



(86) Date de dépôt PCT/PCT Filing Date: 2000/03/28
 (87) Date publication PCT/PCT Publication Date: 2000/10/12
 (85) Entrée phase nationale/National Entry: 2001/08/27
 (86) N° demande PCT/PCT Application No.: DK 2000/000149
 (87) N° publication PCT/PCT Publication No.: 2000/060060
 (30) Priorités/Priorities: 1999/03/31 (PA 1999 00439) DK;
 1999/04/13 (09/290,734) US;
 1999/04/13 (PA 1999 00490) DK

(51) Cl.Int.⁷/Int.Cl.⁷ C12N 9/28, C11D 3/386
 (71) Demandeur/Applicant:
 NOVOZYMES A/S, DK
 (72) Inventeurs/Inventors:
 ANDERSEN, CARSTEN, DK;
 SVENDSEN, ALLAN, DK;
 BISGARD-FRANTZEN, HENRIK, DK;
 NIELSEN, VIBEKE SKOVGAARD, DK;
 BORCHERT, TORBEN VEDEL, DK;
 NIELSEN, BJARNE RONFELDT, DK;
 HOECK, LISBETH HEDEGAARD, DK;
 OUTTRUP, HELLE, DK
 (74) Agent: DIMOCK STRATTON CLARIZIO LLP

(54) Titre : POLYPEPTIDES PRESENTANT UNE ACTIVITE ALCALINE ALPHA-AMYLASE ET ACIDES NUCLEIQUES
 LES CODANT
 (54) Title: POLYPEPTIDES HAVING ALKALINE ALPHA-AMYLASE ACTIVITY AND NUCLEIC ACIDS ENCODING SAME

(57) **Abrégé/Abstract:**

The present invention relates to isolated polypeptides having alpha-amylase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 October 2000 (12.10.2000)

PCT

(10) International Publication Number
WO 00/60060 A3

(51) International Patent Classification⁷: C12N 9/28,
C11D 3/386

Allan; Bakkeledet 28, DK-3460 Birkerød (DK). ANDERSEN, Carsten; Højeloft Vænge 162, DK-3500 Værløse (DK).

(21) International Application Number: PCT/DK00/00149

(22) International Filing Date: 28 March 2000 (28.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 1999 00439 31 March 1999 (31.03.1999) DK
PA 1999 00490 13 April 1999 (13.04.1999) DK
09/290,734 13 April 1999 (13.04.1999) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

Published:

- With international search report.
- With (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description.

(72) Inventors: OUTTRUP, Helle; Skovhaven 89, DK-3500 Værløse (DK). HOECK, Lisbeth, Hedegaard; Anhof, Anhof Allé 14, DK-5871 Frørup (DK). NIELSEN, Bjarne, Rønfeldt; Ligustervænget 27, DK-2830 Virum (DK). BORCHERT, Torben, Vedel; Vordingborggade 6A, 31, DK-2100 Copenhagen Ø (DK). NIELSEN, Vibeke, Skovgaard; Hjortevænget 45, DK-2880 Bagsværd (DK). BISGÅRD-FRANTZEN, Henrik; Elmevænget 8B, DK-2880 Bagsværd (DK). SVENDSEN,

(88) Date of publication of the international search report:
19 April 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYPEPTIDES HAVING ALKALINE ALPHA-AMYLASE ACTIVITY AND NUCLEIC ACIDS ENCODING SAME

(57) Abstract: The present invention relates to isolated polypeptides having alpha-amylase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

WO 00/60060 A3



INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 00/00149

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 96 23873 A (NOVO NORDISK AS) 8 August 1996 (1996-08-08) aa 41-74, 95-131, 187-279, 324-382, 401-436</p>	1-26
X	<p>--- DATABASE AMY BACLI ST. <a &="" -="" 1147-1156,<="" 1985="" 98,="" a="" abstract="" accession="" acid="" alpha-amylase="" alpha-amylases="" amino="" amy="" and="" bacillus="" bacli="" bacterial="" biochem,="" coding="" comparison="" complete="" deduced="" dna="" for="" from="" gene="" heat-="" href="http://srs6.ebi.ac.uk/srs6bin/cgi-bin/wgetz?-e+[sw...:AMY BACLI;1 January 1988 (1988-01-01) YUUKI T ET AL: " j="" licheniformis:="" liquefying="" no.="" nucleotide="" of="" p="" p06278="" pages="" ph-stable="" retrieved="" sequence="" sequences="" sequences"="" standard,="" the="" three="" vol.="" xp002901161=""> </p>	1-26
X	<p>--- TSUKAMOTO A ET AL: "Nucleotide Sequence of the Maltohexaose-Producing Amylase Gene from an Alkalophilic Bacillus sp. #707 and Structural Similarity to Liquefying Type Alpha-Amylases" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMM, vol. 151, no. 1, 29 February 1988 (1988-02-29), pages 25-31, XP002901160 page 28</p> <p>-----</p>	1-27

INTERNATIONAL SEARCH REPORT**Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 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 fee, this Authority did not invite payment of any additional fee.

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.:

2-6,10,14-16,19-22 (completely); 1,7-9,11-13,17,18,23-26 (partially)

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- 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: 2-6,10,14-16,19-22 (completely);1,7-9,11-13,17,18,23-26 (partially)

See additional sheet.

2. Claims: 1,7-9,11-13,17,18,23-26 (partially)

See additional sheet.

3. Claims: 1,7-9,11-13,17,18,23-26 (partially)

See additional sheet.

4. Claims: 1,7-9,11-13,17,18,23-26 (partially)

See additional sheet.

5. Claims: 1,7-9,11-13,17,18,23-26 (partially)

See additional sheet.

6. Claims: 1,7-9,11-13,17,18,23-26 (partially)

See additional sheet.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

According to Article 34 (3) (a-c) and Rule 13.2, an international application shall relate to one invention only or to a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over the prior art. The present application relates to six such groups of inventions, namely:

1. A protein with the amino acid sequence given in SEQ ID N:0 2 or 4, or functional variants thereof, according to claims 2-6, 10, 14-16, 19-22 (all completely) and claims 1 (parts a-d), 7-9, 11-13, 17, 18, 23-26 (all partially)
2. A protein encoded by a DNA sequence complementary to SEQ ID N:OS 1 or 3, according to claims 1 (parts b(i), b(iii), b(iv), c and d), 7-9, 11-13, 17, 18, 23-26 (all partially).
3. An alpha-amylase with optimum pH in the range 8-9, according to claims 1 (part e), 7-9, 11-13, 17, 18, 23-26 (all partially).
4. An alpha-amylase with optimum temperature range 55°C-65°C, according to claims 1 (part f), 7-9, 11-13, 17, 18, 23-26 (all partially).
5. An alpha-amylase with pI 7-8, according to claims 1 (part g), 7-9, 11-13, 17, 18, 23-26 (all partially).
6. An alpha-amylase with improved washing or dishwashing performance in the pH-range 9-11, according to claims 1 (part i), 7-9, 11-13, 17, 18, 23-26 (all partially).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK 00/00149

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9923211 A	14-05-1999	AU 9737398 A	24-05-1999
		BR 9813328 A	22-08-2000
		EP 1027428 A	16-08-2000

WO 9919467 A	22-04-1999	AU 9434398 A	03-05-1999
		EP 1023439 A	02-08-2000

WO 9805748 A	12-02-1998	AU 6716996 A	25-02-1998
		BR 9612702 A	03-08-1999
		EP 0925343 A	30-06-1999
		HU 9903559 A	28-02-2000

WO 9732961 A	12-09-1997	BR 9707951 A	27-07-1999
		CA 2247501 A	12-09-1997
		CN 1217742 A	26-05-1999
		EP 0885285 A	23-12-1998
		JP 2000505502 T	09-05-2000

WO 9623873 A	08-08-1996	AU 4483396 A	21-08-1996
		BR 9607735 A	14-07-1998
		CA 2211405 A	08-08-1996
		CN 1172500 A	04-02-1998
		EP 0815208 A	07-01-1998
		JP 11503003 T	23-03-1999

22. JUN. 2001 15:30

NOVOZYMES PATENTS

NO. 8185 P. 4

5820.204-WO Amended once

1

Claims

What is claimed is:

1. An isolated polypeptide having alpha-amylase activity and
5 one or more characteristics or properties selected from the
group consisting of:
- (a) a polypeptide having an amino acid sequence which has
at least 96% identity with amino acids 1 to 485 of SEQ ID NO:2
or SEQ ID NO: 4;
- 10 (b) an allelic variant of (a); and
- (c) a fragment of (a) or (b), that has alpha-amylase
activity.
2. The polypeptide of claim 1, having an amino acid sequence
15 which has at least 96% identity with amino acids 1 to 485 of
SEQ ID NO: 2 or SEQ ID NO: 4
3. The polypeptide of claim 1, comprising the amino acid
sequence of SEQ ID NO:2 or SEQ ID NO: 4.
- 20 4. The polypeptide of claim 1, consisting of the amino acid
sequence of SEQ ID NO:2 or SEQ ID NO: 4 or a fragment thereof.
5. The polypeptide of claim 1, which consists of amino acids
25 1 to 485 of SEQ ID NO:2 or SEQ ID NO: 4.
6. The polypeptide of claim 1, which is encoded by a nucleic
acid sequence which hybridizes under very high stringency
conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or
30 SEQ ID NO: 3, (ii) the cDNA sequence of SEQ ID NO:1 or SEQ ID
NO: 3, or (iii) a complementary strand of (i) or (ii).
7. The polypeptide of claim 1, which is encoded by the
nucleic acid sequence contained in plasmid pLiH1274 or pTVB299
35 contained in *E. coli* DSM12761 or *E. coli* DSM12764,

AMENDED SHEET

Empf.zeit:22/Jun/2001 15:30

Empf.nr.: 8185 P.1114

22. JUN. 2001 15:30

NOVOZYMES PATENTS

NO. 8185 P. 5

5820.204-WO Amended once

2

respectively.

