

SEQUENCE LISTING

<110> Agency for Science, Technology and Research

<120> Fusion Protein for Bacterial Surface Display

<130> S61015740

<160> 26

<170> PatentIn version 3.5

<210> 1

<211> 860

<212> PRT

<213> Artificial Sequence

<220>

<223> L. plantarum Lys2 autolysin

<400> 1

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			20					25					30		

Phe	Phe	Ser	Phe	Leu	Gly	Gly	Ser	Val	Leu	Asn	Asp	Thr	Asn	Val	His
		35					40					45			

Ala	Asp	Ala	Thr	Asn	Ala	Thr	Thr	Thr	Ser	Ser	Ser	Ala	Ala	Ser	Thr
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Ala	Ser	Ser	Ala	Ser	Val	Ala	Thr	Ser	Ser	Ala	Ala	Ser	Asp	Glu	Thr
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Thr	Lys	Ala	Thr	Ser	Val	Ser	Ser	Ser	Ala	Ala	Thr	Gln	Val	Thr	Ser
				85					90					95	

Ala	Ala	Thr	Thr	Lys	Thr	Thr	Ser	Gln	Ala	Ala	Thr	Ala	Ser	Ser	Ser
				100				105					110		

Ala Ala Thr Ser Gln Ser Thr Ser Thr Ala Ser Ser Ser Thr Ser Gln
115 120 125

Ala Thr Lys Thr Ala Ser Ser Ala Ala Thr Ser Thr Ser Ala Thr Ala
130 135 140

Thr Ser Gln Ala Thr Ser Val Ala Ser Ser Thr Ala Thr Thr Thr Gln
145 150 155 160

Val Ser His Thr Ala Ser Ser Thr Ala Ala Ser Ala Ala Ser Ser Thr
165 170 175

Ala Thr Val Lys Ala Val Lys Ala Ala Ser Ser Thr Val Ser Ser Ala
180 185 190

Ala Thr Thr Ala Thr Lys Ala Thr Ala Lys Val Ala Ala Val Ala Tyr
195 200 205

Thr Ala Ala Thr Thr Ser Ser Thr Asp Val Trp Thr Ile Gly Asp Thr
210 215 220

Thr Arg Pro Arg Val Asp Val Val Asp Val Ala Ser Tyr Gln Ser Thr
225 230 235 240

Met Thr Gln Ser Asp Phe Asn Lys Leu Lys Ala Ala Gly Val Lys Thr
245 250 255

Val Ile Val Lys Ser Thr Glu Gly Thr Asp Tyr Thr Asn Pro Ala Ala
260 265 270

Leu Asn Gln Ala Lys Met Ala Asn Lys Ala Gly Leu Asn Val Asp Phe
275 280 285

Tyr His Tyr Ala Thr Phe Ser Thr Ala Asp Ala Ala Lys Ser Glu Ala
290 295 300

Thr Asn Met Ala Gly Phe Leu Val Lys Asn Asn Val Ser Thr Lys Val
305 310 315 320

Leu Leu Phe Ala Asp Met Glu Asp Ser Ser Ser Tyr Ser Val Asn Ala
325 330 335

Thr Ala Asn Leu Asn Ala Phe Trp Ser Thr Leu Asp Ser Phe Gly Tyr
340 345 350

Lys Asn His Gly Val Tyr Thr Ser Asn Ser Tyr Leu Tyr Arg Asp Ala
355 360 365

Val Val Lys Thr Val Gly Gln Ser Arg Val Trp Arg Ala Gln Tyr Pro
370 375 380

Tyr Thr Pro Ser Ala Asn Asn Leu Trp Asn Thr Asn Asp Gly Ala Trp
385 390 395 400

Gln Phe Ser Asp Thr Ala Leu Leu Pro Ser Gly Ser Asp Tyr Thr Gly
405 410 415

Tyr Ile Asp Val Ser Ile Asp Tyr Asn Gly Leu Thr Glu Asp Ser Ala
420 425 430

Gly Thr Asn Thr Phe Val Thr Thr Thr Ser Ser Asn Asp Thr Thr Thr
