

SEQUENCE LISTING

<110> Coöperatie Koninklijke Avebe U.A.

<120> Novel anti-staling enzyme, and methods, doughs and baked food products relating thereto.

<130> P124569PC00

<140> PCT/NL2022/050048

<141> 2022-02-02

<150> EP 21154734.4

<151> 2021-02-02

<160> 21

<170> PatentIn version 3.5

<210> 1

<211> 706

<212> PRT

<213> Geobacillus sp. 12AMOR1

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Tyr Ser Ser Gly Pro Glu Leu Asp Asn Arg Val Ile Phe Gln Ser Phe
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Ser Leu Tyr Gln Pro Tyr Glu Ser Asn Met Tyr Lys Ile Leu Ala Thr
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Lys Gly Asp Leu Leu Lys Glu Trp Gly Ile Thr Asp Val Trp Leu Pro
35 40 45

Pro Ala Tyr Arg Ser Phe Asn Met Ala Arg Tyr Met Glu Gly Tyr Ala
50 55 60

Ile Ala Asp Arg Tyr Asp Leu Gly Glu Phe Asn Gln Gly Pro Asn Asn
65 70 75 80

Glu Arg Ala Thr Lys Tyr Gly Thr Ser Asp Glu Leu Lys Asp Met Ile
85 90 95

Asp Lys Leu His Ala Ala Gly Leu Lys Val Gln Leu Asp Leu Val Pro
100 105 110

Asn Gln Leu Leu Gly Leu Asn Gly Arg Glu Ala Val Tyr Val Thr Arg
115 120 125

Val Asp Asn Asn Gly Asp Leu Phe Lys Asn Pro Tyr Thr Thr Gly Leu
130 135 140

Thr Thr Arg Ile Arg Ala Asp Leu Tyr Leu Ala Tyr Thr Lys Gly Gly
145 150 155 160

Gly Gln Gly Gln Ala Lys Tyr Gly Tyr Ile Lys Glu Trp Asn Lys Asn
165 170 175

Tyr Phe Asn Gly Thr Ser Leu Gln Gly Gln Gly Met Gly Arg Val Met
180 185 190

Thr Asp Asp Asn Gly Lys Pro Tyr Arg Phe Phe Gly Pro Asn Asp Pro
195 200 205

Arg Asn Tyr Leu Pro Ser Trp Leu Glu Glu Ala Ala Ala Ala Asn Lys
210 215 220

Ile Asn Thr Val Asp Thr Tyr Leu Pro Val Asp Gly Trp Tyr Ala Ala
225 230 235 240

Lys Asp Ala Ala Thr Ser Asp Gln Tyr Trp Lys Pro Met Leu Ile His
245 250 255

Tyr Ala Lys Asp Lys Gly Tyr Leu Ser Phe Met Ser Gln His Gly Phe
260 265 270

Ala Thr Val Asp Asp Ile Ile Asn Gly Asp Asn Ala Glu Ile Ala Lys
275 280 285

Trp Thr Asn Ala Tyr Ile Gln Ser Arg Pro Glu Tyr Gly Phe Gly Ser
290 295 300

Glu Glu Arg Ser Tyr Lys Asn Asp Asn Thr Gly Val Asp Asp Gln Asp
305 310 315 320

Gln Phe Leu Phe Val Glu Glu Asn Gly Ser Thr Lys His Asn Ile His
325 330 335

