

Supplementary information

Title: α -1,3-Glucanase from the gram-negative bacterium *Flavobacterium* sp. EK-14
hydrolyzes fungal cell wall α -1,3-glucan

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Supplementary Table S1. Oligonucleotide primers.

Primer name	Oligonucleotide sequence
Flavo-for	5'-TATGCSCAGCGMGGTTATTATGATGCASCTTATAA-3'
Flavo-rev	5'-TTTACYAAAACCTTTTCTTCCGYTKMTATCWGTAAAC-3'
FlavoEK-f4	5'-TTGCATCTATATTGATTCTTGATTTTTTATTTTGG-3'
FlavoEK-r4	5'-GCACGGATTACAAATCCGCGCTATCGTTGTTGAGC-3'
Flavof1RE	5'-TGCTTCAGGTTCCCATATGCAGCGCGGTTATTATG-3'
Flavo5rRE	5'-CTAAATTTTGGATCCTCATTTAATAATCAATTTTT-3'
T7 promoter	5'-CCCGCGAAATTAATACGACTCACTATAGGG-3'
Cbam4	5'-GGAAGGATTGGATCCTTAGCTAACTACAGTATTCT-3'

Restriction sites in the oligonucleotide sequences are underlined.

Supplementary Table S2. Purification of Agl-EK14

	Volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)
Cell-free extract	50	9500	321	29.6	100
(NH ₄) ₂ SO ₄ precipitation 25–70% fraction	30	6270	171	36.8	66.1
Cellufine A-500	93	5950	63.9	93.1	62.6
HiTrap Butyl 650M	31	4310	32.1	134	45.4

Supplementary Table S3. Substrate specificity of Agl-EK14

Substrate	Main linkage-type	Relative activity (%)
α -1,3-Glucan	α -1,3-	100
Starch	α -1,4-	1.5 ± 0.8
Dextran	α -1,6-	2.0 ± 0.5
Laminarin	β -1,3-	5.0 ± 2.7
Cellulose	β -1,4-	1.3 ± 0.2

ATGAGAAACAAATTACATTTTATTTTTTTGAGAAAAGCCCAATATTACCATTGTATATTA 60
 M R N K L H F I F L R K A Q Y Y H V I L
 TGGCTTTTTTTCTTCTTTTACTTCAGGTTCTATGCCAGCGCGGTTATTATGATGCA 120
 W L F F L L F T S G S Y A Q R G Y Y D A
 CCTTATAAAAGGTACGAAGCAAATTTAGGTCAATTGTCTAATGGTGCTTTAGTTACTGCA 180
 P Y K R Y E A N L G Q L S N G A L V T A
 AAATCATACAATCAGGCCGATCTTCAGTCAGAAGCTTCAGATCAGCAGTGTGTGAATATG 240
 K S Y N Q A D L Q S E A S D Q Q C V N M
 TCTGCCACAAACGCGACCGTTCAATGGACGCTAAGTGAAGCTGCCGATGGATTAGTAATT 300
 S A T N A T V Q W T L S E A A D G L V I
 CGTTATAGTGTCTCTGACGGGCAATCTGGAATATCGGGGTTTATAATGGCAACACAAAG 360
 R Y S V P D G Q S G T I G V Y N G N T K
 CTTACAACGCTTACTTTGACTTCAACTTGGTCTTGGGAATATTTGTGGAGAACGGAAT 420
 L T T L T L T S T W S W E Y L W S N G N
 CCGAACAATAACGGAATTACGAATCAAAACCAAGAATGCGTTTGTATGAAGTTCGTTAT 480
 P N N N G I T N Q N P R M R F D E V R Y
 AAACCTCCTGCTAAAATTGCTGTAAACGGAACATTAAAATTGGTGAGAGAATCTGGTAAT 540
 K L P A K I A V N G T L K L V R E S G N
 GTGCATATTGACTTTGCAGAAATGGAACCAAGTGCCAACAGCTATTACAGCTCCTGCAGGT 600
 V H I D F A E M E P V P T A I T A P A G
 GCCGTAACTTATTCAGGTAACGGAAGCGATCTTCAGACTTTTATTGATGCAAATGGCGGA 660
 A V T Y S G N G S D L Q T F I D A N G G
 AAAAAAATATTTGTTCGAGCGGGGTTTATAATGTTAACCGAGAATTATATTTGGTTCA 720
 K K I F V P S G V Y N V N R E L Y F G S
 GCAAACACTTCGCTAATTGGTGCAGGAATGTGGTACACAGATTAATTTACCAATACT 780
 A N T S L I G A G M W Y T Q I N F T N T
 AGTAGTTTAAATGGCGGATTACGTGCCAATGCAAGTAACATTTTCGTTACAGATTATAT 840
 S S L N G G L R A N A S N I S F T D L Y
 CTCACAACAAATTCAGCTTCGAGAAGTAATCTTATAAAGCAATAAATGGAGTTTTTACA 900
 L T T N S A S R S N S Y K A I N G V F T
 AGTGGTTCTATAGTAAAAATATCTGGGCAGAACATTTTGAATGTGGTGCCTGGATTGCG 960
 S G S I V K N I W A E H F E C G A W I A
 CAATACAATTCTGGTGGGCGGCAATTGCTGACGGATTTACATTATCACACTGTCGTTTT 1020
 Q Y N S G G P A I A D G F T L S H C R F
 AGAAATAATTATGCCGATGGAATCAATCTTTGCAAAGGAACAGCAAATTCATTGTAGAA 1080
 R N N Y A D G I N L C K G T A N S I V E
 CACTGTAATTTTAGAAACAACGAGACGACGATCAGGCGATTGGTCTGCTGACGGACTC 1140
 H C N F R N N G D D D Q A I W S A D G L
 GAATGTATCAATAATACATTTTCGATACAATACTTCAGAAAAGTGTGGCGCGCTGTGGA 1200
 E C I N N T F R Y N T S E N C W R A C G
 CTTGCTATTATGGAGGGAAAAACAACAAAGGCTACAATTTAATTATCAAAGACAATCTG 1260
 L A I Y G G K N N K G Y N L I I K D N L
 GAAGCAGGAATTAGAGTAAGTAATAATTTCCGGGAGCACCGTTTAAACACGATGGTATG 1320
 E A G I R V S N N F P G A P F N N D G M
 CACGAAATTCATGATATTACCGTAACAGCCTGTGGAACCTTTAATGATACCTACAATAAT 1380
 H E I H D I T V T A C G T F N D T Y N N
 CCCGTGGCGGCAATTGATATTTTGTAGTCTACAAATGCCGGAAGTCAGGTTAAGAATGTT 1440
 P V A A I D I F S A T N A G S Q V K N V
 CAGCTTTATAGTATTGATATTATAGATTCAAGAAACGATGCTATTTCTATTAGTAAAAGA 1500
 Q L Y S I D I I D S R N D A I S I S K R
 TCGGGAGATGGCATTATAATCTTCTTTTAAAGACATAACCGTTAACGGAACGGGTAAA 1560
 S G D G I Y N L S F K D I T V N G T G K
 GAATACCAAAATAACATGTATTAAACAGAAACTGGGGAAGAGGCTATTTGTACTTATT 1620
 E Y P N N N V L N R N W G R G Y F V L I