435 440 445

Ser Glu Val Thr Thr Asp Thr Ser Ala Thr Thr Asn Thr Ser Thr Ser
450 455 460

Ser Thr Thr Lys Lys Ala Ser Gly Trp Tyr Thr Phe Thr Lys Asn Thr
465 470 475 480

Ala Ile Lys Ser Ala Ala Ser Asp Ser Ala Lys Thr Val Gly Thr Tyr
485 490 495

Ser Lys Gly Asn Arg Val Tyr Tyr Asn Ala Glu Ile Thr Thr Asn Gly
500 505 510

Glu Thr Trp Leu Arg Tyr Leu Ser Tyr Ser Gly Ser Glu His Phe Val
515 520 525

Lys Ile Ala Ala Ala Lys Thr Thr Thr Thr Lys Pro Ala Ala Ser Thr
530 535 540

Ser Lys Thr Val Thr Lys Asn Glu Thr Gly Thr Tyr Lys Phe Thr Lys
545 550 555 560

Thr Thr Ala Ile Lys Gly Ser Val Ser Asp Ser Ala Lys Thr Leu Gly
565 570 575

Thr Tyr Tyr Lys Gly Asp Thr Val Tyr Tyr Asn Ala Lys Val Thr Lys
580 585 590

Asn Gly Glu Thr Trp Leu Arg Tyr Leu Ser Tyr Ser Gly Ala Gln His
595 600 605

Tyr Val Lys Ile Ser Gly Ala Ala Thr Ser Thr Thr Thr Thr Lys Pro
610 615 620

Ala Thr Ser Ser Ser Lys Thr Val Thr Lys Ala Glu Thr Gly Thr Tyr
625 630 635 640

Lys Phe Thr Gly Thr Thr Ala Ile Lys Gly Ser Val Asn Asp Ser Ala
645 650 655

Lys Thr Leu Gly Thr Tyr Tyr Lys Gly Asp Thr Val Tyr Tyr Asn Ala
660 665 670

Lys Val Thr Lys Asn Gly Gln Thr Trp Leu Arg Tyr Leu Ser Tyr Ser
675 680 685

Gly Ala Gln His Tyr Val Lys Ile Ser Gly Ala Ala Thr Thr Thr Thr
690 695 700

Thr Ser Ser Lys Ser Thr Ala Thr Ala Ser Ala Lys Thr Val Ala Gln
705 710 715 720

Ser Gly Thr Tyr Lys Phe Ala Lys Thr Thr Ala Ile Lys Ser Ser Ala
725 730 735

Ser Asp Ala Ala Ser Thr Val Gly Thr Tyr Tyr Lys Gly Asn Thr Val
740 745 750

Asn Tyr Asn Ala Lys Val Thr Thr Asn Gly Gln Thr Trp Leu Arg Tyr
755 760 765

Thr Ser Tyr Ser Gly Ala Gln His Tyr Val Lys Val Ser Gly Gly Ala
770 775 780

Ala Thr Thr Thr Ser Ser Ser Ala Ser Lys Thr Thr Ala Ala Ala Gly
785 790 795 800

Thr Tyr Thr Phe Lys Thr Thr Thr Asn Ile Arg Thr Ala Ala Ser Leu
805 810 815

Asn Ala Ser Val Val Gly Gln Tyr Ser Ala Gly Glu Ser Val Tyr Tyr
820 825 830

Ile Gly Thr Val Ser Ala Asp Gly Tyr Gln Trp Leu Lys Tyr Leu Ser
835 840 845

Asn Ser Gly Ala Tyr His Tyr Val Ala Lys Ile Gly
850 855 860

<210> 2
<211> 82
<212> PRT
<213> Artificial Sequence

<220>
<223> R1 of Lys2 CWT

<400> 2

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

Leu Ser Tyr Ser Gly Ser Glu His Phe Val Lys Ile Ala Ala Ala Lys
50 55 60

Thr Thr Thr Thr Lys Pro Ala Ala Ser Thr Ser Lys Thr Val Thr Lys
65 70 75 80

Asn Glu

<210> 3

<211> 84

<212> PRT

<213> Artificial Sequence

<220>

<223> R2 of Lys2 CWT (SEQ ID NO: 3)

