution at position 304 of SEQ ID NO: 1 with the substitution being 304I, and

an amino acid substitution at position 323 of SEQ ID NO: 1 with the substitution being 323I,

an amino acid substitution at position 487 of SEQ ID NO: 1 with the substitution being 487A, and

an amino acid substitution at position 550 of SEQ ID NO: 1 with the substitution being 550I, and

an amino acid substitution at position 556 of SEQ ID NO: 1 with the substitution being 556T;

with the numbering referring to an aligning position in SEQ ID NO: 1.

16. The trehalose phosphorylase variant of claim 15, wherein the variant comprises an amino acid sequence, wherein the polypeptide comprises an amino acid substitution at two or more of amino acid positions selected from the group consisting of amino acid position 383, 225, 304, 323, 487, and 556, wherein the amino acid numbering refers to an aligning position in SEQ ID NO: 1.

17. The trehalose phosphorylase variant of claim 15, wherein the variant comprises an amino acid sequence, wherein the polypeptide comprises an amino acid substitution at two or more of amino acid positions selected from the group consisting of amino acid position 383, 225, 304, 323, and 556, wherein the amino acid numbering refers to an aligning position in SEQ ID NO: 1.

18. The trehalose phosphorylase variant of any one of claims 16 to 17, wherein the variant comprises at least one further amino acid substitution at amino acid positions selected from amino acid position 712, 118, 349, 487, 590, and 649, preferably selected from amino acid position 712M, 118V, 118I, 349Y, 487A, 487G, 487L, 487M, 590N, and 649E, wherein the amino acid numbering refers to an aligning position in SEQ ID NO:1.

19. A thermally stable trehalose phosphorylase variant, wherein the variant retains at least 30% of its initial activity after incubation for 15 minutes at 52 °C in a buffer containing 1 M sucrose, and the initial activity is determined after incubation for 15 minutes at room temperature and/or wherein the variant retains at least 50% of its initial activity after incubation for 15 minutes at 52 °C in a buffer containing 1 M sucrose, and the initial activity is determined after incubation for 15 minutes at room temperature, wherein said trehalose phosphorylase variant is any of the trehalose phosphorylase variant of claims 1 to 18.

20. A thermally stable variant of trehalose phosphorylase from *Schizophyllum commune*, wherein the variant has a residual activity of at least 30 % after incubation at 52° C for 15 minutes, in a buffer containing 1 M sucrose, preferably wherein the variant has a residual activity of at least 50 % of its initial activity after incubation for 15 minutes at 52 °C, in a buffer containing 1 M sucrose, and the initial activity is determined after incubation for 15 minutes at room temperature, wherein said trehalose phosphorylase variant is any of the trehalose phosphorylase variant of claims 1 to 18.

21. A thermally stable variant of a trehalose phosphorylase comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, wherein the variant has a residual activity of at least 30 % after incubation at 52° C for 15 minutes, in a buffer containing 1 M sucrose, preferably wherein the variant has a residual activity of at least 50 % of its initial activity after incubation for 15 minutes at 52 °C, in a buffer containing 1 M sucrose, and the initial activity is determined after incubation for 15 minutes at room temperature, wherein said trehalose phosphorylase variant is any of the trehalose phosphorylase variant of claims 1 to 18.

22. A method for reacting a glucosyl monosaccharide and alpha-D-glucose-1 phosphate, wherein the method comprises reacting the glucosyl monosaccharide and alpha-D-glucose-1 phosphate with a trehalose phosphorylase as defined in any one of claims 1 to 20.

23. A method for preparing trehalose comprising reacting glucose and alpha-D-glucose-1 phosphate at a temperature of at least 40° C in the presence of a trehalose phosphorylase, wherein the trehalose phosphorylase

(i) retains at least 30% of its activity after incubation for 15 minutes at 52 °C in a buffer containing 1 M sucrose compared to its activity without thermal treatment; and/or

(ii) retains at least 50% of its activity after incubation for 15 minutes at 52 °C in a buffer containing 1 M sucrose compared to its activity without thermal treatment; and/or

(iii) has a ratio of activity at 100 mM glucose to activity at 500 mM glucose of at least 0.65,

wherein the trehalose phosphorylase is any of the trehalose phosphorylase variant of claims 1 to 18.