Signal peptide (SP)

GH87

Catalytic domain (CAT)

GCAGGTTCTCCAAGCGGAAATGGTACTTATTGCAATATGAATTATTCTAATAGAGGCGGA 1680
A G S P S G N G T Y C N M N Y S N R G G
AATGCCACAATAATGAAGAAATAGTGCAATTGGTACATTTTCATGGACGCAGGCGGA 1720
N A T T N E E I S A I G T F S W T Q G G
AACTGTTCCTACTACAAATGTCCCGTTTCTGGTGAACACTTCGCCAGGAGCAGTAACT 1780
N C S T T N V P V S G V T L S P G A V T
CTAGGTGTTGGTGCTACACAGCAATTAACCTGCTGTTTCTCCTGCCAATGCTACCAAT 1860
L G V G A T Q Q L T P A V S P A N A T N
AAAACAGTAAGTTATAGTTCAAATAACTGGTGTGCTACAGTAAGTGGTTCAGGTTTA 1920
K T V S Y S S N N T G V A T V S G S G L
GTAAGTGCAGTGTCTCGGGATCTGCTACAATTACCGTAACGACTCAGGATGGTAATAAA 1980
V T A V A S G S A T I T V T T Q D G N K
ACGGCGACTTCTGTAATAACAGTAAATTCATCAAATGTAGCAGTAACAAGTGTGAGTTTA 2040
T A T S V I T V N S S N V A V T S V S L
AGTCCTTCATCAGCTACATTAGCGGTAGGCGGAACACAGCAGCTAACACCAACAGTTTGT 2100
S P S S A T L A V G G T Q Q L T P T V L
CCTTCAAACGCAACTAATAAATCGGTTAATTATGCTTCAAGCAATACAGGTGTGGCAACG 2160
P S N A T N K S V N Y A S S N T G V A T
GTTAATTCATCAGGATTAGTCACTGCAGTTTCAGCAGGTACCGTACCATTACGTAACA 2220
V N S S G L V T A V S A G T A T I T V T
ACAGTTGATGGAAATAAAACGAGTACTGCAGCAATTACTGTAATGCCGCAACAGGAAGC 2280
T V D G N K T S T A A I T V N A A T G S
TATTTTACAATAAAAACAAATGGACAGGTAATTATTTATATGATGCCGGAACAATGTA 2340
Y F T I K N K W T G N Y L Y D A G N N V
GGATACGGTCCAACAGTTGCAGACAATACTTATAAATGGGAAAAAGTGCCATTGATGTT 2400
G Y G P T V A D N T Y K W E K V A I D G
ACTTACTATATGATTAATAATGTAGGTACAGGAGATGTAATGCATATTGAGAATTAAAC 2460
T Y Y M I K N V G T G D V M H I E N L T
GGTGCAGTACAATGTACAGCAGGTCTAGCTGGTGGAGCGCACAATGGTCTTCCGAA 2520
G A V Q C T A G Q S S W W S A Q W S S E
AATGTAGACGCAACCTGGGTAAGAATCAAAAACAGATGGCAGACAACAGCAATGATTCAT 2580
N V D A T W V R I K N R W Q T T A M I H
ATTGAAAACTAATGGTCTGCAACAATATCTTGGTGGTCAAAATAGTTGGGAAAGTGCA 2640
I E N L N G S A Q Y L G G Q N S W E S A
CAATGGCAGTTTCAAAACACTTCGACATCGAAAAAAGTGAATCCTGTTGAAGTTGCGGTT 2700
Q W Q F Q N T S T S K K V N P V E V A V
GAGAATACTGTAGTTAGCATTATCCAAATCCTTCCATTGATAATGAATTTAATATTGTT 2760
E N T V V S I Y P N P S I D N E F N I V
TTGCCCACTGGAAGCAGGTGATACAGCAACTGTAACAGTTACTGATAGCAGCGGAAGA 2820
L P P L E A G D T A T V T V T D S S G R
AAAGTTTGGTAAACAAAATAGTTTCATCTTCAAAAATCAGTCATCATTTAGCCCTGGA 2880
K V L V N K I S S S S K I S H H L A P G
ATATATATTGTGACAATTAACCTCTAATACATTTAATGTTTCTAAAAAATTGATTATAAA 2940
I Y I V T I N S N T F N V S K K L I I K
TGA 2943
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First immunoglobulin-like domain (Ig1)

Second immunoglobulin-like domain (Ig2)

Ricin B-like lectin domain (Ricin B)

Carboxy-terminal domain (CTD)

Supplementary Fig. S1. Nucleotide and amino acid sequences of GH87 α -1,3-glucanase (Agl-EK14). The amino acid sequence of the GH87 catalytic domain is shown in light gray; Ig1 and Ig2 are shown in gray; Ricin B is shown in a white box; and CTD is shown in black.

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      10      20      30      40      50      60      70      80      90      100
EK-14      1 : MRNKLFFFLKACVYHETWFFFLFSSGYACRGYDAPYKRYEANLGQLSNGAVTEKSYSCADLCSEASDQCVNMSANATVQWTLSEADGLVI : 100
anhuiense 1 : MRNKLFFFLKACVYHETWFFFLFSSGYACRGYDAPYKRYEANLGQLSNGAVTEKSYSCADLCSEASDQCVNMSANATVQWTLSEADGLVI : 100
johnsoniae 1 : MRKCLGCLFRRKESRFFIMIFFFLFSSNYACRGYDAPYKRYEANLGQLSNGAVTEKSYSCADLCSEASDQCVNMSANATVQWTLSEADGLVI : 100

      110      120      130      140      150      160      170      180      190      200
EK-14     101 : RYSVPDQSGGTIGVYNGNTKLTTLTSTWSWEYLWSNGNPNNGITNCNPRMRFDEVRYKLPKRIYVNTGLKLVRESGNVHIDFAEMEFPVPIATAPAG : 200
anhuiense 101 : RYSVPDQSGGTIGVYNGNTKLTTLTSTWSWEYLWSNGNPNNGITNCNPRMRFDEVRYKLPKRIYVNTGLKLVRESGNVHIDFAEMEFPVPIATAPAG : 200
johnsoniae 101 : RYSVPDQSGGTIGVYNGNTKLTTLTSTWSWEYLWSNGNPNNGITNCNPRMRFDEVRYKLPKRIYVNTGLKLVRESGNVHIDFAEMEFPVPIATAPAG : 200