Asn Thr Ile His Gly Asn Tyr Glu Phe Leu Val Gly Leu Asp Ile Asp
340 345 350

Asn Ser Asn Pro Thr Val Arg Lys Glu Gln Ile His Trp Met Asn Trp
355 360 365

Leu Leu Asp Thr Tyr Lys Phe Asp Gly Phe Arg Ile Asp Ala Ala Thr
370 375 380

His Phe Asp Lys Gln Val Leu Leu Asp Glu Ala Asp Val Arg Lys Ala
385 390 395 400

His Phe Gly Asn Asp Leu Asn Asn His Leu Ser Tyr Ile Glu Ser Tyr
405 410 415

Thr Ser Lys Ala Glu Lys Phe Glu Asn Glu Asn Gly Asn Pro His Leu
420 425 430

Thr Met Asp Trp Ala Leu Tyr Tyr Thr Leu Gln Asp Thr Leu Gly Lys
435 440 445

Gly Thr Pro Ser Gln Lys Leu Ser Thr Ile Ala Thr Asn Ser Val Val
450 455 460

Asn Arg Ser Gly Ser Gly Ser Ala His Ala Ile Pro Asn Trp Ser Phe
465 470 475 480

Val Asn Asn His Asp Gln Glu Lys Asn Arg Val Asn Thr Ile Met Leu
485 490 495

Asp Leu Tyr Gly Ile Lys Thr Gly Glu Lys Tyr Thr Thr Thr Pro Pro
500 505 510

Lys Ser Phe Ala Asp Leu Tyr Asp Lys Glu Thr Glu Lys Lys Ala Leu
515 520 525

Ala Ile Tyr Lys Asp Asp Met Lys Arg Val Asp Lys Lys Tyr Ala Pro
530 535 540

Asn Asn Val Val Ser Gln Tyr Ala Phe Leu Leu Thr Asn Lys Asp Thr
545 550 555 560

Val Pro Thr Ile Tyr Tyr Gly Asp Leu Tyr Gln Thr Asp Ala Ser Tyr
565 570 575

Met Ser Lys Pro Thr Leu Tyr Tyr Glu Pro Ile Thr Lys Leu Leu Lys
580 585 590

Met Arg Lys Ala Tyr Ala Tyr Gly Gly Gln Lys Ile Thr Gly Tyr Thr
595 600 605

Ser Asn Thr Ser Pro Glu Thr Ala Gly Gln Asp Leu Ile Ala Ser Val
610 615 620

Arg Tyr Gly Lys Asp Arg Tyr Thr Gly Val Ala Val Val Ile Gly Thr
625 630 635 640

Asn Pro Lys Thr Asp Thr Thr Ile Lys Val Asp Met Gly Thr Lys His
645 650 655

Ala Asn Gln Val Phe Lys Asp Ala Thr Gly Phe His Ser Glu Lys Leu
660 665 670

Val Ala Asp Asn Lys Gly Val Leu Thr Ile Arg Val Lys Gly Thr Ala
675 680 685

Asn Ala Leu Val Lys Gly Tyr Leu Gly Val Trp Val Pro Thr Lys Asp
690 695 700

Lys Ala
705

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<212> PRT
<213> Geobacillus sp. 12AMOR1

<400> 2

Met Ile Lys Lys Tyr Val His Arg Thr Val Ala Leu Ala Val Ala Leu
1 5 10 15

Leu Ile Ile Phe Gly Gln Ile Gly Val Phe Pro Gly Lys Ala His Ala
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Tyr Ser Ser Gly Pro Glu Leu Asp Asn Arg Val Ile Phe Gln Ser Phe
35 40 45