8. A polypeptide having the same alpha-amylase activity as the polypeptide of any of claims 1-7.

5

9. An isolated nucleic acid sequence comprising a nucleic acid sequence which encodes the polypeptide of any of claims 1-8.

10. An isolated nucleic acid sequence comprising a nucleic acid sequence having at least one mutation in the mature polypeptide coding sequence of SEQ ID NO:1 or SEQ ID NO: 3, in which the mutant nucleic acid sequence encodes a polypeptide consisting of amino acids 1 to 485 of SEQ ID NO: 2 or SEQ ID
15 NO: 4.

11. The isolated nucleic acid sequence of claim 9 or 10 produced by (a) hybridizing a DNA under very high stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1,
20 (ii) the cDNA sequence of SEQ ID NO:1, or (iii) a complementary strand of (i), or (ii); and (b) isolating the nucleic acid sequence.

12. A recombinant expression vector comprising the nucleic acid construct of claims 9-11.
25

13. A recombinant host cell comprising the nucleic acid construct of claim 12.

14. A method for producing a mutant nucleic acid sequence, comprising (a) introducing at least one mutation into the mature polypeptide coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3, wherein the mutant nucleic acid sequence encodes a polypeptide consisting of amino acids 1 to 485 of SEQ ID NO: 2
35 or SEQ ID NO: 4; and (b) recovering the mutant nucleic acid

AMENDED SHEET

Empf.zeit: 22/06/2001 15:31

Empf.nr.: 8185 P. 111

22. JUN. 2001 15:30

NOVOZYMES PATENTS

NO. 8185 P. 6

5820.204-WO Amended once

3

sequence.

15. A mutant nucleic acid sequence produced by the method of claim 14.

5

16. A method for producing a polypeptide, comprising (a) cultivating a strain comprising the mutant nucleic acid sequence of claim 15 encoding the polypeptide to produce a supernatant comprising the polypeptide; and (b) recovering the polypeptide.

10

17. A method for producing the polypeptide of any of claims 1-8 comprising (a) cultivating a strain to produce a supernatant comprising the polypeptide; and (b) recovering the polypeptide.

15

18. A method for producing the polypeptide of any of claims 1-8 comprising (a) cultivating a host cell comprising a nucleic acid construct comprising a nucleic acid sequence encoding the polypeptide under conditions suitable for production of the polypeptide; and (b) recovering the polypeptide.

20

19. A method for producing a polypeptide comprising (a) cultivating a host cell under conditions conducive for production of the polypeptide, wherein the host cell comprises a mutant nucleic acid sequence having at least one mutation in the mature polypeptide coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3, wherein the mutant nucleic acid sequence encodes a polypeptide consisting of amino acids 1 to 485 of SEQ ID NO: 2 or SEQ ID NO: 4, and (b) recovering the polypeptide.

25

20. A mutant of a polypeptide with alpha-amylase activity of any of claims 1 to 8, wherein said alpha-amylase has one or more mutations, in particular substitution or deletions, in the following positions (relative to SEQ ID NO: 2):

30

1: R181*, G182*, D183*, G184*;

AMENDED SHEET

Empf.zeit:22/06/2001 15:37

Empf. nr. 885 D 006

22. JUN. 2001 15:30

NOVOZYMES PATENTS

NO. 8185 P. 7

5820.204-WO Amended once

- 2: N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- 3: I206A, R, D, N, C, E, Q, G, H, L, K, M, F, P, S, T, W, Y, V;
- 4: E212A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- 5: E216A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- 5 6: K269A, R, D, N, C, E, Q, G, H, I, L, M, F, P, S, T, W, Y, V;
- 7: R181A, N, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V.

21. The mutant of claim 20, selected from the group of mutants with the following mutantions:

- 10 -R181*/G182*/N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- G182*/T183*/N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- T183*/G184*/R181A, N, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- T183*/G184*/N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- R181*/G182*/V206A, R, D, N, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y;
- 15 -G182*/T183*/V206A, R, D, N, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y;
- T183*/G184*/V206A, R, D, N, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y;
- R181*/G182*/E212A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- G182*/T183*/E212A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- T183*/G184*/E212A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- 20 -R181*/G182*/E216A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- G182*/T183*/E216A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- T183*/G184*/E216A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- R181*/G182*/K269A, R, D, N, C, E, Q, G, H, I, L, M, F, P, S, T, W, Y, V;
- G182*/T183*/K269A, R, D, N, C, E, Q, G, H, I, L, M, F, P, S, T, W, Y, V;
- 25 -T183*/G184*/K269A, R, D, N, C, E, Q, G, H, I, L, M, F, P, S, T, W, Y, V;
- N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- /V206A, R, D, N, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y;
- N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V
- /E212A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- 30 -N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V
- /E216A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;
- N195A, R, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V
- /K269A, R, D, N, C, E, Q, G, H, I, L, M, F, P, S, T, W, Y, V;
- V206A, R, D, N, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y
- 35 /E212A, R, D, N, C, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V;

22. JUN. 2001 15:30

NOVOZYMES PATENTS

NO. 8185 P. 8

5820.204-WO Amended once

5

-V206A,R,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y
/E216A,R,D,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
-V206A,R,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y
/K269A,R,D,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
5 -E212A,R,D,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V
/E216A,R,D,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
E212A,R,D,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V
/K269A,R,D,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
-E216A,R,D,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V
10 /K269A,R,D,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V.

22. The mutant of claims 20 or 21, further comprise a substitution in position E216Q.

15 23. Use of the polypeptide of any of claims 1-8 or variant of claims 20-22 in a detergent composition, in particular a laundry detergent composition and a dishwash detergent composition.

20 24. Use of the polypeptide of any of claims 1-8 or variant of claims 20-22 in a desizing composition.

25 25. Use of the polypeptide of any of claims 1-8 or variant of claims 20-22 for liquefaction of starch.

26. Use of the polypeptide of any of claims 1-8 or variant of claims 20-22 for ethanol production.

80

707amy HHNGTNGTMMQYFEWYLPNDGNHWNRLNSDASNLSKSGITAVWI PPAWKGASQNDVGYGAYDLYDLGEFNQKGTVRTKYG
AA349 HHNGTNGTMMQYFEWYLPNDGNHWNRLRSDASNLDKGI SAVWI PPAWKGASQNDVGYGAYDLYDLGEFNQKGT IRTKYG
AA560 HHNGTNGTMMQYFEWYLPNDGNHWNRLRSDASNLDKGI SAVWI PPAWKGASQNDVGYGAYDLYDLGEFNQKGT IRTKYG
BAN . . . VNGTLMQYFEWYTPNDGQHWKRLQNDAEHLSDIGITAVWI PPAWKGLSQSDNGYGPYDLYDLGEFQKGTVRTKYG
BSG A . APFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGITALWLPPAYKGTSRSDVGYGVYDLYDLGEFNQKGTVRTKYG
AP1378 HHNGTNGTMMQYFEWHL PNDGNHWNRLRDDAANLKS KGITAVWI PPAWKGTSQNDVGYGAYDLYDLGEFNQKGTVRTKYG
SP690 HHNGTNGTMMQYFEWYLPNDGNHWNRLRDDAANLKS KGITAVWI PPAWKGTSQNDVGYGAYDLYDLGEFNQKGTVRTKYG
SP722 HHNGTNGTMMQYFEWHL PNDGNHWNRLRDDASNLRNRGITAIWI PPAWKGTSQNDVGYGAYDLYDLGEFNQKGTVRTKYG
Term AN . . LNGTLMQYFEWYMPNDGQHWRRRLQND SAYLAEHGITAVWI PPAWKGTSQADVGYGAYDLYDLGEFHQKGTVRTKYG