      210      220      230      240      250      260      270      280      290      300
EK-14     201 : SVTYGNGSDLCQTFIDANGGKKIFVPSGVNVNRELIFPGSANTSLIGAGMWYTCINFNTSSINGGLRANASNISFTDLYLTNSASRSNSYKAINGVFT : 300
anhuiense 201 : SVTYGNGSDLCQTFIDANGGKKIFVPSGVNVNRELIFPGSANTSLIGAGMWYTCINFNTSSINGGLRANASNISFTDLYLTNSASRSNSYKAINGVFT : 300
johnsoniae 201 : SVTYGNGSDLCQTFIDANGGKKIFVPSGVNVNRELIFPGSANTSLIGAGMWYTCINFNTSSINGGLRANASNISFTDLYLTNSASRSNSYKAINGVFT : 300

      310      320      330      340      350      360      370      380      390      400
EK-14     301 : SGSTYKNIWAHEFECCGAWIACYNVGGPAIADGFTLSHCRFRNNYADGINLCKGTANIVEHCNFRNNGDDQCAIWSAGGECINNTFFRYTSENCWRACG : 400
anhuiense 301 : SGSTYKNIWAHEFECCGAWIACYNVGGPAIADGFTLSHCRFRNNYADGINLCKGTANIVEHCNFRNNGDDQCAIWSAGGECINNTFFRYTSENCWRACG : 400
johnsoniae 301 : SGSTYKNIWAHEFECCGAWIACYNVGGPAIADGFTLSHCRFRNNYADGINLCKGTANIVEHCNFRNNGDDQCAIWSAGGECINNTFFRYTSENCWRACG : 400

      410      420      430      440      450      460      470      480      490      500
EK-14     401 : LAIYGGKNNRYNLIKDNLEAGIRVSNNFGAPFNNDGHEHIDITVTCCTFNDTYNNPVAAIDIFSAWAGSCVKNVQLYSIDIDSRNDAISISKE : 500
anhuiense 401 : LAIYGGKNNRYNLIKDNLEAGIRVSNNFGAPFNNDGHEHIDITVTCCTFNDTYNNPVAAIDIFSAWAGSCVKNVQLYSIDIDSRNDAISISKE : 500
johnsoniae 401 : LAIYGGKNNRYNLIKDNLEAGIRVSNNFGAPFNNDGHEHIDITVTCCTFNDTYNNPVAAIDIFSAWAGSCVKNVQLYSIDIDSRNDAISISKE : 500

      510      520      530      540      550      560      570      580      590      600
EK-14     501 : SGDGIYNLSFRNITVNGTGKEYPNNNLNRNWGRGYFVLIAGSPGNGTYCNMNYSNRGGNAITNEEISAIGFSWTGSGNCSITNVVSGVTLSPGTVI : 600
anhuiense 501 : SGDGIYNLSFRNITVNGTGKEYPNNNLNRNWGRGYFVLIAGSPGNGTYCNMNYSNRGGNAITNEEISAIGFSWTGSGNCSITNVVSGVTLSPGTVI : 600
johnsoniae 501 : SGDGIYNLSFRNITVNGTGKEYPNNNLNRNWGRGYFVLIAGSPGNGTYCNMNYSNRGGNAITNEEISAIGFSWTGSGNCSITNVVSGVTLSPGTVI : 600

      610      620      630      640      650      660      670      680      690      700
EK-14     601 : LCVGATCQLTPTVSPANATNKTIVSYSSNNTGVATVNSGSLVTAVASGSATITVTTQDGNKTATSVITVNSSNVAVTSVSLSPSSAHLVGGTQQLTPTVI : 700
anhuiense 601 : LCVGATCQLTPTVSPANATNKTIVSYSSNNTGVATVNSGSLVTAVASGSATITVTTQDGNKTATSVITVNSSNVAVTSVSLSPSSAHLVGGTQQLTPTVI : 700
johnsoniae 601 : LCVGATCQLTPTVSPANATNKTIVSYSSNNTGVATVNSGSLVTAVASGSATITVTTQDGNKTATSVITVNSSNVAVTSVSLSPSSAHLVGGTQQLTPTVI : 700

      710      720      730      740      750      760      770      780      790      800
EK-14     701 : PSNATNKSVMNASSNTGVATVNSGSLVTAVASGATITVTTVDGDKTSTAITVNTATGSYFTIKNKWTGNLYLDAGNVVGYGPTVANNYKRWKVAID : 800
anhuiense 701 : PSNATNKSVMNASSNTGVATVNSGSLVTAVASGATITVTTVDGDKTSTAITVNTATGSYFTIKNKWTGNLYLDAGNVVGYGPTVANNYKRWKVAID : 800
johnsoniae 701 : PSNATNKSVMNASSNTGVATVNSGSLVTAVASGATITVTTVDGDKTSTAITVNTATGSYFTIKNKWTGNLYLDAGNVVGYGPTVANNYKRWKVAID : 800

      810      820      830      840      850      860      870      880      890      900
EK-14     801 : TVYMIKNVGTGDVMHIENTLGVCCCTACGSDWISACWSENVDATWVRIKNRWCTGSMIHIENTLSACQYIGCNWESACWQFNTSTAKKNTPVEVA : 900
anhuiense 801 : TVYMIKNVGTGDVMHIENTLGVCCCTACGSDWISACWSENVDATWVRIKNRWCTGSMIHIENTLSACQYIGCNWESACWQFNTSTAKKNTPVEVA : 900
johnsoniae 801 : TVYMIKNVGTGDVMHIENTLGVCCCTACGSDWISACWSENVDATWVRIKNRWCTGSMIHIENTLSACQYIGCNWESACWQFNTSTAKKNTPVEVA : 900

      910      920      930      940      950      960      970      980
EK-14     901 : ENKAVSYIYPNPSVNNNEFNILPHELEAGLHATITVTDSSGRKVLVNTSSSKINHLGAGIYVVTINSNGLVSKKLLIK : 980
anhuiense 901 : ENKAVSYIYPNPSVNNNEFNILPHELEAGLHATITVTDSSGRKVLVNTSSSKINHLGAGIYVVTINSNGLVSKKLLIK : 980
johnsoniae 901 : ENKAVSYIYPNPSVNNNEFNILPHELEAGLHATITVTDSSGRKVLVNTSSSKINHLGAGIYVVTINSNGLVSKKLLIK : 981

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Supplementary Fig. S2. Multiple alignments of amino acid sequences of Agl-EK14 with the putative α -1,3-glucanase of *Flavobacterium*. A black box indicates identical amino acids, and a gray box indicates amino acids conserved in at least two of the three sequences. Abbreviations: EK-14, Agl-EK14 of *Flavobacterium* sp. EK-14; anhuiense, putative α -1,3-glucanase of *F. anhuiense* M168 (WP_119790483.1); johnsoniae, putative α -1,3-glucanase of *F. johnsoniae* UW101 (ABQ06220.1).

