(19) (KR) (11) 10-2010-0040438
(12) (A) (43) 2010 04 20

(51) Int. Cl.
C12N 9/42 (2006.01) *C12N 15/56* (2006.01)
C12N 15/09 (2006.01)
(21) 10-2008-0099538
(22) 2008 10 10
2008 10 10

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(71)
5 1
(72)
3 8-502
4 18-703
( )
(74)

(54)

(57)

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(72)
2 32-403
LG 103-1502
7 3-1003
651

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: 1 D- (D-galactose) 3,6- -L- (3,6-anhydro-L-galactose) β-1,4- (β-1,4- glycosidic bond) .

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[0001]
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[0002]
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[0003] .
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13,754 .(, 2006,
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[0004] (70%) (30%) D-
(D-galactose) 3,6- -L- (3,6-anhydro-L-galactose) α-1,3- (α-1,3-glycosidic bond) β-1,4- (β-1,4- glycosidic bond)
. ,
(pyruvic acid), (glucuronic acid) .(Duckwort.M, Yaphe, W. 1971 Carbohydr. Res. 16: 435-445)

[0005]
. immune system
.(Nomura, K., Naitoh, Y., Muramatsu, S., Yoshizawa, Y., Tsunehiro, J., Fukui, F., Itoh, M. 1997 Biosci. Biotechnol. Biochem. 62: 1190-1195)
(Kobayashi, R., Takisada, M., Suzuki, T., Kirimura, K., Usami, S. 1997 Biosci. Biotechnol. Biochem. 61: 162-163). prebiotics effect .(Hu, B., Gong, Q. H., Wang, Y., Ma, Y. M., Li, J. B., Yu, W. G. 2006 Anaerobe 12: 260-266). neoagarobiose neoagaro-oligosaccharide
.

[0006] .
.
.
.(Jingbao L., Feng H., Xinzhi L., Xiaoyan F., cuiping M., Yan C., Wengong Y. 2007 Carbohydr. Res. 342: 1030-1033).
.(Pickering, T. D., Sladden, V. H., Furneaux, R. H., Hemmingson, J. A., Redfearn, P. 1993 J. Appl. Phycol. 5: 85-91)

[0007] ,

,
. , 2002-9735
.

[0008] .
.(Araki C. 1959
Pergamon Press London, pp 15-30).
(furfural), HMF( ) (Boussaid, A., Robinson, J., Cai, Y.j., Gregg, D.J., Saddler, J.N., 1999. Biotechnol. Bioeng. 64: 284.289; Lohmeier-Vogel, E., Sopher, C., Lee, H., 1998. J. Ind. Microbiol. Biotechnol. 20: 75-81)
(degree of polymerization, DP) .
DP
,
.

[0009] , (*Pseudomonas*) (Ha, J. C., G. T. Kim, S. K. Kim, T. K. Oh, J. H. Yu, and I. S. Kong. 1997. Biotechnol. Appl. Biochem. 26:1-6), (*Alteromonas*) (Potin, P., C. Richard, C. Rochas, and B. Kloareg. 1993. Eur. J. Biochem. 214:599-607), (*Agarivorans*) (Ohta, Y., Y. Hatada, S. Ito, and K. Horikoshi. 2005. Biotechnol. Appl. Biochem. 41:183-191), (*Pseudoalteromonas*) (Belas, R. 1989. J. Bacteriol. 171:602-605), (*Microsilla*) (Zhong, Z., A. Toukdarian, D. Helinski, V. Knauf, S. Sykes, J. E. Wilkinson, C. O'Bryne, T. Shea, C. DeLoughery, and R. Caspi. 2001. Appl. Environ. Microbiol. 67:5771-5779) (*Vibrio*) (Aoki, T., T. Araki, and M. Kitamikado. 1990. Eur. J. Biochem. 187:461-465) .

[0010] ,
.

[0011] (Algae) . (US Department of Energy; DOE) 1978 1996
.

[0012]
.
. (Michael Briggs)
, (algae pond)
.
. 2006 6 Solix Biofuels
,
. 10
.(Current Biology Vol 18 No 2, R46).
22
(, 2008. 9. 22,
).

[0013] ,
.

[0014] (algae)

(agar polymer), (high DP agaro-oligosaccharides) one-step .