81 160

707amy TRSQLQAAVTSLKNNGIQVYGDVVMNHKGGADATEMVR AVEVNPNNRNQEV TGEYTI EAWTRFDFPGRGNTHSSFKWRWY
AA349 TRNQLQAAVNALKSNGIQVYGDVVMNHKGGADATEMVR AVEVNPNNRNQEVSGEYTI EAWTKFDFPGRGNTHSNFKWRWY
AA560 TRNQLQAAVNALKSNGIQVYGDVVMNHKGGADATEMVR AVEVNPNNRNQEVSGEYTI EAWTKFDFPGRGNTHSNFKWRWY
BAN TKSELQDAIGSLHSRNVQVYGDVVLNKHKAGADATEDVT AVEVNPANRNQETSEEYQIKAWTDFRFPGRGNTYSDFKWHWY
BSG TKAQYLQAIQAHAAGMQVYADVVDVFDHKGADGTEWVDA VEVNPSDRNQEI SGTYQIQAWTKFDFPGRGNTYSDFKWRWY
AP1378 TRSQLQGAVTSLKNNGIQVYGDVVMNHKGGADGTE MVAVEVNRNRNQEISGEYTI EAWTKFDFPGRGNTHSNFKWRWY
SP690 TRNQLQAAVTSLKNNGIQVYGDVVMNHKGGADGTEI VNAVEVNRNRNQETSGEYAI EAWTKFDFPGRGNTHSNFKWRWY
SP722 TRSQLESAIHALKNNGVQVYGDVVMNHKGGADATE NVLAVEVNPNNRNQEI SGEYTI EAWTKFDFPGRGNTYSDFKWRWY
Term TKGELQSAIKSLHSRDINVYGDVVINHKGGADATEDVT AVEVDPADRNRV I SGEHLIKAWTHFHFPGRGSTYSDFKWHWY

161 240

707amy HFDGVDWDQSRRLNNRIYKFRGHGKAWDWEVDTENGN YDYL MYADIDMDHPEVVNELRNWGVWYTN TLGLDGFRI DAVKH
AA349 HFDGVDWDQSRKLNRIYKFRGDGKGDWEVDTENGN YDYL MYADIDMDHPEVVNELRNWGVWYTN TLGLDGFRI DAVKH
AA560 HFDGVDWDQSRKLNRIYKFRGDGKGDWEVDTENGN YDYL MYADIDMDHPEVVNELRNWGVWYTN TLGLDGFRI DAVKH
BAN HFDGADWDESRKIS . RIFKFRGEGKAWDWEVSS ENGN YDYL MYADVVDYDHPDVVAETKKWGIWYAN ELSLDGFRI DAAKH
BSG HFDGVDWDESRKLS . RIYKFRGIGKAWDWEVDTEN GN YDYL MYADLDMDHPEVVTELKNWGWYVNTN IDGFR L DAVKH
AP1378 HFDGTDWDQSRQLQNKIYKFRGTGKAWDWEVDI ENGN YDYL MYADIDMDHPEV INELRNWGVWYTN TLNLDGFR I DAVKH
SP690 HFDGTDWDQSRQLQNKIYKFRGTGKAWDWEVDI ENGN YDYL MYADVMDHPEV IHEL RNWGVWYTN TLNLDGFR I DAVKH
SP722 HFDGVDWDQSRQFNRIYKFRGDGKAWDWEVDS ENGN YDYL MYADVMDHPEVVNELRRWGEWYTN TLNLDGFR I DAVKH
Term HFDGTDWDESRKLN . RIYKF . . QGKAWDWEVSN ENGN YDYL MYADIDYDHPDVAAE I KRWGTWYAN ELQLDGFRL DAVKH

241 320

707amy IKYSFTRDWINHVR SATGKNMFAVAEFWKNDLGA IENYLQKT NWNH SVFDVPLHYNLYNASKSGGNYDMRNI FNGTVVQR
AA349 IKYSFTRDWINHVR SATGKNMFAVAEFWKNDLGA IENYLNKT NWNH SVFDVPLHYNLYNASKSGGNYDMRQI FNGTVVQR
AA560 IKYSFTRDWINHVR SATGKNMFAVAEFWKNDLGA IENYLNKT NWNH SVFDVPLHYNLYNASKSGGNYDMRQI FNGTVVQR
BAN IKFSFLRDWVQAVRQATGKEMFTVAEYWQNNAGKLE NYLNKTSFNQSVFDVPLHFNLQAASSQGGGYDMRRL LDGTVVSR
BSG IKFSFFPDWLSYVRSQTGKPLFTVGEYWSYDINKL HNYITKTDGTMSLFDAPLHNKFYTASKSGGAFDMRTLMTNTLMKD
AP1378 IKYSYTRDWLTHVRNTTGKPMFAVAEFWKNDLAA IENYLNKT SWNH SVFDVPLHYNLYNASNSGGYFDMRNI L NGSVVQK
SP690 IKYSFTRDWLTHVRNTTGKPMFAVAEFWKNDLGA IENYLNKT SWNH SVFDVPLHYNLYNASNSGGYFDMRNI L NGSVVQK
SP722 IKYSFTRDWLTHVRNATGKEMFAVAEFWKNDLGA LENYLNKT NWNH SVFDVPLHYNLYNASNSGGNYDMAKLLNGTVVQK
Term IKFSFLRDWVNHVREKTGKEMFTVAEYWQNDLGA LENYLNKT NFNH SVFDVPLHYQFHAAS TQGGGYDMRKL L NGSVVSK

Fig. 1

	321	400
707amy	HPSHAVTFVDNHDSQPEEALESFVEEWFKPLAYALTLTREQGYPSVIFYGDYYGI . . . PTHGVPAMRSKIDPILEARQKYA	
AA349	HPMHAVTFVDNHDSQPEEALESFVEEWFKPLAYALTLTREQGYPSVIFYGDYYGI . . . PTHGVPAMKSKIDPILEARQKYA	
AA560	HPMHAVTFVDNHDSQPEEALESFVEEWFKPLAYALTLTREQGYPSVIFYGDYYGI . . . PTHGVPAMKSKIDPILEARQKYA	
BAN	HPEKAVTFVENHDTQPGQSLESTVQTFWKPLAYAFILITRESGYPQVIFYGDMYGTGKTSPEIIPSLKDNIEPILKARKEYA	
BSG	QPTLAVTFVDNHDTQPGQALQSWVDPWFKPLAYAFILITRQEGYPCVIFYGDYYGI . . . PQYNIIPSLKSKIDPLLIARRDYA	
AP1378	HPIHAVTFVDNHDSQPGEALESFVQSWFKPLAYALILITREQGYPSVIFYGDYYGI . . . PTHGVPMSKSKIDPLLQARQTYA	
SP690	HPTHAVTFVDNHDSQPGEALESFVQSWFKPLAYALVLTREQGYPSVIFYGDYYGI . . . PTHGVPAMKSKIDPLLQARQTFY	
SP722	HPMHAVTFVDNHDSQPGEALESFVQSWFKPLAYALILITREQGYPSVIFYGDYYGI . . . PTHSVPAMKAKIDPILEARQNFA	
Term	HPLKSVTFVDNHDTQPGQSLESTVQTFWKPLAYAFILITRESGYPQVIFYGDMYGTGKDSQREIPALKHKIEPILKARKQYA	
	401	480
707amy	YGKQNDYLDHHNIIGWTREGNTAHPNSGLATIMSDGAGGSKWMFVGRNKAGQVWSDITGNRTGTVTINADGWGNFSVNGG	
AA349	YGRQNDYLDHHNIIGWTREGNTAHPNSGLATIMSDGAGGNKWMFVGRNKAGQVWTDITGNRAGTVTINADGWGNFSVNGG	
AA560	YGRQNDYLDHHNIIGWTREGNTAHPNSGLATIMSDGAGGNKWMFVGRNKAGQVWTDITGNRAGTVTINADGWGNFSVNGG	
BAN	YGPQHDYIDHPDVIIGWTREGDSSAAKSGLAALITDGPGGSKRMYAGLKNAGETWYDITGNRSDTVKIGSDGWGEFHVNDG	
BSG	YGTQHDYLDHSDIIGWTREGGTEKPGSGLAALITDGPGGSKWMYVKGKQHAGKVFYDLTGNRSDTVINSIDGWGEFKVNGG	
AP1378	YGTQHDYFDHDIIGWTREGDSSHPNSGLATIMSDGPGGNKWMYVKGKQVWRDITGNRSVTINADGWGNFTVNGG	
SP690	YGTQHDYFDHDIIGWTREGNSSHHPNSGLATIMSDGPGGNKWMYVKGKQVWRDITGNRTGTVTINADGWGNFSVNGG	
SP722	YGTQHDYFDHDIIGWTREGNTTHHPNSGLATIMSDGPGGKWMYVQNKAGQVWHDITGNKPGTVTINADGWANFSVNGG	
Term	YGAQHDYFDHDIIGWTREGDSSVANSGLAALITDGPGGAKRMYVGRQVWHDITGNRSEPVVINSEGWGEFHVNGG	
	481	520
707amy	SVSIWVVK	
AA349	SVSIWVVK	
AA560	SVSIWVVK	
BAN	SVSIYVQK	
BSG	SVSVWVPRKTTVSTIARPITTRPWTGEFVRWTEPRLVAWP	
AP1378	AVSVWVKQ	
SP690	SVSVWVKQ	
SP722	SVSIWVKR	
Term	SVSIYVQR	

Fig. 1 (cont.)