(A)

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      10      20      30      40      50      60      70      80
Agl-EK14 Ig1  589 : VSEVTLSSGAVTLGVGATCCLTPAVSEANATNKKVSYSSNNTGVATVSGSLVTAVALSGSALIVVTCDGNKTATSIVTVNS : 670
Agl-EK14 Ig2  675 : VMSVSLSSSSATLAVGTCCLTPVLESNATNKSVMYASSNTGVATVNSSLVTAVALSGSALIVVTCDGNKTATSIVTVNS : 756
EOL45850.1    567 : -TCVVITLENKELLVGETCCLKAEVAFANATNKNVIRSSNEAIAITVDQNGIVTAKKGBAILIVETRDGKGSASCKI---- : 643
OJG55201.1    567 : -TCVTIDLKKNELLVGETRCINAEVTPANATNKNRVMSSNEAIAITVDQNGIVTAKKGBAILIKVETEDGRESASCKI---- : 643
WP_106604633.1 646 : -TCVSVSEATASLDINNTCCLTATVTPESNATNKNVNTSSAPAIATVNSNGLVTALACRATITATTADGGF----- : 716
WP_101262190.1 649 : -TCVSVSEATASLDINNTCCLTATVTPESNATNKNVNTSSAPAIATVNSNGLVTALACRATITATTADGGF----- : 719
ETT67022.1    693 : -TCVTLNETSAQVEVGQSTICINAGIAESNATNKNVNTSSGASVSVSQEGVVVGLAPGTAIVATSVDCQKTASSTI---- : 769
WP_213578890.1 693 : -TCVTLNETSAQVEVGQSTICINAGIAESNATNKNVNTSSGASVSVSQEGVVVGLAPGTAIVATSVDCQKTASSTI---- : 769
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(B)

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      10      20      30      40      50      60      70
Agl-EK14 Ricin B 761 : -YFTLNKNTGNYLYTAGNNVGYGPTVADNTYKWEKVA-IDGTYIMIKN-VGIGDVMHIEINLTGAVQCTA : 827
Q47899.1        297 : GTYNTTTSLAGDKNIDITGSSTADGTDVLYSATTGNN-QKFIFRKSEHGYFTIKSILDSTKVLTVRNNG : 365
P02879.1        322 : -EPIVRIIVGRNGLCVYVRDGR-FHNGNAQLWPCKSNT-DANCLATIKR-DNTIRSNKGCLTTYGYSPGV : 387
AAB23029.1      1 : -EPTVRIGGRDGMQVDVYDNG-YHNGYRIIMKCKDRL-EENQLATIKS-DKTIIRSNKGCLTTYGYAPGS : 66
B2ZRS9.1        6 : GTYNTTNVAYTNRLIDITGSNPAENTLIIGHHLNKTSPSGYGNQQTIVQLPHITTIYTMQAVNPQSVVRVR : 75

      80      90      100     110     120     130     140
Agl-EK14 Ricin B 828 : GQSSNWS-AQWSSENVDAITVR-IKNRWQTAMTHIE-----NLNGSAQYLGGQNSWESACWQFQNT : 887
Q47899.1        366 : TANGTAVELRTNADTDACKLLFNLGNEGFGFAPKNA-----PSLRLEVKDGLTTNLTPIVI----- : 422
P02879.1        388 : YVMIYDCNTAATDATRWQIDNGTIINPRSSLVTAAT-----SGNSGTTLTVQNTIYAVSCGWLPT- : 448
AAB23029.1      67 : YVMIYDCTSAVAEATYWEIDNGTIINPKSALVSAE-----SSSMGGTTLTVQNTIYLMRCGWRTG- : 127
B2ZRS9.1        76 : DDNLIVDGAALVGSQQPTPVSTIESAGNSGQFRIKPNLGLALTLPSDANSTPIVLGEVETSTNCLWAFE- : 144
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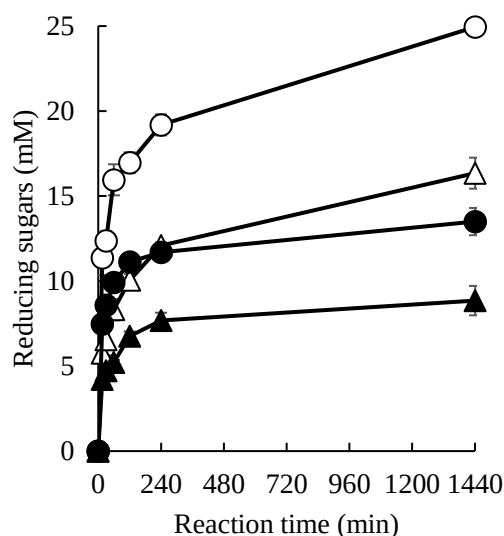
Supplementary Fig. S3. Multiple alignments of amino acid sequences of the immunoglobulin-like domain of Agl-EK14 with other enzymes (A) and Ricin B of Agl-EK14 with other enzymes (B). Amino acid sequences were aligned by ClustalW. The residue numbers of the first and last amino acids in each line are shown on the left and right. (A) A black box indicates completely identical amino acids. Amino acids conserved in at least six of the seven sequences are indicated by a gray box. Amino acids conserved in at least five of the seven sequences are indicated by a light gray box. Agl-EK14 Ig1, Agl-EK14 of *Flavobacterium* sp. EK-14 (589–670 amino acids); Agl-EK14 Ig2, Agl-EK14 of *Flavobacterium* sp. EK-14 (675–756 amino acids); EOL45850.1, metalloprotease of *Enterococcus* *caccae* ATCC BAA-1240; OJG55201.1, metalloprotease of *Enterococcus* *haemoperoxidus*; WP_106604633.1, GH9 protein of *Labililabaculum* *filiforme*; ETT67022.1, GH family protein of *Paenibacillus* sp. FSL H8-457; WP_213578890.1, glycosylhydrolase of *Paenibacillus* *lautus*. (B) A black box indicates all similar amino acids. Amino acids similar to at least four of the five sequences are indicated by a gray box. Amino acids similar to at least three of the five sequences are indicated by a light gray box. Abbreviations: Agl-EK14 Ricin B, Agl-EK14 of *Flavobacterium* sp. EK-14; Q47899.1, flavastacin of *Flavobacterium* *meningoseticum*;

P02879.1, Ricin B chain lectin domain; AAB23029.1, abrin-a B-chain lectin domain of *Abrus precatorius*; B2ZRS9.1, CNL of *Clitocybe nebularis*.