24. A method for increasing thermal stability of a trehalose phosphorylase, wherein the method comprises:

- aligning an amino acid sequence of a first trehalose phosphorylase with an amino acid sequence of a second trehalose phosphorylase,

- identifying one or more amino acid positions of the amino acid sequence of the second trehalose phosphorylase which correspond to one or more amino acid positions of the amino acid sequence of the first trehalose phosphorylase, wherein substitution of an amino acid residue at the one or more amino acid position of the amino acid sequence of the first trehalose phosphorylase increases thermal stability of the first trehalose phosphorylase,

- substituting an amino acid residue at the one or more amino acid positions of the second trehalose phosphorylase corresponding to the one or more amino acid positions of the amino acid sequence of the first trehalose phosphorylase, wherein substitution of an amino acid residue at the one or more amino acid position of the amino acid sequence of the first trehalose phosphorylase increases thermal stability of the first trehalose phosphorylase,

wherein the first trehalose phosphorylase is a trehalose phosphorylase comprising an amino acid sequence according to SEQ ID NO: 1

wherein said substitution of an amino acid residue in the second trehalose phosphorylase is done in one or more of the amino acids positions corresponding to

an amino acid substitution at position 712 of SEQ ID NO: 1 with the substitution being 712M;

an amino acid substitution at position 383 of SEQ ID NO: 1 with the substitution being 383V, 383G, 383M, 383A, 383N, 383C, 383Q, 383S or 383T, preferably 383A, 383G, 383V, 383C, 383S or 383T;

an amino acid substitution at position 225 of SEQ ID NO: 1 with the substitution being 225V, 225L, 225M, or 225I, preferably, 225L, 225M or 225V;

an amino acid substitution at position 304 of SEQ ID NO: 1 with the substitution being 304I or 304L;

an amino acid substitution at position 323 of SEQ ID NO: 1 with the substitution being 323I or 323V, preferably 323I;

an amino acid substitution at position 487 of SEQ ID NO: 1 with the substitution being 487A, 487G, 487L, or 487M;

an amino acid substitution at position 550 of SEQ ID NO: 1 with the substitution being 550I;

an amino acid substitution at position 556 of SEQ ID NO: 1 with the substitution being 556T;

an amino acid substitution at position 705 of SEQ ID NO: 1 with the substitution being 705N.

25. A method for increasing the relative activity expressed as 100/500-ratio of a trehalose phosphorylase, wherein the method comprises:

- aligning an amino acid sequence of a first trehalose phosphorylase with an amino acid sequence of a second trehalose phosphorylase,

- identifying one or more amino acid positions of the amino acid sequence of the second trehalose phosphorylase which correspond to one or more amino acid positions of the amino acid sequence of the first trehalose phosphorylase, wherein substitution of an amino acid residue at the one or more amino acid position of the amino acid sequence of the first trehalose phosphorylase increases the relative activity expressed as 100/500-ratio of the first trehalose phosphorylase,

- substituting an amino acid residue at the one or more amino acid positions of the second trehalose phosphorylase corresponding to the one or more amino acid positions of the amino acid sequence of the first trehalose phosphorylase, wherein substitution of an amino acid residue at the one or more amino acid position of the amino acid sequence of the first trehalose phosphorylase increases the relative activity expressed as 100/500-ratio of the first trehalose phosphorylase;

wherein the first trehalose phosphorylase is a trehalose phosphorylase comprising an amino acid sequence according to SEQ ID NO: 1,

wherein said substitution of an amino acid residue in the second trehalose phosphorylase is done in one or more of the amino acids positions corresponding to

an amino acid substitution at position 712 of SEQ ID NO: 1 with the substitution being 712M;

an amino acid substitution at position 383 of SEQ ID NO: 1 with the substitution being 383V, 383G, 383M, 383A, 383N, 383C, 383Q, 383S or 383T, preferably 383A, 383G, 383V, 383C, 383S or 383T;

an amino acid substitution at position 225 of SEQ ID NO: 1 with the substitution being 225V, 225L, 225M, or 225I;

an amino acid substitution at position 304 of SEQ ID NO: 1 with the substitution being 304I or 304L, preferably 304I;

an amino acid substitution at position 323 of SEQ ID NO : 1 with the substitution being 323I or 323V, preferably 323I;

an amino acid substitution at position 487 of SEQ ID NO: 1 with the substitution being 487A, 487G, 487L, or 487M;

an amino acid substitution at position 550 of SEQ ID NO: 1 with the substitution being 550I;

an amino acid substitution at position 556 of SEQ ID NO: 1 with the substitution being 556T;

an amino acid substitution at position 705 of SEQ ID NO: 1 with the substitution being 705N.

26. Use of a trehalose phosphorylase according to any one of claims 1 to 18 for producing trehalose, preferably for producing trehalose according to any one of claims 22 to 23.