Ser Leu Tyr Gln Pro Tyr Glu Ser Asn Met Tyr Lys Ile Leu Ala Thr
50 55 60

Lys Gly Asp Leu Leu Lys Glu Trp Gly Ile Thr Asp Val Trp Leu Pro
65 70 75 80

Pro Ala Tyr Arg Ser Phe Asn Met Ala Arg Tyr Met Glu Gly Tyr Ala
85 90 95

Ile Ala Asp Arg Tyr Asp Leu Gly Glu Phe Asn Gln Gly Pro Asn Asn
100 105 110

Glu Arg Ala Thr Lys Tyr Gly Thr Ser Asp Glu Leu Lys Asp Met Ile
115 120 125

Asp Lys Leu His Ala Ala Gly Leu Lys Val Gln Leu Asp Leu Val Pro
130 135 140

Asn Gln Leu Leu Gly Leu Asn Gly Arg Glu Ala Val Tyr Val Thr Arg
145 150 155 160

Val Asp Asn Asn Gly Asp Leu Phe Lys Asn Pro Tyr Thr Thr Gly Leu
165 170 175

Thr Thr Arg Ile Arg Ala Asp Leu Tyr Leu Ala Tyr Thr Lys Gly Gly
180 185 190

Gly Gln Gly Gln Ala Lys Tyr Gly Tyr Ile Lys Glu Trp Asn Lys Asn
195 200 205

Tyr Phe Asn Gly Thr Ser Leu Gln Gly Gln Gly Met Gly Arg Val Met
210 215 220

Thr Asp Asp Asn Gly Lys Pro Tyr Arg Phe Phe Gly Pro Asn Asp Pro

225		230		235		240									
Arg	Asn	Tyr	Leu	Pro	Ser	Trp	Leu	Glu	Glu	Ala	Ala	Ala	Ala	Asn	Lys
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Ile	Asn	Thr	Val	Asp	Thr	Tyr	Leu	Pro	Val	Asp	Gly	Trp	Tyr	Ala	Ala
			260				265						270		
Lys	Asp	Ala	Ala	Thr	Ser	Asp	Gln	Tyr	Trp	Lys	Pro	Met	Leu	Ile	His
		275					280					285			
Tyr	Ala	Lys	Asp	Lys	Gly	Tyr	Leu	Ser	Phe	Met	Ser	Gln	His	Gly	Phe
	290					295					300				
Ala	Thr	Val	Asp	Asp	Ile	Ile	Asn	Gly	Asp	Asn	Ala	Glu	Ile	Ala	Lys
305					310					315					320
Trp	Thr	Asn	Ala	Tyr	Ile	Gln	Ser	Arg	Pro	Glu	Tyr	Gly	Phe	Gly	Ser
			325						330					335	
Glu	Glu	Arg	Ser	Tyr	Lys	Asn	Asp	Asn	Thr	Gly	Val	Asp	Asp	Gln	Asp
			340					345					350		
Gln	Phe	Leu	Phe	Val	Glu	Glu	Asn	Gly	Ser	Thr	Lys	His	Asn	Ile	His
		355					360					365			
Asn	Thr	Ile	His	Gly	Asn	Tyr	Glu	Phe	Leu	Val	Gly	Leu	Asp	Ile	Asp
	370					375					380				
Asn	Ser	Asn	Pro	Thr	Val	Arg	Lys	Glu	Gln	Ile	His	Trp	Met	Asn	Trp
385					390					395					400
Leu	Leu	Asp	Thr	Tyr	Lys	Phe	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Ala	Thr
			405						410					415	
His	Phe	Asp	Lys	Gln	Val	Leu	Leu	Asp	Glu	Ala	Asp	Val	Arg	Lys	Ala
			420					425					430		
His	Phe	Gly	Asn	Asp	Leu	Asn	Asn	His	Leu	Ser	Tyr	Ile	Glu	Ser	Tyr
		435					440					445			