3 / 8

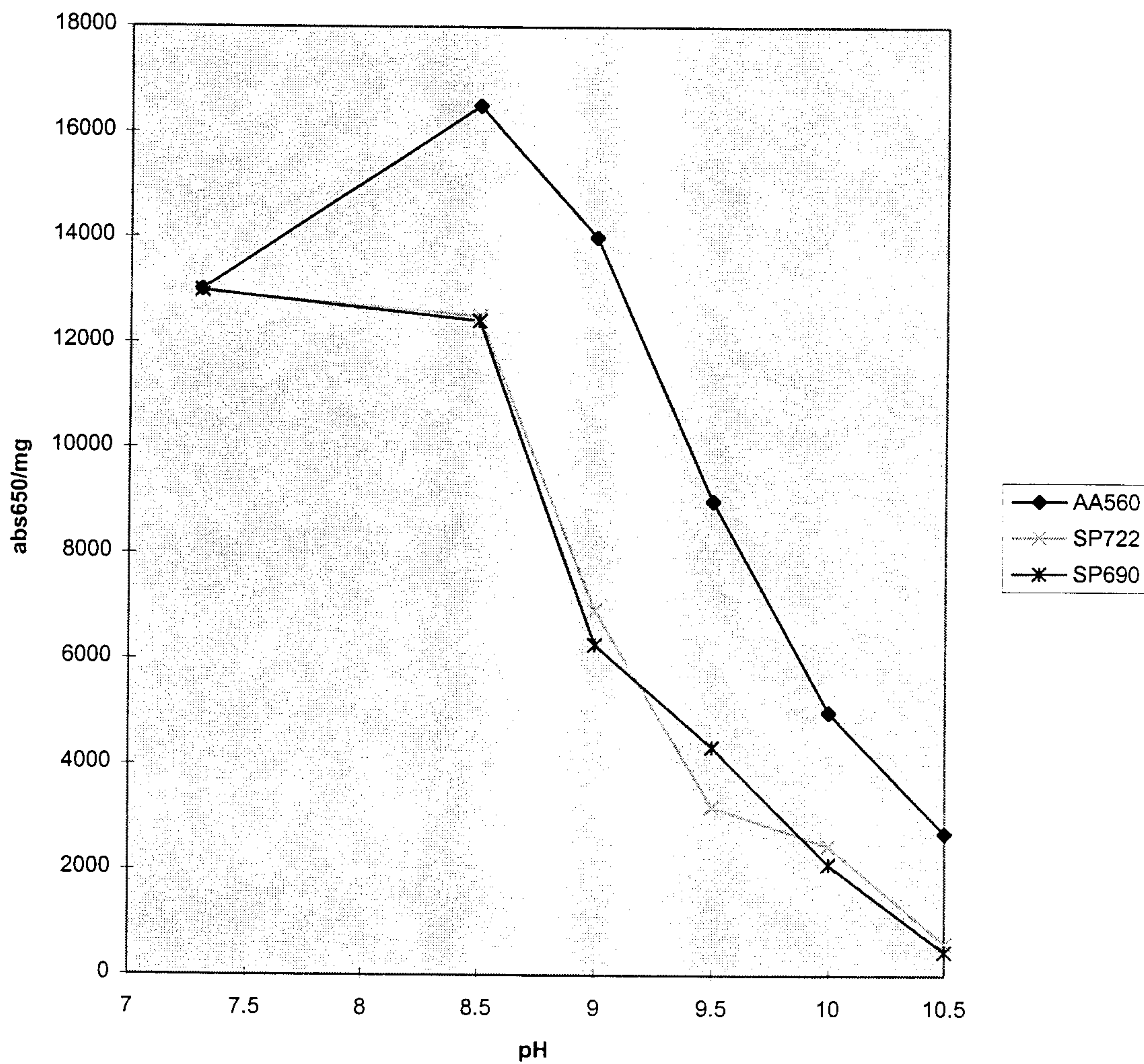


Fig. 2

4/8

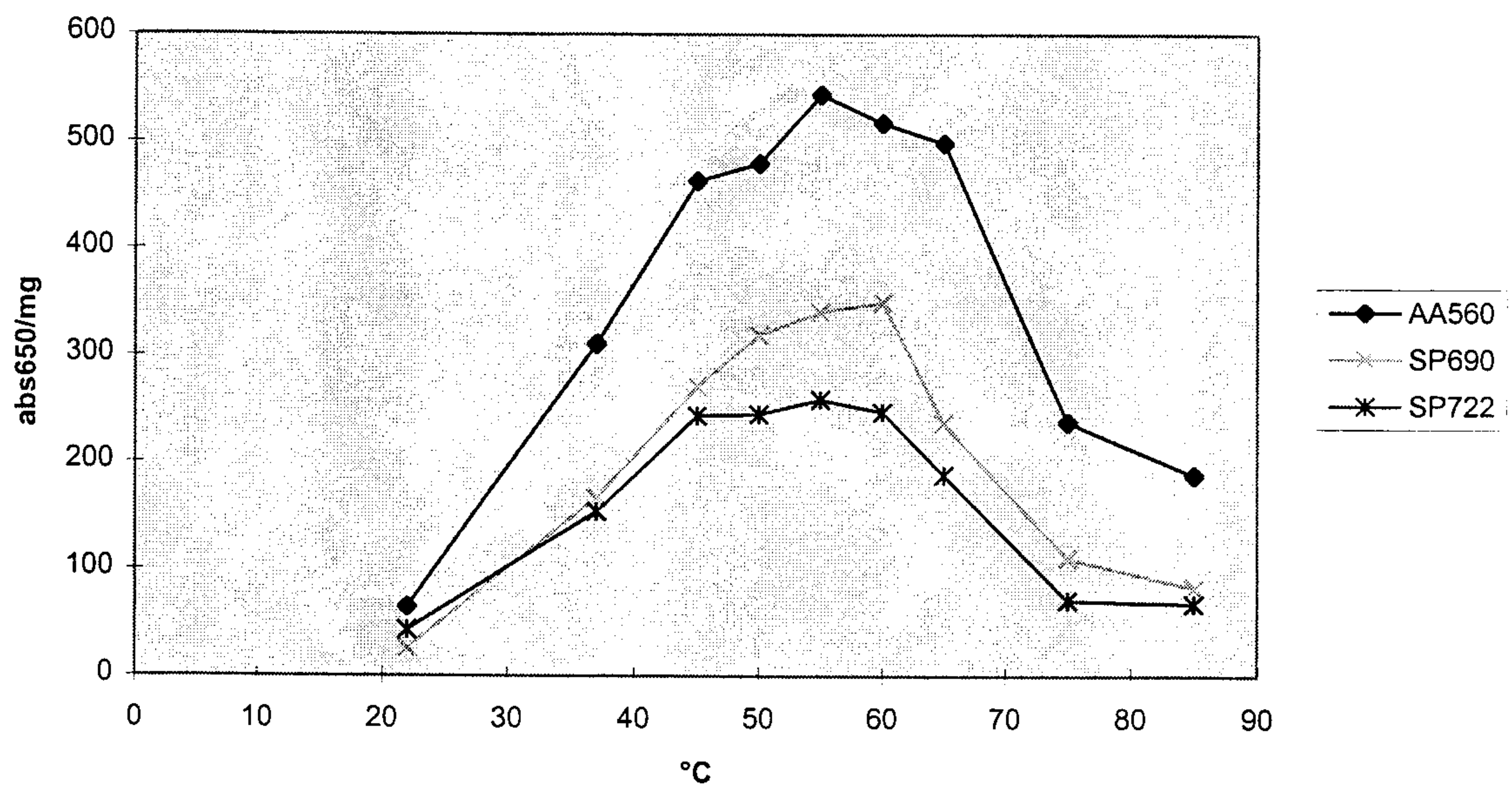


Fig. 3

5/8

Delta Remission

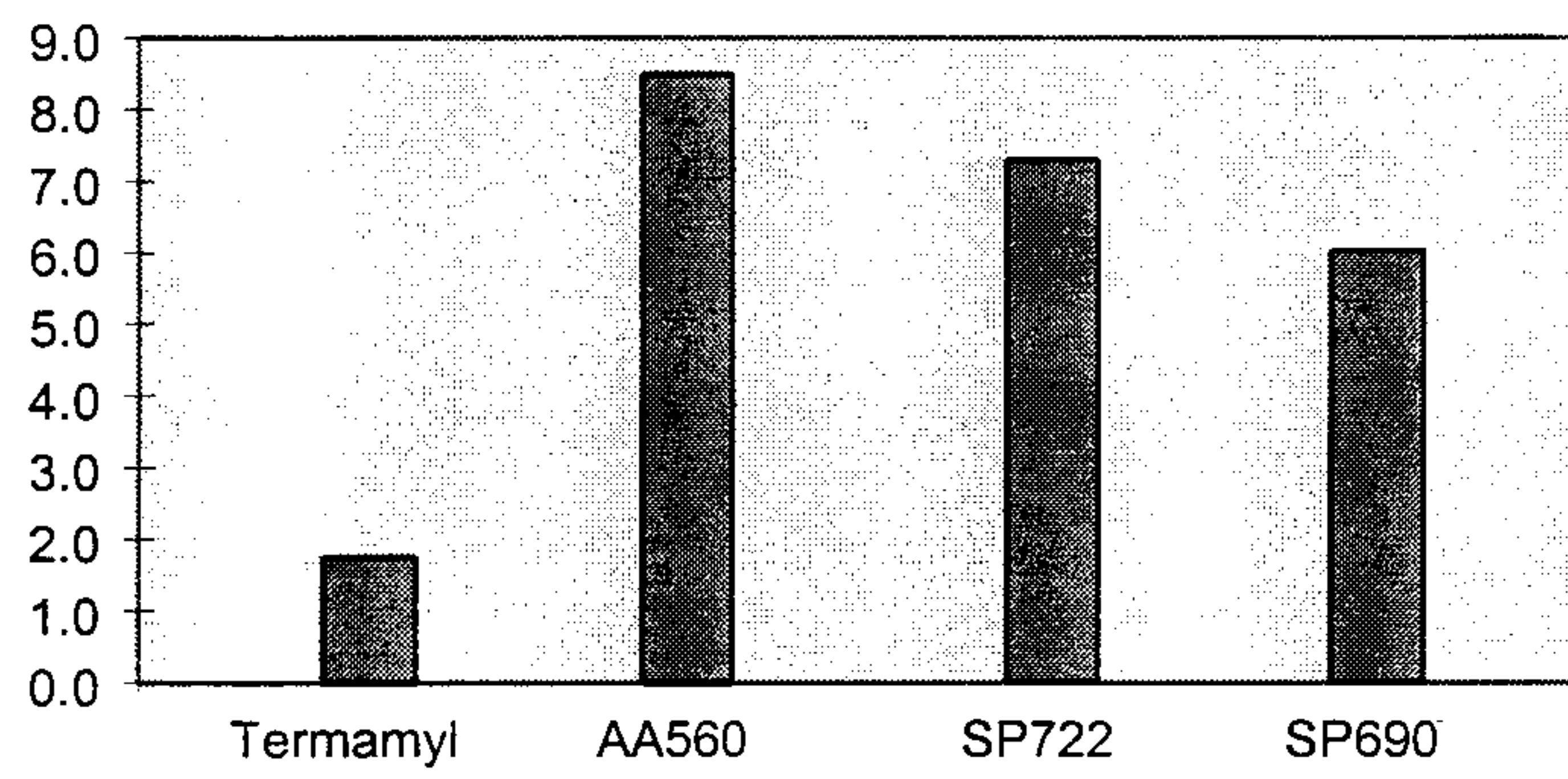


Fig. 4

6/8

Delta Remission

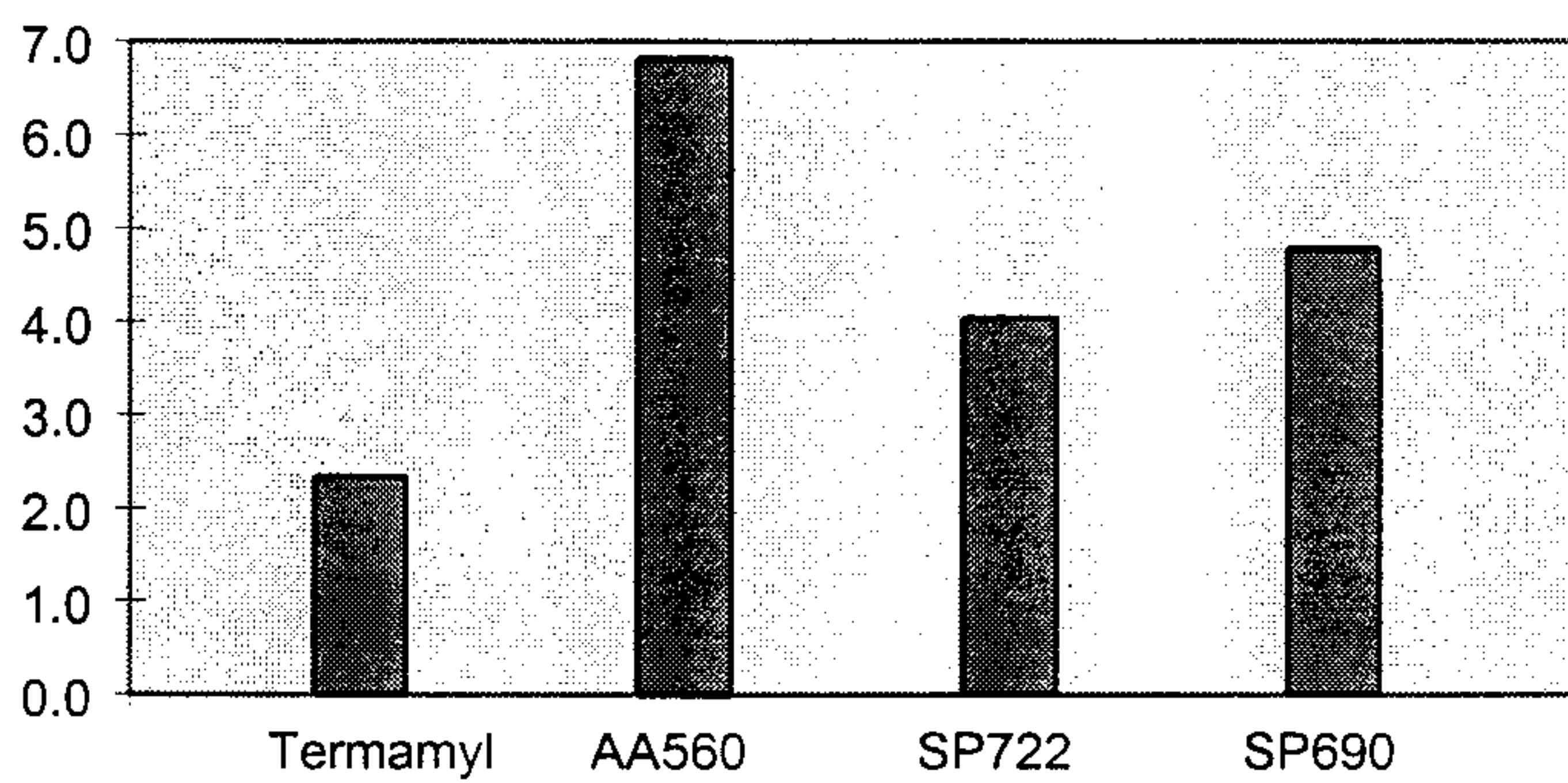


Fig. 5

7/8

Delta Remission

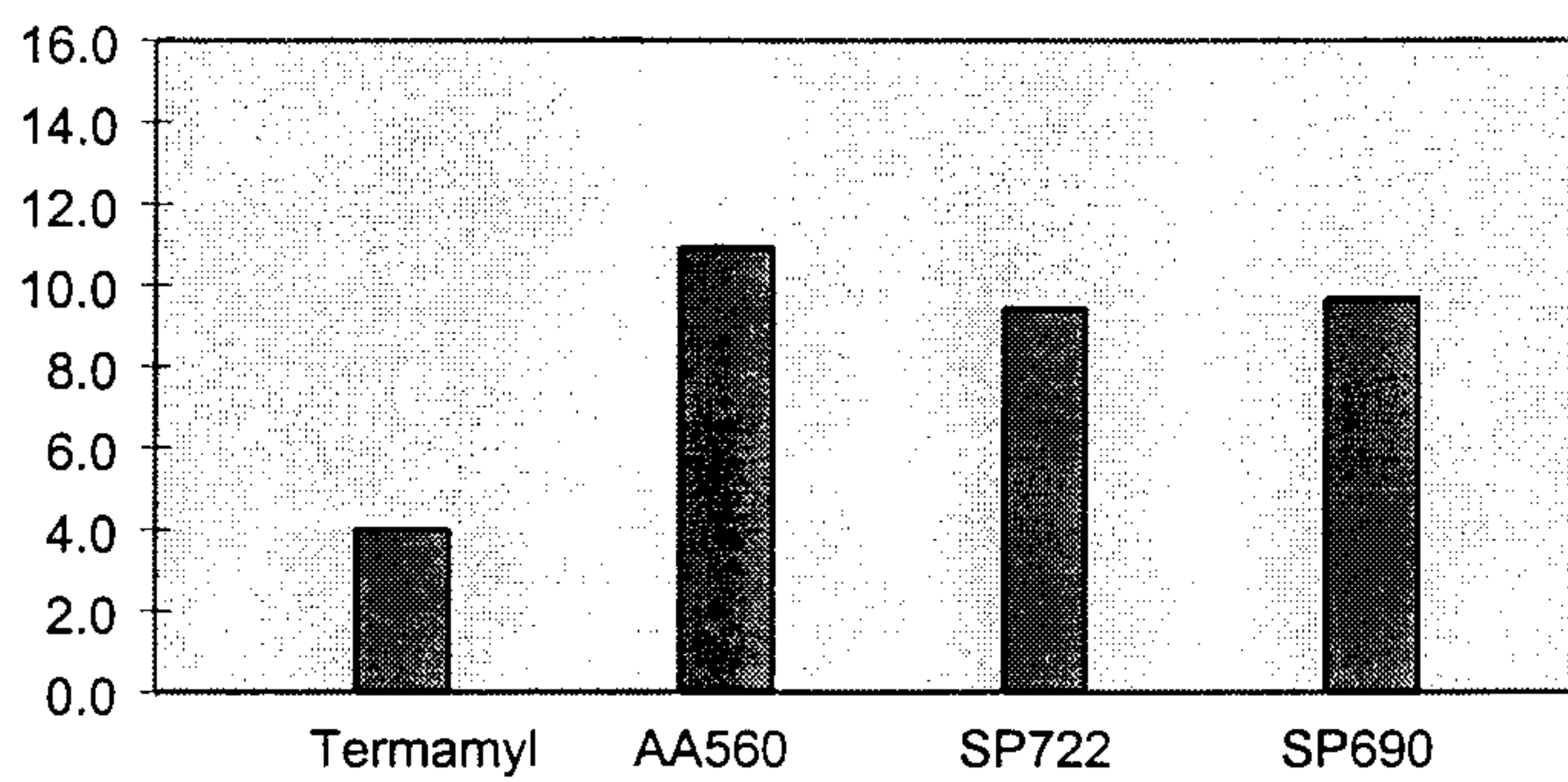


Fig. 6

8/8

Delta Remission

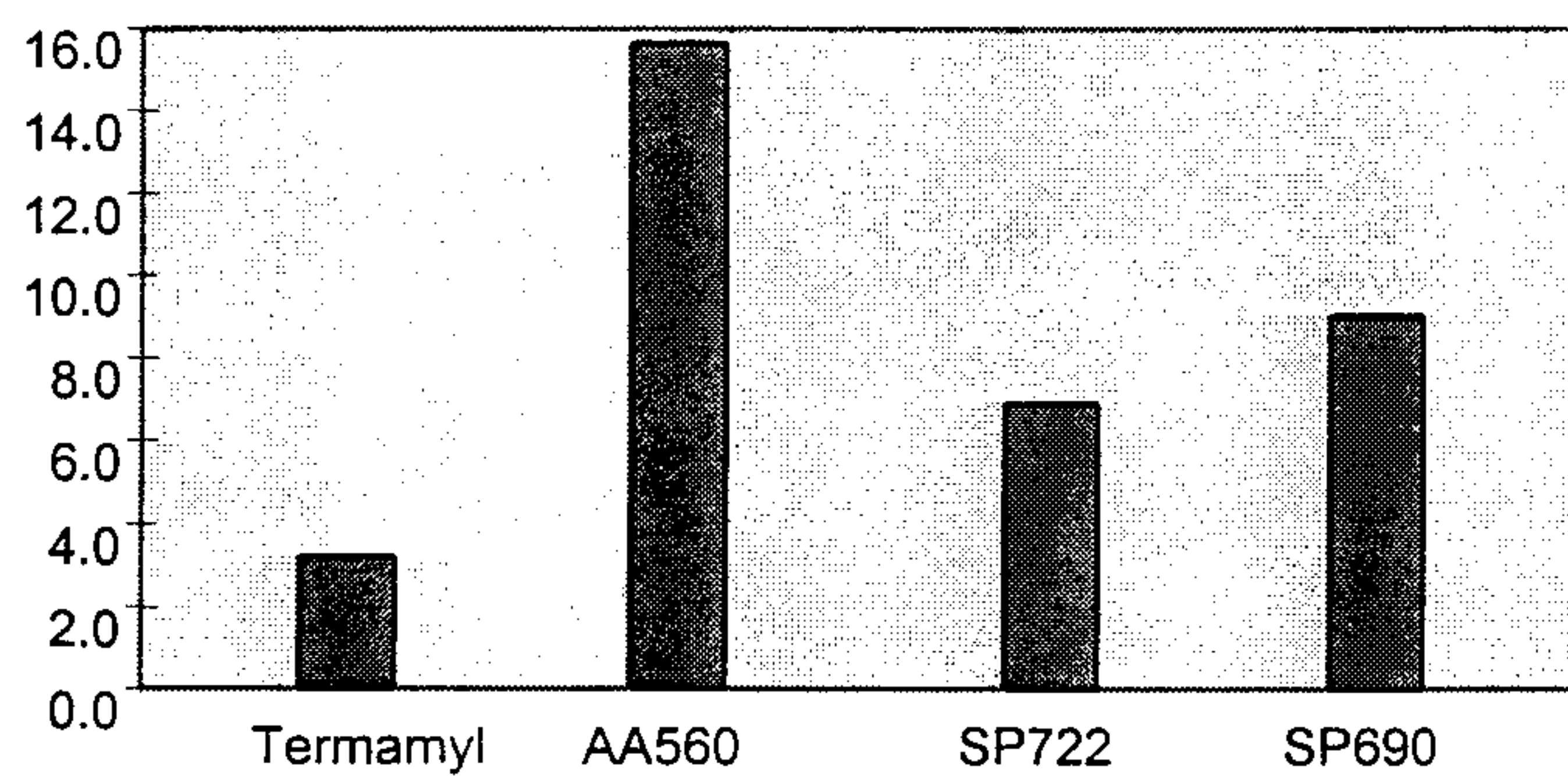


Fig. 7

SEQUENCE LISTING

5 <110> Novo Nordisk A/S

<120>

<130>

10 <160> 16

<170> PatentIn Ver. 2.1

15 <210> 1
<211> 1458
<212> DNA
<213> Bacillus sp.

20 <220>
<221> CDS
<222> (1)..(1458)

<220>

25 <221> mat_peptide
<222> (1)..(1458)

<400> 1

30 cac cat aat ggt acg aac ggc aca atg atg cag tac ttt gaa tgg tat 48
His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
1 5 10 15

cta cca aat gac gga aac cat tgg aat aga tta agg tct gat gca agt 96
Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Ser Asp Ala Ser
35 20 25 30

aac cta aaa gat aaa ggg atc tca gcg gtt tgg att cct cct gca tgg 144
Asn Leu Lys Asp Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp
40 35 40 45

aag ggt gcc tct caa aat gat gtg ggg tat ggt gct tat gat ctg tat 192
Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
50 55 60

gat tta gga gaa ttc aat caa aaa gga acc att cgt aca aaa tat gga 240
Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly
65 70 75 80

acg cgc aat cag tta caa gct gca gtt aac gcc ttg aaa agt aat gga 288
Thr Arg Asn Gln Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly
50 85 90 95

att caa gtg tat ggc gat gtt gta atg aat cat aaa ggg gga gca gac 336
Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
55 100 105 110

WO 00/60060

PCT/DK00/00149

2

	gct acc gaa atg gtt agg gca gtt gaa gta aac ccg aat aat aga aat	384
	Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn	
	115 120 125	
5	caa gaa gtg tcc ggt gaa tat aca att gag gct tgg aca aag ttt gac	432
	Gln Glu Val Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp	
	130 135 140	
10	ttt cca gga cga ggt aat act cat tca aac ttc aaa tgg aga tgg tat	480
	Phe Pro Gly Arg Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr	
	145 150 155 160	
15	cac ttt gat gga gta gat tgg gat cag tca cgt aag ctg aac aat cga	528
	His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg	
	165 170 175	
20	att tat aaa ttt aga ggt gat gga aaa ggg tgg gat tgg gaa gtc gat	576
	Ile Tyr Lys Phe Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp	
	180 185 190	
25	aca gaa aac ggt aac tat gat tac cta atg tat gca gat att gac atg	624
	Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met	
	195 200 205	
30	gat cac cca gag gta gtg aat gag cta aga aat tgg ggt gtt tgg tat	672