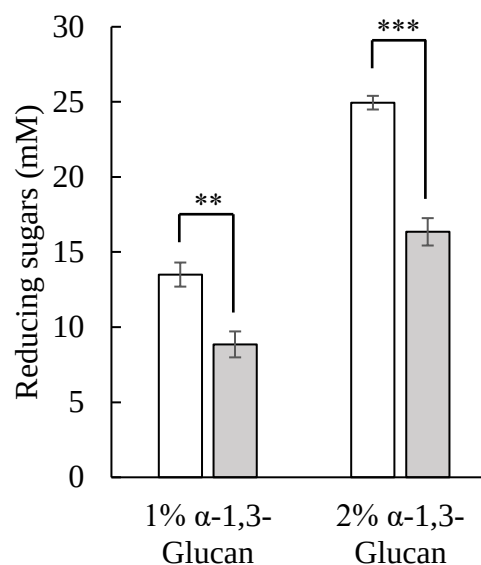
GH87 α -1,3-glucanases and mycodextranases were given by the National Center for Biotechnology Information protein database. An arrow indicates Agl-EK14. Uncharacterized GH87 α -1,3-glucanases and mycodextranases indicated by asterisks are as follows: α -1,3-glucanase from *Capsulimonas corticalis* AX-7 (BDI29047.1), α -1,3-glucanase from *Luteibacter anthropi* SM7.4 (URX61610.1), α -1,3-glucanase from *Micromonospora chokoriensis* DSM 45160 (SCF30157.1), α -1,3-glucanase from *Micromonospora krabiensis* DSM 45344 (SBV25278.1), α -1,3-glucanase from *Streptomyces subutilus* ATCC 27467 (QEU82241.1), α -1,3-glucanase from *Cellulosimicrobium protaetiae* BI34 (QJW35263.1), α -1,3-glucanase from *Streptomyces griseus* subsp. *griseus* NBRC 13350 (BAG23451.1), α -1,3-glucanase from *Streptomyces lividans* TK23 (BDE43507.1), α -1,3-glucanase from *Streptomyces solisilvae* HNM0141 (QPI55097.1), α -1,3-glucanase from *F. johnsoniae* UW101 (ABQ06220.1), α -1,3-glucanase from *F. anhuiense* M168 (WP_119790483.1), α -1,3-glucanase from *Streptococcus salivarius* ATCC 25975 (ARI60364.1), α -1,3-glucanase from *Streptococcus sobrinus* 10919 (AWN21666.1), α -1,3-glucanase from *Chitinophaga agri* H33E-04 (QHS58067.1), α -1,3-glucanase from *Fulvivirga ligni* W9P-11 (UII24169.1), α -1,3-glucanase from *Prevotella melaninogenica* F0299 (QUB68149.1), α -1,3-glucanase from *Anaerocolumna* sp. CTTW (BCJ98831.1), α -1,3-glucanase from *Cystobacter fuscus* Cbf 10 (WNG18114.1), α -1,3-glucanase from *Cohnella abietis* HS21 (BBI30922.1), α -1,3-glucanase from *Paenibacillus albicereus* UniB2 (QJC50342.1), α -1,3-glucanase from *Dactylosporangium fulvum* NRRL B-16292 (UWP84950.1), α -1,3-glucanase from *Dactylosporangium vinaceum* NRRL B-16297 (UAB99015.1), α -1,3-glucanase from *Polymorphospora rubra* NBRC 101157 (BCJ68800.1), α -1,3-glucanase from *Verrucosipora* sp. WMMA2044 (WBB48106.1), α -1,3-glucanase from *Kribbella qitaiheensis* SPB151 (QNE20272.1), α -1,3-glucanase from *Actinoplanes ianthinogenes* NBRC 13996 (BCJ42003.1), α -1,3-glucanase from *Streptomyces cavourensis* 1AS2a (ATY94307.1), α -1,3-glucanase from *Amycolatopsis albisporea* WP1 (AXB48709.1), α -1,3-glucanase from *Nonomuraea gerenzanensis* L70 (UBU15042.1), α -1,3-glucanase from *Glycomyces* sp. TRM65418 (QZD55419.1), α -1,3-glucanase from *Cellvibrio* sp. PSBB006 (ARU29420.1), mycodextranase from *Streptomyces* sp. PBH53 (AKN70696.1), mycodextranase from *Streptomyces* sp. NRRL 30748 (ABC87502.1), mycodextranase from *Streptomyces* sp. Tue6028 (PBC59932.1), mycodextranase from

Amycolatopsis vastitatis (OXM62597.1), mycodextranase from *Peterkaempferia bronchialis* DSM 106435 (AXI76714.1), mycodextranase from *Agromyces aureus* AR33 (ANJ26885.1), mycodextranase from *Ktedonobacteria bacterium* brp13 (BCL81308.1), mycodextranase from *Melittangium boletus* DSM 14713 (ATB32882.1), mycodextranase from *Cystobacter fuscus* DSM 52655 (ATB43383.1), mycodextranase from *Curtobacterium* sp. MCBA15_004 (WIA96983.1), and mycodextranase from *Actinoplanes* sp. L3-i22 (BCY08438.1). Characterized GH87 α -1,3-glucanases and mycodextranases are as follows: Agl-KA from *B. circulans* KA-304 (BAE98302.1), MuE from *Paenibacillus* sp. KSM-M318 (BAH10514.1), mutanase RM1 from *Paenibacillus* sp. RM1 (E16590), MuB from *Paenibacillus* sp. KSM-M86 (BAF56208.1), Mut from *Paenibacillus humicus* NA1123 (BAI23187.1), Agl-FH2 from *P. glycanilyticus* FH11 (BAP10900.2), MuC1 from *Paenibacillus* sp. KSM-M126 (BAG15879.1), MutP from *P. curdlanolyticus* MP-1 (ADT91063.1), Agl-FH1 from *P. glycanilyticus* FH11 (BAP10900.1), MutC2 from *Paenibacillus* sp. KSM-M126 (BAG15880.1), MuA from *Paenibacillus* sp. KSM-M35 (BAG15878.1), Myd from *Streptomyces* sp. J-13-3 (BAB62749.1), and Agl-ST from *S. thermodiastaticus* HF 3-3 (BCK59654.1).