[0015]

[0016] : 1 D- (D-galactose) 3,6- -L- (3,6-anhydro-L-galactose) β-1,4- (β-1,4- glycosidic bond) (, 'Aga50D') .

[0017] , : 1 80% , 85% , 90% , 93% , 94% , 95% , 96% , 97% , 98% 99% .

[0018] (, 80%, 85%, 90%, 95%, 99%) , , . , , [CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel (eds) 1987 Supplement 30 section 7.7.18)] . , GCG Pileup , FASTA(Pearson 1988 Proc. Natl Acad. Sci USA85:2444-2448), BLAST (BLAST Manual, Altschul , Natl. Cent. Biotechnol. Inf., Natl Lib. Med. (NCIB NLM NIH), Bethesda, MD, Altschul 1997 NAR25:3389-3402) . ALIGN Plus(Scientific and Educational Software, PA) , . Sequence Software Package Version 6.0 (Genetics Computer Group, University of Wisconsin, Madison, WI) TFASTA Data Searching Program .

[0019] , .

[0020] (*Saccharophagus degradans* ATCC 43961) .

[0021] .

[0022] , 2 .

[0023] , .

[0024] ; , .

[0025] ; .

[0026] , .

[0027] .

[0028] , .

[0029] .

[0030] Agar 50D .

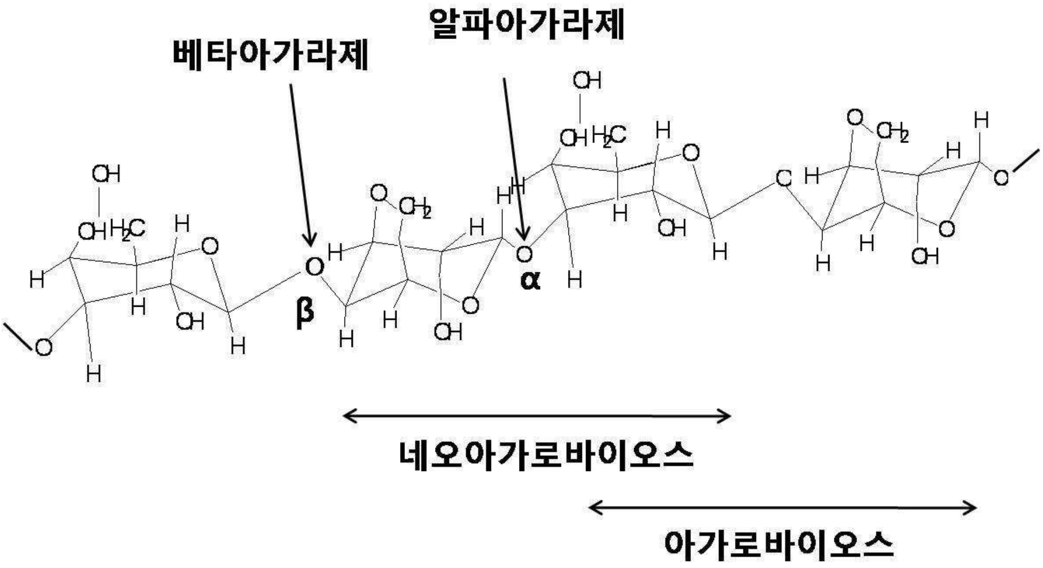
[0031] *Saccharophagus degradans 2-40*('*Microbulbifer degradans*

[0032] 2-40') (Chesapeake Bay) (saltwater cord grass)

Spartina alterniflora (Andrykovich, G., and I. Marx 1988 Appl. Microbiol. Biotechnol. 54: 1061-1062). *S. degradans* (Ekborg, N. A., J. M. Gonzalez, M. B. Howard, L. E. Taylor, S. W. Hutcheson, and R. M. Weiner. 2005. Int. J. Syst. Evol.Microbiol. 55: 1545-1549;Gonzalez, J. M., and R. M. Weiner. 2000. Int. J.Syst. Evol. Microbiol. 50:831-834) - (proteobacteria) . *S.degradans* 2-40 , , , , - , , , , , , (Andrykovich, G., and I. Marx. 1988. Appl. Microbiol. Biotechnol. 54: 1061-1062;Ensor, L., S. Stosz, and R. Weiner. 1999 J. Ind. Microbiol. Biotechnol. 23: 123-126;Howard, M. B., N. A. Ekborg, L. E. Taylor, R. M. Weiner, and S. W.Hutcheson 2003 J. Bacteriol. 185: 3352-33360; NKelly, S. K., V. Coyne, D. Sledjeski, C. Fuqua, and R. Weiner. 1990. FEMS Microbiol.Lett. 67: 275-280). (Howard, M. B., N. A. Ekborg, L. E. Taylor, R. M. Weiner, and S. W.Hutcheson. 2003 J. Bacteriol.185: 3352-33360).