	Asp His Pro Glu Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr	
	210 215 220	
35	ata aaa tac agc ttt act cgt gat tgg att aat cat gtt aga agt gca	720
	Ile Lys Tyr Ser Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala	
	245 250 255	
40	act ggc aaa aat atg ttt gcg gtt gcg gaa ttt tgg aaa aat gat tta	816
	Thr Gly Lys Asn Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu	
	260 265 270	
45	ggt gct att gaa aac tat tta aac aaa aca aac tgg aac cat tca gtc	864
	Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val	
	275 280 285	
50	ttt gat gtt ccg ctg cac tat aac ctc tat aat gct tca aaa agc gga	912
	Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Lys Ser Gly	
	290 295 300	
55	ggg aat tat gat atg agg caa ata ttt aat ggt aca gtc gtg caa aga	960
	Gly Asn Tyr Asp Met Arg Gln Ile Phe Asn Gly Thr Val Val Gln Arg	
	305 310 315 320	
55	cat cca atg cat gct gtt aca ttt gtt gat aat cat gat tcg caa cct	1008
	His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro	

3

				325					330					335			
5																	
10																	
15																	
20																	
25																	
30																	
35																	
40																	
45																	
50																	
55																	

<210> 2
 <211> 485
 <212> PRT
 <213> Bacillus sp.