(A)

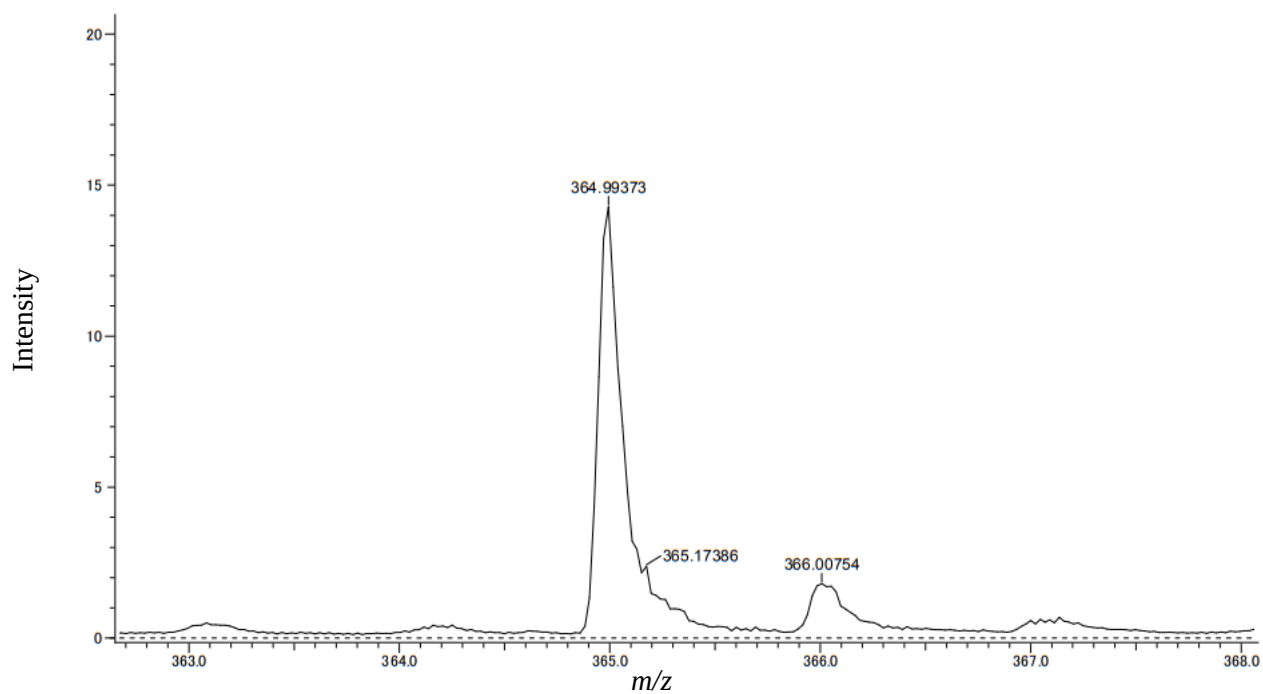


(B)

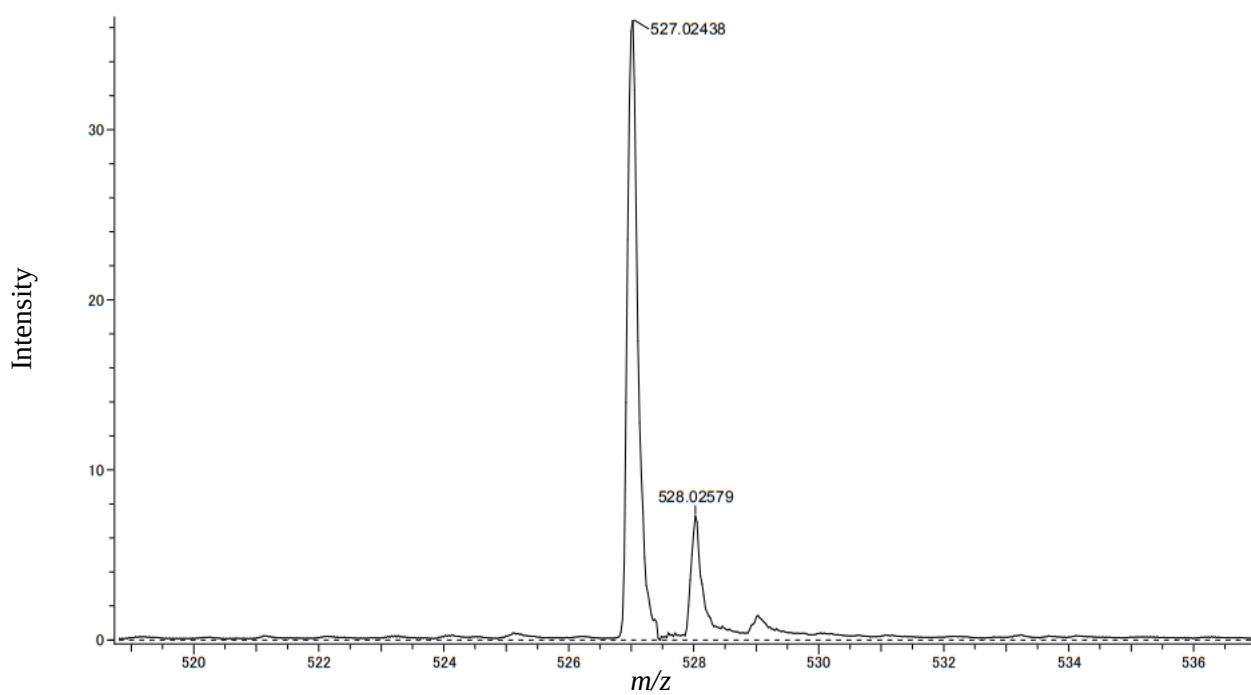


Supplementary Fig. S5. Comparison of α -1,3-glucan hydrolysis of Agl-EK14 with Agl-KA (A), and the amounts of reducing sugars released by Agl-EK14 and Agl-KA after 24 h of reaction (B). The reaction mixtures containing 1.0 nmol/ml α -1,3-glucanase, 50 mM potassium phosphate (pH 6.5), and α -1,3-glucan were incubated at 37°C. (A) Each data point represents the mean $\pm$ SD from triplicate experiments. Symbols: solid circles, 1% α -1,3-glucan hydrolyzed by Agl-EK14; open circles, 2% α -1,3-glucan hydrolyzed by Agl-EK14; solid triangles, 1% α -1,3-glucan hydrolyzed by Agl-KA; open triangles, 2% α -1,3-glucan hydrolyzed by Agl-KA. (B) White bars, Agl-EK14; gray bars, Agl-KA. The values are the means $\pm$ SD from triplicate experiments. Student's *t*-test was used to compare the amounts of reducing sugar released by Agl-EK14 and Agl-KA. Asterisks indicate significant differences (** $p < 0.01$, *** $p < 0.001$).