Thr Ser Lys Ala Glu Lys Phe Glu Asn Glu Asn Gly Asn Pro His Leu
450 455 460

Thr Met Asp Trp Ala Leu Tyr Tyr Thr Leu Gln Asp Thr Leu Gly Lys
465 470 475 480

Gly Thr Pro Ser Gln Lys Leu Ser Thr Ile Ala Thr Asn Ser Val Val
485 490 495

Asn Arg Ser Gly Ser Gly Ser Ala His Ala Ile Pro Asn Trp Ser Phe
500 505 510

Val Asn Asn His Asp Gln Glu Lys Asn Arg Val Asn Thr Ile Met Leu
515 520 525

Asp Leu Tyr Gly Ile Lys Thr Gly Glu Lys Tyr Thr Thr Thr Pro Pro
530 535 540

Lys Ser Phe Ala Asp Leu Tyr Asp Lys Glu Thr Glu Lys Lys Ala Leu
545 550 555 560

Ala Ile Tyr Lys Asp Asp Met Lys Arg Val Asp Lys Lys Tyr Ala Pro
565 570 575

Asn Asn Val Val Ser Gln Tyr Ala Phe Leu Leu Thr Asn Lys Asp Thr
580 585 590

Val Pro Thr Ile Tyr Tyr Gly Asp Leu Tyr Gln Thr Asp Ala Ser Tyr
595 600 605

Met Ser Lys Pro Thr Leu Tyr Tyr Glu Pro Ile Thr Lys Leu Leu Lys
610 615 620

Met Arg Lys Ala Tyr Ala Tyr Gly Gly Gln Lys Ile Thr Gly Tyr Thr
625 630 635 640

Ser Asn Thr Ser Pro Glu Thr Ala Gly Gln Asp Leu Ile Ala Ser Val
645 650 655

Arg Tyr Gly Lys Asp Arg Tyr Thr Gly Val Ala Val Val Ile Gly Thr

660

665

670

Asn Pro Lys Thr Asp Thr Thr Ile Lys Val Asp Met Gly Thr Lys His
 675 680 685

Ala Asn Gln Val Phe Lys Asp Ala Thr Gly Phe His Ser Glu Lys Leu
 690 695 700

Val Ala Asp Asn Lys Gly Val Leu Thr Ile Arg Val Lys Gly Thr Ala
 705 710 715 720

Asn Ala Leu Val Lys Gly Tyr Leu Gly Val Trp Val Pro Thr Lys Asp
 725 730 735

Lys Ala Pro Gly Leu Ser Trp Asn Ser Ala Lys Thr Val Tyr Gln Gly
 740 745 750

Lys Thr Val Lys Leu Ser Val Lys Leu Thr Asn Ser Ala Ser Lys Ile
 755 760 765

Lys Thr Val Thr Phe Thr Ser Ser Asn Pro Ser Ile Ala Ser Val Asp
 770 775 780

Lys Tyr Gly Asn Val Lys Gly Asn Lys Lys Thr Gly Lys Val Asn Ile
 785 790 795 800

Tyr Ala Thr Val Thr Thr Ala Asp Asn Phe Val Leu Tyr Ser Ser Lys
 805 810 815

Pro Ile Asp Val Lys Ala Asn Gln Val Thr Leu Lys Ala Asn Arg Ala
 820 825 830

Thr Val Lys Arg Gly His Ser Thr Lys Ile Gln Ile Lys Ser Ser Thr
 835 840 845

Asp Lys Ile Lys Ser Ala Ala Tyr Lys Ser Ser Asn Thr Arg Val Ala
 850 855 860

Thr Val Ser Lys Ser Gly Val Val Thr Gly Lys Arg Pro Gly Lys Ala
 865 870 875 880

Thr Ile Thr Ile Tyr Tyr Lys Thr Gln Gly Gly Tyr Thr Val Lys Lys
885 890 895

Tyr Phe Ala Val Thr Val Arg
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<213> Arti f i c i a l Sequence

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<223> Moti f I

<220>
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<222> (1) . (1)
<223> X i s M, E or L

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<210> 4
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<220>
<223> Moti f I

<400> 4

Leu Asp Leu Val Pro Asn Gln
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<210> 5
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<223> X is S, G, or T

<220>
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<223> X is Y or F

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Gly Phe Arg Ile Asp Ala Ala Xaa His Xaa Asp
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<211> 11
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<213> Arti f i c i a l Sequence

<220>
<223> Moti f I I

<400> 6

Gly Phe Arg Ile Asp Ala Ala Thr His Phe Asp
1 5 10

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<213> Arti f i c i a l Sequence

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<223> X is S or N

<220>
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<223> X is E, V, A or K

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

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<400> 8

His Leu Ser Tyr Ile Glu Ser Tyr Thr Ser Lys
1 5 10

<210> 9
<211> 14
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<223> X is V or I

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<223> X is Q, N or T

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Phe Val Xaa Asn His Asp Gln Glu Lys Asn Arg Xaa Asn Xaa
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<210> 10
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<223> Motif IV

<400> 10

Phe Val Asn Asn His Asp Gln Glu Lys Asn Arg Val Asn Thr
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<210> 11

<211> 20

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<223> N-terminal His-tag sequence

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Met Ala His His His His His His Ser Ala Ala Leu Glu Val Leu Phe
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Gln Gly Pro Gly
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<210> 12

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<212> DNA

<213> Artificial Sequence

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<223> primer Fw1AMOR

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33

<210> 13

<211> 33

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<223> primer Fw33AMOR

<400> 13

cagggacccg gttatagctc cggcccggaa ttg

33

<210> 14

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<212> DNA

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 <223> primer Rv738AMOR

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 <223> primer Rv748AMOR

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 <210> 18
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 <400> 18
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