[0033] -1,4 (agarobiose) , (agarotetraose), (agarohexaose) . -1,4 - (agarase) , -1,4 - . ' (agarobiose)' ' ' .

[0034]



[0035] , *Saccharophagus degradans* , *Saccharophagus degradans* .

[0036] , , , , . *Saccharophagus degradans* , .

[0037] , , " " , , , , . , ,

(( )) ,
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[0038] " " " " .
1 3 .

[0039] " " ,
, DNA .

[0040] " " DNA, RNA, .
" " " " .
, ,
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[0041] " "
. , , , , , , .

[0042] " " DNA
DNA DNA . ,
,

[0043] (operator) , mRNA ,
.

[0044] " " RNA
. (constitutive) .

[0045] " " "~ ", " " "~ ", "~
" , ,
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[0046] " " .
, ( )
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[0047] " ", " " " "
, , , .

[0048] " ", " " "
(transgenic)"
( ) (,) .

[0049] " " .
.

[0050] " " " (transfection)", " " "
(transduction)" , ,
(, , , , DNA) ,
, .

[0051] D- (D-galactose) 3,6- -L-
(3,6-anhydro-L-galactose) β-1,4- (β-1,4- glycosidic bond)
(, ,) ,

,
,
.

[0052] (algae)
(agar polymer), (high DP agaro-oligosaccharides)
one-step
, , .

[0053] .

[0054] .

[0055] 1:

[0056] DNA ((,)
DNA),
.

[0057] aga50d (
(EMBL) : CP000282, 2382 nt)
. . 1: 5ʻ-
atgttattcgattttgaaaacgatcaag-3ʼ(28mer)(:3), 2: 5ʻ- tttgctgcctagcctttcgg-3ʼ(20mer)(
:4). 1 2 (:
95 (30)- 58 (30)- 72 (2), 25), .

(pET21a (,)) .
6
.

[0058] 2:

[0059] E. coli BL21 (DE3)(,)
LB broth OD 600nm 1.0 0.1mM isopropyl- -D-thiogalactopyranoside
. ( )
(1). , 5,000rpm 15 lysis
buffer (20 mM sodium phosphate 300mM sodium chloride, pH 7.4)
. 16,000 rpm 1 4
. SDS-PAGE . His-trap column (GE
Healthcare, Piscataway, NJ, USA) . Amicon Ultra-15 Centrifugal Device
(Millipore, Billerica, MA, USA) 60mg/ml .

[0060] 3:

[0061] TLC

[0062] Aga50D 0.25% Tris-HCL(pH6.8)
. 50ul 1ul 2
TLC . TLC n-butanol:ethanol:water 3:2:2
(10% v/v in ethanol) .

[0063] 2 .

[0064] HPLC

[0065] TLC GPC(gel permeation column; Shodex KS-802, Shodex, Kawasaki, Japan) (Agilent 1100, Agilent Technologies, Waldbronn, Germany) . 0.5ml/min 80
RID detector . 3 ,
HPLC retention time 16.7 18.4
. Aga50D ,
9.4 16.0 . 16.0
retention time Aga50D
(3).

[0066] Aga50D 30 , 1 , 2 , 12 GPC HPLC
.
(4).

[0067] Molecular size marker 9.4

[0068] (poly molecular wight standard kit, waters corpoation,Milford, Massachusetts)
106, 222, 434, 626, 985, 1,400Da f = 86,154×
exp(-0.6877× x) (caibration curve) .(R square=0.99)(5).
retention time 16.0 (elution volume) 8.0ml 346.50
Da . 324Da (dimer) .
6ml
3,400Da DP 20
. DP 2 DP 10
- (Lee, D.-G. Lee, M. K. Jang, O.-H Lee, N. Y. Kim, S.-A. Ju, S.-H. Lee. 2008. Biotechnol. Lett. 30: 911-918; Dong, J., Y. Tamaru, and T. Araki. 2007. Biosci. Biotechnol. Biochem. 71: 38-46; Ohta, Y., Y. Hatada, S. Ito, and K. Horikoshi. 2005. Biotechnol. Appl. Biochem. 41: 183-191)

.