<400> 3

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Ser Asp Ser Ala Lys Thr Leu Gly Thr Tyr Tyr Lys Gly Asp Thr Val
20 25 30

Tyr Tyr Asn Ala Lys Val Thr Lys Asn Gly Glu Thr Trp Leu Arg Tyr
35 40 45

Leu Ser Tyr Ser Gly Ala Gln His Tyr Val Lys Ile Ser Gly Ala Ala
50 55 60

Thr Ser Thr Thr Thr Thr Lys Pro Ala Thr Ser Ser Ser Lys Thr Val
65 70 75 80

Thr Lys Ala Glu

<210> 4
<211> 84
<212> PRT
<213> Artificial Sequence

<220>
<223> R3 of Lys2 CWT

<400> 4

Thr Gly Thr Tyr Lys Phe Thr Gly Thr Thr Ala Ile Lys Gly Ser Val
1 5 10 15

Asn Asp Ser Ala Lys Thr Leu Gly Thr Tyr Tyr Lys Gly Asp Thr Val
20 25 30

Tyr Tyr Asn Ala Lys Val Thr Lys Asn Gly Gln Thr Trp Leu Arg Tyr
35 40 45

Leu Ser Tyr Ser Gly Ala Gln His Tyr Val Lys Ile Ser Gly Ala Ala
50 55 60

Thr Thr Thr Thr Thr Ser Ser Lys Ser Thr Ala Thr Ala Ser Ala Lys
65 70 75 80

Thr Val Ala Gln

<210> 5
<211> 79
<212> PRT
<213> Artificial Sequence

<220>
<223> R4 of Lys2 CWT

<400> 5

Ser Gly Thr Tyr Lys Phe Ala Lys Thr Thr Ala Ile Lys Ser Ser Ala
1 5 10 15

Ser Asp Ala Ala Ser Thr Val Gly Thr Tyr Tyr Lys Gly Asn Thr Val
20 25 30

Asn Tyr Asn Ala Lys Val Thr Thr Asn Gly Gln Thr Trp Leu Arg Tyr
35 40 45

Thr Ser Tyr Ser Gly Ala Gln His Tyr Val Lys Val Ser Gly Gly Ala
50 55 60

Ala Thr Thr Thr Ser Ser Ser Ala Ser Lys Thr Thr Ala Ala Ala
65 70 75

<210> 6
<211> 61
<212> PRT
<213> Artificial Sequence

<220>
<223> R5 of Lys2 CWT

<400> 6

Gly Thr Tyr Thr Phe Lys Thr Thr Thr Asn Ile Arg Thr Ala Ala Ser
1 5 10 15

Leu Asn Ala Ser Val Val Gly Gln Tyr Ser Ala Gly Glu Ser Val Tyr
20 25 30

Tyr Ile Gly Thr Val Ser Ala Asp Gly Tyr Gln Trp Leu Lys Tyr Leu
35 40 45

Ser Asn Ser Gly Ala Tyr His Tyr Val Ala Lys Ile Gly
50 55 60

<210> 7
<211> 228
<212> PRT
<213> Artificial Sequence

<220>

<223> CAD4a

<400> 7

Ser Gly Trp Tyr Thr Phe Thr Lys Asn Thr Ala Ile Lys Ser Ala Ala
1 5 10 15

Ser Asp Ser Ala Lys Thr Val Gly Thr Tyr Ser Lys Gly Asn Arg Val
20 25 30

Tyr Tyr Asn Ala Glu Ile Thr Thr Asn Gly Glu Thr Trp Leu Arg Tyr
35 40 45

Leu Ser Tyr Ser Gly Ser Glu His Phe Val Lys Ile Ala Ala Ala Lys
50 55 60

Thr Thr Thr Thr Lys Pro Ala Ala Ser Thr Ser Lys Thr Val Thr Lys
65 70 75 80

Asn Glu Thr Gly Thr Tyr Lys Phe Thr Lys Thr Thr Ala Ile Lys Gly
85 90 95

Ser Val Ser Asp Ser Ala Lys Thr Leu Gly Thr Tyr Tyr Lys Gly Asp
100 105 110

Thr Val Tyr Tyr Asn Ala Lys Val Thr Lys Asn Gly Glu Thr Trp Leu
115 120 125

Arg Tyr Leu Ser Tyr Ser Gly Ala Gln His Tyr Val Lys Ile Ser Gly
130 135 140

Ala Ala Thr Ser Thr Thr Thr Thr Lys Pro Ala Thr Ser Ser Ser Lys
145 150 155 160

Thr Val Thr Lys Ala Glu Thr Gly Thr Tyr Lys Phe Thr Gly Thr Thr
165 170 175

Ala Ile Lys Gly Ser Val Asn Asp Ser Ala Lys Thr Leu Gly Thr Tyr
180 185 190

Tyr Lys Gly Asp Thr Val Tyr Tyr Asn Ala Lys Val Thr Lys Asn Gly
195 200 205