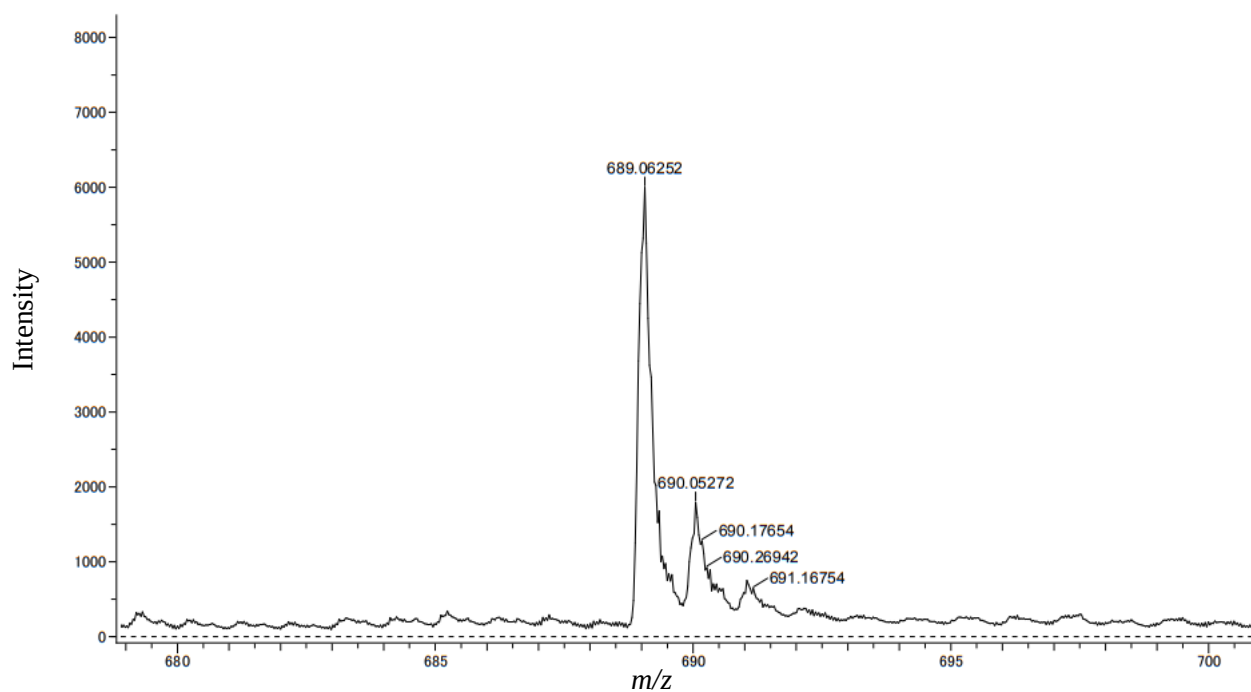
Peak 2



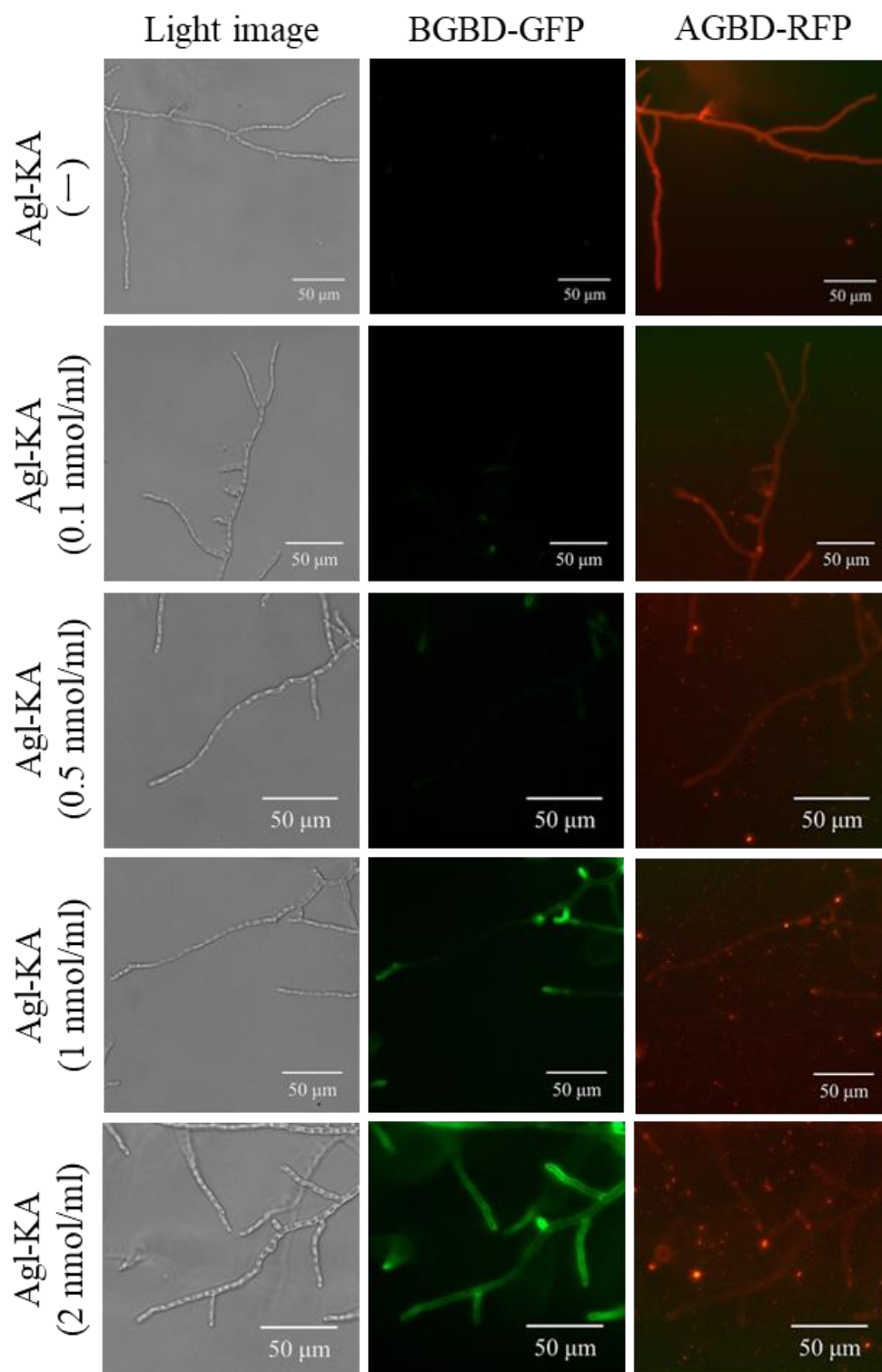
Peak 3



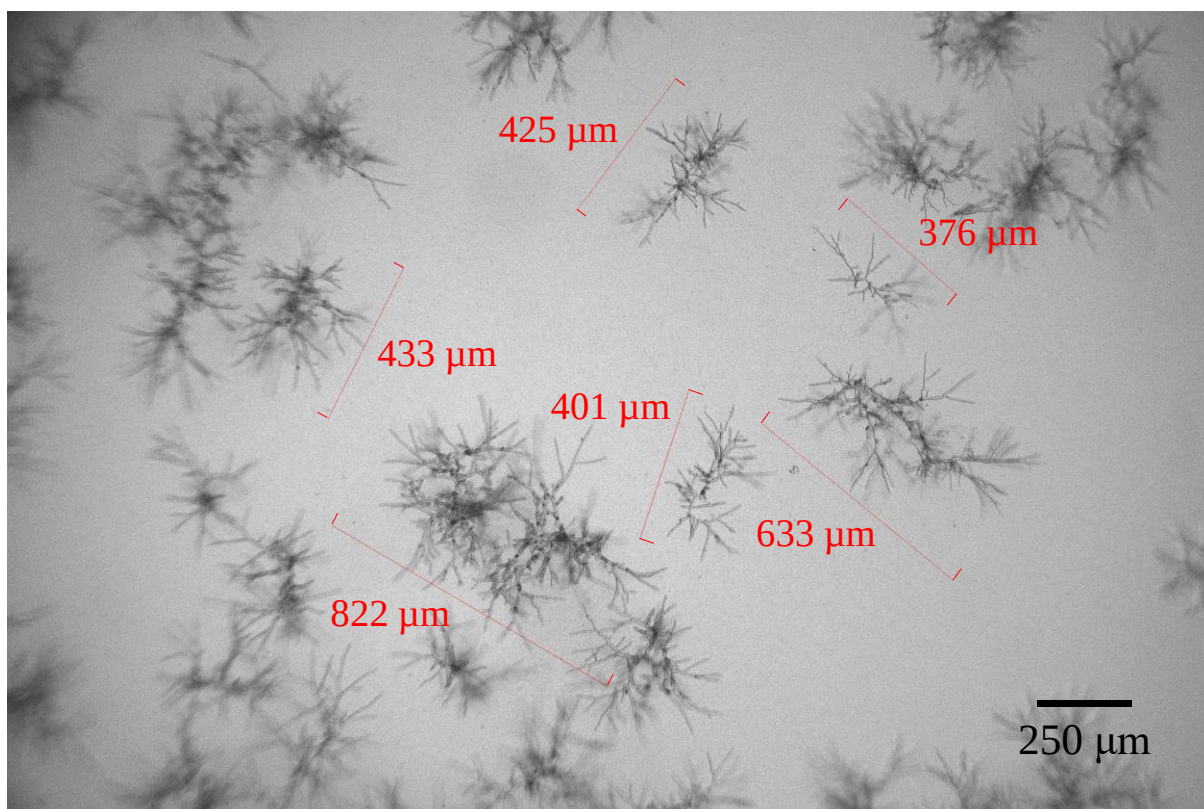
Peak 4



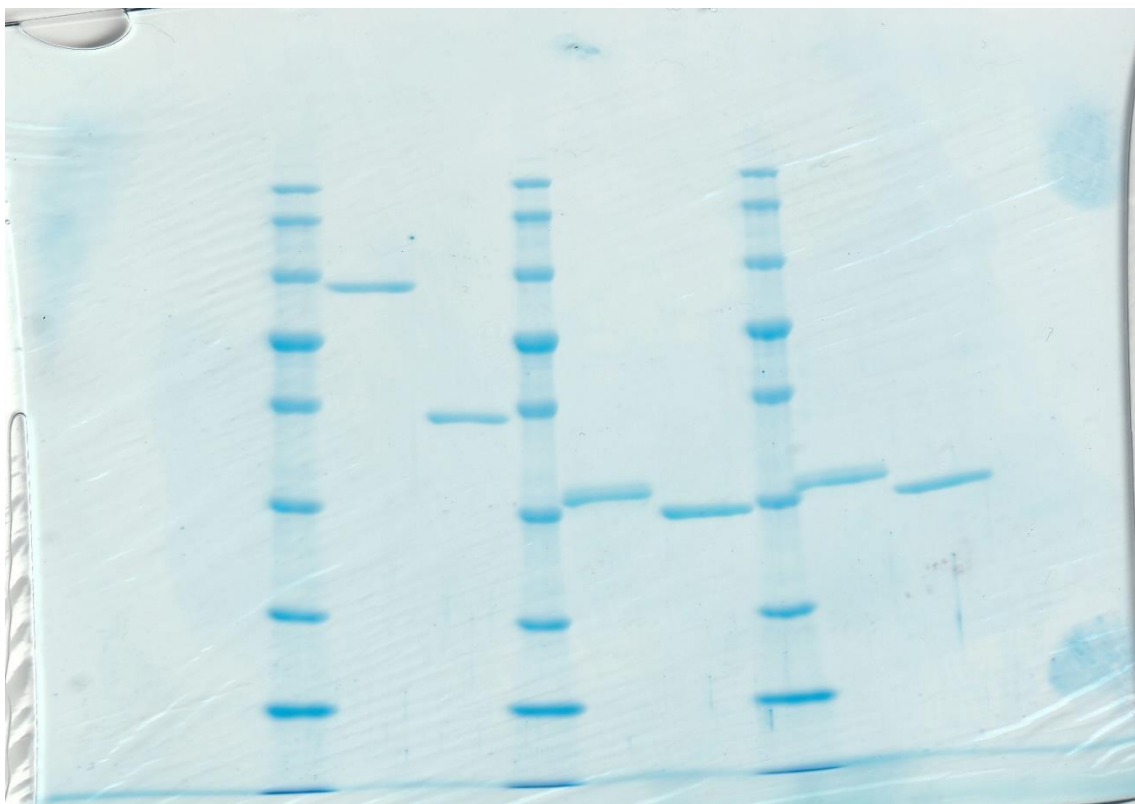
Supplementary Fig. S6. ESI-MS analysis of oligosaccharides in peaks 2 to 4 separated by HPLC. After separation by HPLC with an amide column, peaks 2 to 4 were collected, and collected samples were directly injected into the mass spectrometer (AccuTOF™ JMS-T100 LC, Jeol, Japan).



Supplementary Fig. S7. Cell wall binding assay of BGBD-GFP and AGBD-RFP for *A. oryzae* live mycelia. *A. oryzae* live mycelia were treated with Agl-KA at 0–2 nmol/ml. After 30-h treatment, mycelia were stained with AGBD-RFP (1.0 nmol/ml) and BGBD-GFP (1.0 nmol/ml).



Supplementary Fig. S8. The diameters of colonies formed by *Aspergillus oryzae* treated with Agl-EK14, Chi19MK, and BgluC16MK. The colony diameters were measured for several colonies located in the center of the lower right photograph in Fig. 7. The software “MicroStudio” (WRAYMER INC.; Osaka, Japan) was used for size measurement.



Supplementary Fig. S9. The original image of the SDS-PAGE analysis of the purified proteins used to construct Figure 1B.