[0069] - (Q-Tof micro, Waters, Milford, Massachusetts)

[0070] Aga50D - (Q-Tof micro,
Waters, Milford, Massachusetts) . - -3,6-
324 4 , 6 , 8 630, 936, 1242 LC-MS
323, 647, 970, 1294m/z 324 .
323 m/z LC/MS
.
. GPC HPLC Aga50D 3,6- -L-
- -1,3-D-)( ) .(6).

[0071] NMR Aga 50D

[0072] Aga 50D neoagarobiose agarobiose (Varian NMR Systems 500MHz, Varian,Inc., Palo Alto, CA) . 97.110 93.124ppm
resonance resonance reducing end D-galactose β α anomeric carbon
.(CYRILLE ROCHAS, MARC LAHAYE, WILFRED YAPHE 1986 Carbohydr. Res. 148: 199-207).
agarooligosaccharide reducing end 3,6-anhydro-L-galactose resonance 90.72 ppm
. (Cyrille Rochas, Philippe Potin, and Bernard Kloareg 1994 Carbohydr. Res. 253: 69-77)
aga50D . (7)

[0073] .

[0074] 1 12% SDS-polyacrylamide gel eletrophoresis ; 1 , (aga50D) .

[0075] 2 (Aga50D) TLC(Thin layer chromatography) . 20 mM Tris-HCl(pH6.8) 40 , 0.25% (w/v) . , 1 2 . A: ; B: Aga50D (30); C: Aga50D (2) .

[0076] 3 GPC(gel permeation column; Shodex KS-802, Shodex, Kawasaki, Japan) (Agilent 1100, Agilent Technologies, Waldbronn, Germany) . 0.5ml/min 80 RID detector . 3a , 3b HPLC .

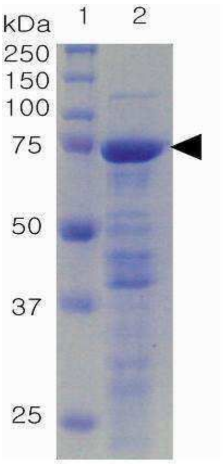
[0077] 4 Aga50D 30 , 1 , 2 , 12 GPC HPLC . 4a 30 , 1 , 2 4b 12 .

[0078] 5 (poly molecular wight standard kit, waters corpoation,Milford, Massachusetts) 106, 222, 434, 626, 985, 1,400 Da .

[0079] 6 LC mass .

[0080] 7 aga50D neoagarobiose NMR .

1



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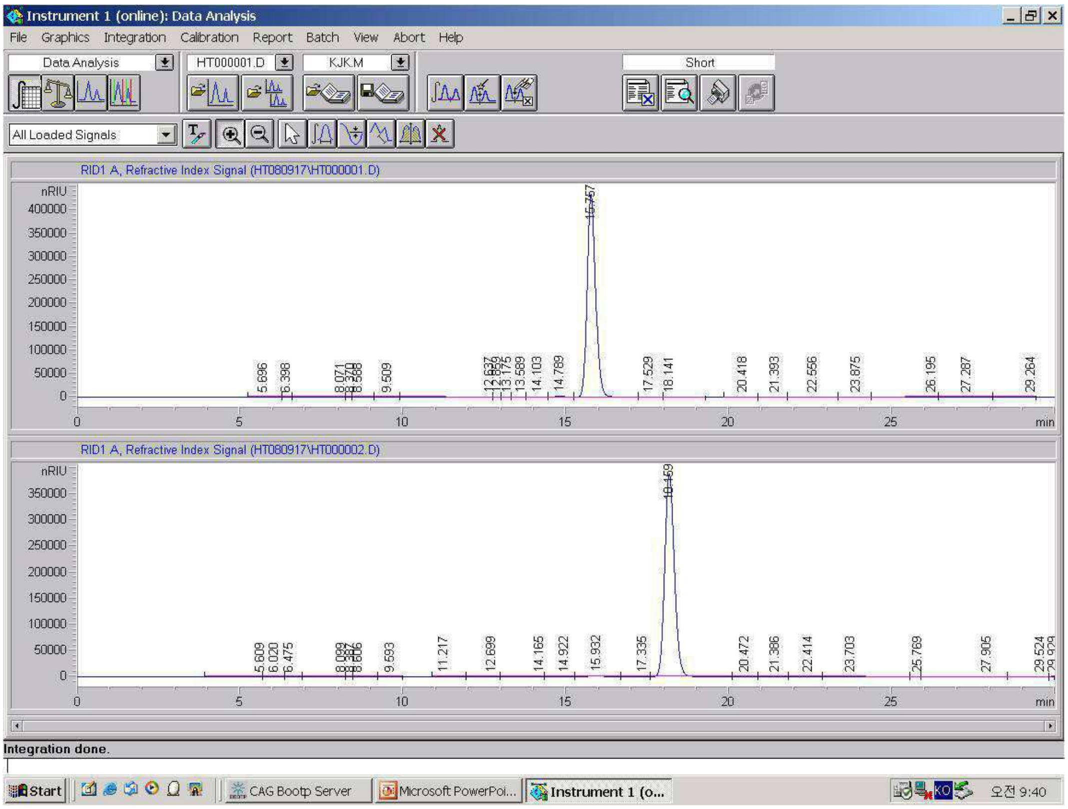
3a