<400> 2
 His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
 1 5 10 15
 Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Ser Asp Ala Ser
 20 25 30

WO 00/60060

PCT/DK00/00149

4

	Asn	Leu	Lys	Asp	Lys	Gly	Ile	Ser	Ala	Val	Trp	Ile	Pro	Pro	Ala	Trp
			35					40					45			
5	Lys	Gly	Ala	Ser	Gln	Asn	Asp	Val	Gly	Tyr	Gly	Ala	Tyr	Asp	Leu	Tyr
		50					55					60				
	Asp	Leu	Gly	Glu	Phe	Asn	Gln	Lys	Gly	Thr	Ile	Arg	Thr	Lys	Tyr	Gly
	65					70					75					80
10	Thr	Arg	Asn	Gln	Leu	Gln	Ala	Ala	Val	Asn	Ala	Leu	Lys	Ser	Asn	Gly
					85						90				95	
	Ile	Gln	Val	Tyr	Gly	Asp	Val	Val	Met	Asn	His	Lys	Gly	Gly	Ala	Asp
15				100					105					110		
	Ala	Thr	Glu	Met	Val	Arg	Ala	Val	Glu	Val	Asn	Pro	Asn	Asn	Arg	Asn
			115						120					125		
	Gln	Glu	Val	Ser	Gly	Glu	Tyr	Thr	Ile	Glu	Ala	Trp	Thr	Lys	Phe	Asp
20		130					135					140				
	Phe	Pro	Gly	Arg	Gly	Asn	Thr	His	Ser	Asn	Phe	Lys	Trp	Arg	Trp	Tyr
	145					150					155					160
25	His	Phe	Asp	Gly	Val	Asp	Trp	Asp	Gln	Ser	Arg	Lys	Leu	Asn	Asn	Arg
					165					170						175
	Ile	Tyr	Lys	Phe	Arg	Gly	Asp	Gly	Lys	Gly	Trp	Asp	Trp	Glu	Val	Asp
30				180					185					190		
	Thr	Glu	Asn	Gly	Asn	Tyr	Asp	Tyr	Leu	Met	Tyr	Ala	Asp	Ile	Asp	Met
			195					200					205			
	Asp	His	Pro	Glu	Val	Val	Asn	Glu	Leu	Arg	Asn	Trp	Gly	Val	Trp	Tyr
35		210					215					220				
	Thr	Asn	Thr	Leu	Gly	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His
	225					230					235					240
40	Ile	Lys	Tyr	Ser	Phe	Thr	Arg	Asp	Trp	Ile	Asn	His	Val	Arg	Ser	Ala
					245					250						255
	Thr	Gly	Lys	Asn	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu
45				260					265					270		
	Gly	Ala	Ile	Glu	Asn	Tyr	Leu	Asn	Lys	Thr	Asn	Trp	Asn	His	Ser	Val
			275					280					285			
	Phe	Asp	Val	Pro	Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Lys	Ser	Gly
50		290					295					300				
	Gly	Asn	Tyr	Asp	Met	Arg	Gln	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg
	305					310					315					320
55	His	Pro	Met	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro

WO 00/60060

PCT/DK00/00149

5

				325						330					335		
	Glu	Glu	Ala	Leu	Glu	Ser	Phe	Val	Glu	Glu	Trp	Phe	Lys	Pro	Leu	Ala	
				340					345					350			
5																	
	Tyr	Ala	Leu	Thr	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	
			355					360					365				
10	Gly	Asp	Tyr	Tyr	Gly	Ile	Pro	Thr	His	Gly	Val	Pro	Ala	Met	Lys	Ser	
		370					375					380					
	Lys	Ile	Asp	Pro	Ile	Leu	Glu	Ala	Arg	Gln	Lys	Tyr	Ala	Tyr	Gly	Arg	
	385					390					395					400	
15	Gln	Asn	Asp	Tyr	Leu	Asp	His	His	Asn	Ile	Ile	Gly	Trp	Thr	Arg	Glu	
					405					410					415		
	Gly	Asn	Thr	Ala	His	Pro	Asn	Ser	Gly	Leu	Ala	Thr	Ile	Met	Ser	Asp	
				420					425					430			
20																	
	Gly	Ala	Gly	Gly	Asn	Lys	Trp	Met	Phe	Val	Gly	Arg	Asn	Lys	Ala	Gly	
			435					440					445				
	Gln	Val	Trp	Thr	Asp	Ile	Thr	Gly	Asn	Arg	Ala	Gly	Thr	Val	Thr	Ile	
25		450					455					460					
	Asn	Ala	Asp	Gly	Trp	Gly	Asn	Phe	Ser	Val	Asn	Gly	Gly	Ser	Val	Ser	
	465					470					475					480	
30	Ile	Trp	Val	Asn	Lys												
					485												
35	<210>	3															
	<211>	1458															
	<212>	DNA															
	<213>	Bacillus sp.															
40	<220>																
	<221>	CDS															
	<222>	(1)..(1458)															
	<220>																
45	<221>	mat_peptide															
	<222>	(1)..(1458)															
	<400>	3															
	cac	cat	aat	ggt	acg	aac	ggc	aca	atg	atg	cag	tac	ttt	gaa	tgg	tat	48
50	His	His	Asn	Gly	Thr	Asn	Gly	Thr	Met	Met	Gln	Tyr	Phe	Glu	Trp	Tyr	
	1				5					10					15		
	cta	cca	aat	gac	gga	aac	cat	tgg	aat	aga	tta	agg	tct	gat	gca	agt	96
	Leu	Pro	Asn	Asp	Gly	Asn	His	Trp	Asn	Arg	Leu	Arg	Ser	Asp	Ala	Ser	
55				20					25					30			

6

	aac cta aaa gat aaa ggg atc tca gcg gtt tgg att cct cct gca tgg	144
	Asn Leu Lys Asp Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp	
	35 40 45	
5	aag ggt gcc tct caa aat gat gtg ggg tat ggt gct tat gat ctg tat	192
	Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr	
	50 55 60	
10	gat tta gga gaa ttc aat caa aaa gga acc att cgt aca aaa tat gga	240
	Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly	
	65 70 75 80	
15	acg cgc aat cag tta caa gct gca gtt aac gcc ttg aaa agt aat gga	288
	Thr Arg Asn Gln Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly	
	85 90 95	
20	att caa gtg tat ggc gat gtt gta atg aat cat aaa ggg gga gca gac	336
	Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp	
	100 105 110	
25	gct acc gaa atg gtt agg gcg gtt gaa gta aac ccg aat aat aga aat	384
	Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn	
	115 120 125	
30	caa gaa gtg tcc ggt gaa tat aca att gag gct tgg aca aag ttt gac	432
	Gln Glu Val Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp	
	130 135 140	
35	ttt cct gga cga ggt aat acc cat tca aac ttc aaa tgg aga tgg tat	480
	Phe Pro Gly Arg Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr	
	145 150 155 160	
40	cac ttt gat gga gta gat tgg gat cag tca cgt aag ctg aac aat cga	528
	His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg	
	165 170 175	
45	att tat aaa ttt aga ggt gat gga aaa ggg tgg gat tgg gaa gtc gat	576
	Ile Tyr Lys Phe Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp	
	180 185 190	
50	aca gaa aac ggt aac tat gat tac cta atg tat gca gat att gac atg	624
	Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met	
	195 200 205	
55	gat cac cca gag gta gtg aat gag cta aga aat tgg ggt gtt tgg tat	672
	Asp His Pro Glu Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr	
	210 215 220	
60	acg aat aca tta ggc ctt gat ggt ttt aga ata gat gca gta aaa cat	720
	Thr Asn Thr Leu Gly Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His	
	225 230 235 240	
65	ata aaa tac agc ttt act cgt gat tgg atc aat cat gtt aga agt gca	768
	Ile Lys Tyr Ser Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala	