Gln Thr Trp Leu Arg Tyr Leu Ser Tyr Ser Gly Ala Gln His Tyr Val
210 215 220

Lys Ile Ser Gly
225

<210> 8
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Lys2 spacer

<400> 8

Val Thr Thr Thr Ser Ser Asn Asp Thr Thr Thr Ser Glu Val Thr Thr
1 5 10 15

Asp Thr Ser Ala Thr Thr Asn Thr Ser Thr Ser Ser Thr Thr Lys Lys
20 25 30

Ala

<210> 9
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic spacer

<400> 9

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<210> 10
<211> 152
<212> PRT
<213> Artificial Sequence

<220>
<223> SOD

<400> 10

Met Ala Lys Gly Val Ala Val Leu Ser Ser Ser Glu Gly Val Ala Gly
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Thr Ile Leu Phe Thr Gln Glu Gly Asp Gly Pro Thr Thr Val Thr Gly
20 25 30

Asn Ile Ser Gly Leu Lys Pro Gly Leu His Gly Phe His Val His Ala
35 40 45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Phe Asn
50 55 60

Pro Ala Gly Lys Glu His Gly Ser Pro Glu Asp Glu Thr Arg His Ala
65 70 75 80

Gly Asp Leu Gly Asn Ile Thr Val Gly Asp Asp Gly Thr Ala Cys Phe
85 90 95

Thr Ile Val Asp Lys Gln Ile Pro Leu Thr Gly Pro His Ser Ile Ile
100 105 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
115 120 125

Gly His Glu Leu Ser Lys Ser Thr Gly Asn Ala Gly Gly Arg Ile Ala
130 135 140

Cys Gly Ile Ile Gly Leu Gln Gly
145 150

<210> 11
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> F1 primer

<400> 11
tgagatccgg ctgctaaca agcccgaaag 30

<210> 12
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> F15 primer

<400> 12
ggcgggtccg gcggtggaag tggcggtggt agtggcggag gttcgggggg tggcagcg 58

<210> 13
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> F18 primer

<400> 13
ccacggtggg tctggggggg gatccggcat ggcaaaggga gtggcggtac tgt 53

<210> 14
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> R1 primer

<400> 14
gccttggttt tctaattttg gttcaaagaa agct 34

<210> 15
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> R4 primer

<400> 15
gccggatccc cccccagacc cac 23

<210> 16
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> R5 primer

<400> 16
ctttcgggct ttgttagcag ccggatctca 30

<210> 17
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> R6 primer

<400> 17
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ccccgaacct cccttgtaga gctcgtccat gccgagagtg 100

<210> 18
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> R7 primer

<400> 18
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agtg 64

<210> 19
 <211> 55
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> R24 primer

 <400> 19
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 <210> 20
 <211> 45
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> R25 primer

 <400> 20
 cgctgccacc ccccgaacct cctccttgaa ggccaatgat gccac 45

 <210> 21
 <211> 64
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> R26 primer