7

				245					250					255			
	act	ggc	aaa	aat	atg	ttt	gcg	gtt	gcg	gaa	ttt	tgg	aaa	aat	gat	tta	816
	Thr	Gly	Lys	Asn	Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	
5				260					265					270			
	ggg	aat	tat	gat	atg	agg	caa	ata	ttt	aat	ggg	aca	gtc	gtg	caa	aga	960
	Gly	Asn	Tyr	Asp	Met	Arg	Gln	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg	
	305					310					315					320	
	cat	cca	atg	cat	gct	gtt	aca	ttt	gtt	gat	aat	cat	gat	tcg	caa	cct	1008
	His	Pro	Met	His	Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	
20				325						330				335			
	gaa	gaa	gct	tta	gag	tct	ttt	gtt	gaa	gaa	tgg	ttc	aaa	cca	tta	gcg	1056
	Glu	Glu	Ala	Leu	Glu	Ser	Phe	Val	Glu	Glu	Trp	Phe	Lys	Pro	Leu	Ala	
25				340					345					350			
	tat	gct	ttg	aca	tta	aca	cgt	gaa	caa	ggc	tac	cct	tct	gta	ttt	tat	1104
	Tyr	Ala	Leu	Thr	Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	
				355				360					365				
30																	
	gga	gat	tat	tat	ggc	att	cca	acg	cat	ggg	gta	cca	gcg	atg	aaa	tcg	1152
	Gly	Asp	Tyr	Tyr	Gly	Ile	Pro	Thr	His	Gly	Val	Pro	Ala	Met	Lys	Ser	
	370						375					380					
	aaa	att	gac	ccg	att	cta	gaa	gcg	cgt	caa	aag	tat	gca	tat	gga	aga	1200
	Lys	Ile	Asp	Pro	Ile	Leu	Glu	Ala	Arg	Gln	Lys	Tyr	Ala	Tyr	Gly	Arg	
	385					390					395					400	
	caa	aat	gac	tac	tta	gac	cat	cat	aat	atc	att	ggg	tgg	aca	cgt	gaa	1248
	Gln	Asn	Asp	Tyr	Leu	Asp	His	His	Asn	Ile	Ile	Gly	Trp	Thr	Arg	Glu	
40					405					410					415		
	ggg	aat	aca	gca	cac	ccc	aac	tct	ggg	tta	gct	act	atc	atg	tcc	gat	1296
	Gly	Asn	Thr	Ala	His	Pro	Asn	Ser	Gly	Leu	Ala	Thr	Ile	Met	Ser	Asp	
				420					425					430			
45																	
	gga	gca	gga	gga	aat	aag	tgg	atg	ttt	gtt	ggg	cgt	aat	aaa	gct	ggg	1344
	Gly	Ala	Gly	Gly	Asn	Lys	Trp	Met	Phe	Val	Gly	Arg	Asn	Lys	Ala	Gly	
				435				440					445				
50																	
	caa	ggt	tgg	acc	gat	atc	act	gga	aat	cgt	gca	ggg	act	ggt	acg	att	1392
	Gln	Val	Trp	Thr	Asp	Ile	Thr	Gly	Asn	Arg	Ala	Gly	Thr	Val	Thr	Ile	
				450				455				460					
55																	
	aat	gct	gat	gga	tgg	ggg	aat	ttt	tct	gta	aat	gga	gga	tca	ggt	tct	1440

WO 00/60060

PCT/DK00/00149

8

Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480

att tgg gta aac aaa taa 1458
 5 Ile Trp Val Asn Lys
 485

<210> 4
 10 <211> 485
 <212> PRT
 <213> Bacillus sp.

<400> 4
 15 His His Asn Gly Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr
 1 5 10 15

Leu Pro Asn Asp Gly Asn His Trp Asn Arg Leu Arg Ser Asp Ala Ser
 20 20 25 30

Asn Leu Lys Asp Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp
 25 35 40 45

Lys Gly Ala Ser Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr
 25 50 55 60

Asp Leu Gly Glu Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly
 65 70 75 80

30 Thr Arg Asn Gln Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly
 85 90 95

Ile Gln Val Tyr Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp
 35 100 105 110

Ala Thr Glu Met Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn
 115 120 125

40 Gln Glu Val Ser Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp
 130 135 140

Phe Pro Gly Arg Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr
 145 150 155 160

45 His Phe Asp Gly Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg
 165 170 175

Ile Tyr Lys Phe Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp
 50 180 185 190

Thr Glu Asn Gly Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met
 195 200 205

55 Asp His Pro Glu Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr
 210 215 220

Thr Asn Thr Leu Gly Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His
 225 230 235 240
 5 Ile Lys Tyr Ser Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala
 245 250 255
 Thr Gly Lys Asn Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu
 260 265 270
 10 Gly Ala Ile Glu Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val
 275 280 285
 Phe Asp Val Pro Leu His Tyr Asn Leu Tyr Asn Ala Ser Lys Ser Gly
 15 290 295 300
 Gly Asn Tyr Asp Met Arg Gln Ile Phe Asn Gly Thr Val Val Gln Arg
 305 310 315 320
 20 His Pro Met His Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro
 325 330 335
 Glu Glu Ala Leu Glu Ser Phe Val Glu Glu Trp Phe Lys Pro Leu Ala
 340 345 350
 25 Tyr Ala Leu Thr Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr
 355 360 365
 Gly Asp Tyr Tyr Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser
 30 370 375 380
 Lys Ile Asp Pro Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Arg
 385 390 395 400
 35 Gln Asn Asp Tyr Leu Asp His His Asn Ile Ile Gly Trp Thr Arg Glu
 405 410 415
 Gly Asn Thr Ala His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp
 420 425 430
 40 Gly Ala Gly Gly Asn Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly
 435 440 445
 Gln Val Trp Thr Asp Ile Thr Gly Asn Arg Ala Gly Thr Val Thr Ile
 45 450 455 460
 Asn Ala Asp Gly Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser
 465 470 475 480
 50 Ile Trp Val Asn Lys
 485
 55 <210> 5

10

<211> 7
<212> PRT
<213> Artificial Sequence

5 <220>
<221> PEPTIDE
<222> (1)..(9)

<220>
10 <223> Description of Artificial Sequence: degenerated
Primer region

<400> 5
Gly Ile Thr Ala Xaa Trp Xaa
15 1 5

<210> 6
20 <211> 9
<212> PRT
<213> Artificial Sequence

<220>
25 <221> PEPTIDE
<222> (1)..(9)

<220>
30 <223> Description of Artificial Sequence: degenerated
Primer region

<400> 6
Val Tyr Xaa Asp Xaa Val Xaa Asn His
35 1 5

<210> 7
40 <211> 10
<212> PRT
<213> Artificial Sequence

<220>
45 <223> Description of Artificial Sequence: degenerated
primer region

<220>
<221> PEPTIDE
<222> (1)..(10)

50 <400> 7
Asp Gly Xaa Arg Xaa Asp Ala Xaa Lys His
1 5 10

55

WO 00/60060

PCT/DK00/00149

11

<210> 8
<211> 10
<212> PRT
5 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: degenerated
primer region
10

<220>
<221> PEPTIDE
<222> (1)..(10)

15 <400> 8
Asp Gly Xaa Arg Xaa Asp Ala Xaa Lys His
1 5 10

20

<210> 9
<211> 8
<212> PRT
<213> Artificial Sequence

25

<220>
<223> Description of Artificial Sequence: degenerated
Primer region

30

<220>
<221> PEPTIDE
<222> (1)..(8)

35 <400> 9
Val Thr Phe Val Xaa Asn His Asp
1 5

40

<210> 10
<211> 6
<212> PRT
<213> Artificial Sequence

45

<220>
<223> Description of Artificial Sequence: degenerated
Primer region

50

<220>
<221> PEPTIDE
<222> (1)..(6)

55 <400> 10
Gly Trp Thr Arg Glu Gly
1 5

5 <210> 11
 <211> 22
 <212> DNA
 <213> Artificial Sequence

10 <220>
 <223> Description of Artificial Sequence: Primer 188(Pst-)

15 <400> 11
 ggcgттаacc gcagcttgta ac 22

20 <210> 12
 <211> 51
 <212> DNA
 <213> Artificial Sequence

25 <220>
 <223> Description of Artificial Sequence: Primer 188cloningC

30 <400> 12
 ccgagctcgg ccggctgggc cgtcgactta ttgtttacc caaatagaaa c 51

35 <210> 13
 <211> 37
 <212> DNA
 <213> Artificial Sequence

40 <220>
 <223> Description of Artificial Sequence: Primer
 188cloningN

45 <400> 13
 cattctgcag cagcggcgca ccataatggt acgaacg 37

50 <210> 14
 <211> 24
 <212> DNA
 <213> Artificial Sequence

55 <220>
 <223> Description of Artificial Sequence: Primer B1

<400> 14
 cgattgctga cgctgttatt tgcg 24

<210> 15
 <211> 24

13

<212> DNA
<213> Artificial Sequence

<220>

5 <223> Description of Artificial Sequence: Primer Y2

<400> 15

cttgttccct tgcagaacc aatg

24

10

<210> 16

<211> 30

<212> DNA

<213> Artificial Sequence

15

<220>

<223> Description of Artificial Sequence: Primer 101458

<400> 16

20 gtcatagttg ccgaaatctg tatcgacttc

30