 <400> 21
 gccactacca cgcgcacttc caccgccgga cccgcccttg tacagctcgt ccatgccgag 60
 agtg 64

 <210> 22
 <211> 59
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> R27 primer

 <400> 22
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<210> 23
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> R37 primer

<400> 23
ctttcgggct ttgttagcag ccg gatctca tccttgaagg ccaatgatgc cacatgc 57

<210> 24
<211> 767
<212> DNA
<213> Artificial Sequence

<220>
<223> G5 (Sirius) gene fragment

<400> 24
gtgggtctgg ggggggatcc ggcgtgagca agggcgagga gctgttcacc ggggtggtgc 60
ccatcctggt cgagctggac ggcgacgtaa acggccacag gttcagcgtg tccggcgagg 120
gcgagggcgga tgccacctac ggcaagctga ccctgaagct catctgcacc accggcaagc 180
tgcccgtgcc ctggcccacc ctctgtacca ccctgcaatt cggcgtgctg tgcttcgccc 240
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tccaggagcg taccatcttc ttcaaggacg acggcaacta caagaccgc gccgaggtga 360
agttcgaggg cgacaccctg gtgaaccgca tcgagctgaa gggcatcgac ttcaaggagg 420
acggcaacat cctggggcac aagctggagt acaacgggat aagctcaaac gtatatatca 480
ccgccgacaa gcagaagaac ggcacatagg ccacttcaa gatccgccac aacatcgagg 540
acggcggcgt gcagctcgcc gaccactacc agcagaacac cccatcggc gacggccccg 600
tgctgctgcc cgacaaccac tacctgagcg tccagtcaa gctgagcaaa gacccaacg 660
agaagcgaga tcacatggtc ctgctggagt ccgtgaccgc cgccgggatc actctcgga 720
tggaagagct gtacaagtga gatccggctg ctaacaaagc ccgaaag 767

<210> 25
<211> 736
<212> DNA
<213> Artificial Sequence

<220>
<223> G10 (CAD4a) gene fragment

<400> 25
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ttacaatgcg gagattacaa ctaacggcga gacatggctt cgctacctta gctattcggg 180
ctctgaacac ttcgtgaaga ttgctggctgc taaaactaca accacaaagc cagctgcattc 240
tacctctaaa actgtttacca agaacgagac aggaacttat aaattcacca aaaccacggc 300
catcaagggc tcggttttcgg attccgccaa gaccctggga acttactaca aaggggacac 360
cgtatattat aatgccaaag ttactaaaaa tggagagacg tggcttcgtt acctgtcgta 420
ttccggcgct caacactatg tgaagatttc tggcgcagcc acgtcgacta ccacgacaaa 480
acccgcaacg tcttcgagta agaccgtcac gaaagctgag actggcacat ataaatttac 540
aggcactacg gctattaagg gcagcgtgaa cgattcggcc aaaaccttag gaacatacta 600
taagggggac accgtttatt acaatgcgaa agttacaaaa aatgggcaga cctggttacg 660
ctatttgtcg tattccgggg cacagcacta cgtgaaaatc tctggttgag atccggctgc 720
taacaaagcc cgaaag 736

<210> 26
<211> 456
<212> DNA
<213> Artificial Sequence

<220>
<223> G29 (SOD) gene fragment

<400> 26
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acacaggaag gtgatggacc gacaactgtc actgggaaca ttagtggact gaaaccggga 120
ttacatgggt tccacgtcca tgcccttggc gacaccacca acggatgtat gtcaacagga 180

ccacatttca atccggcggg caaagaacat ggggtcccctg aggacgaaac ccgccatgcg	240
ggcgacttgg gtaacatcac ggtgggagat gacggaacgg cgtgttttac tatcgtagat	300
aaacagattc ctttaaccgg accgcactca attatcggtc gggccgtcgt ggtgcacgcc	360
gacccggacg acttaggcaa ggggggacac gagctttcaa aatctacggg aaatgctgga	420
ggtcggatcg catgtggcat cattggcctt caagga	456