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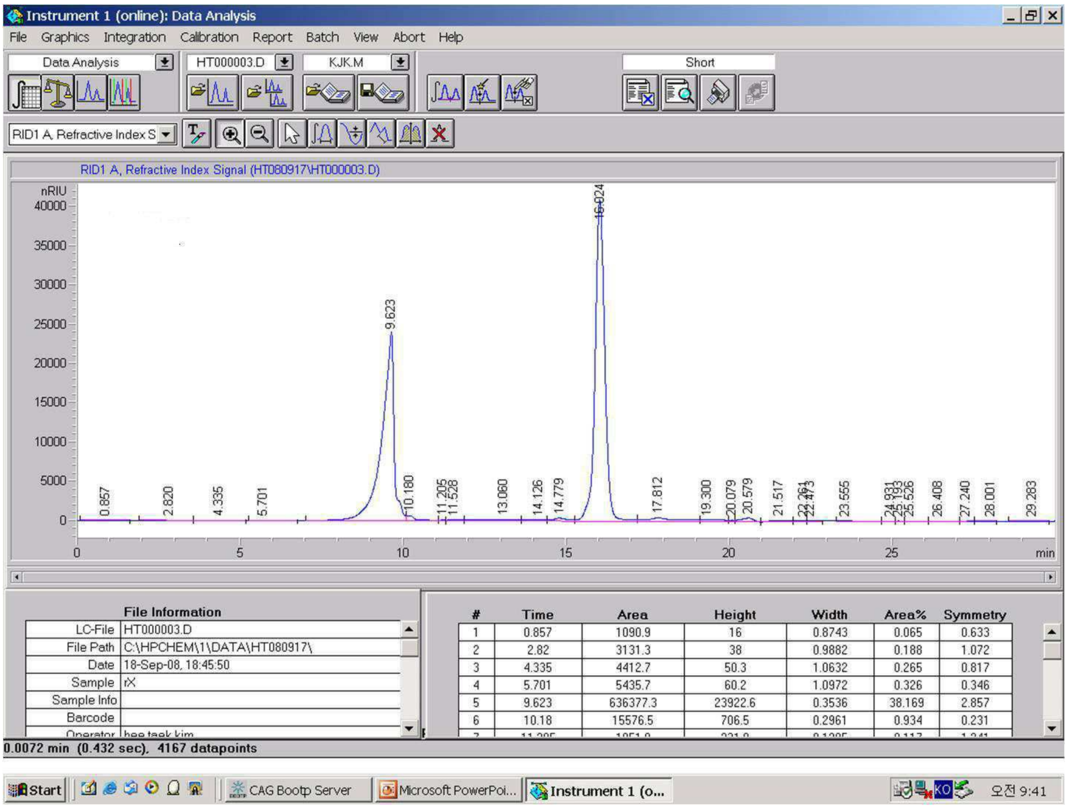
3b



File Information

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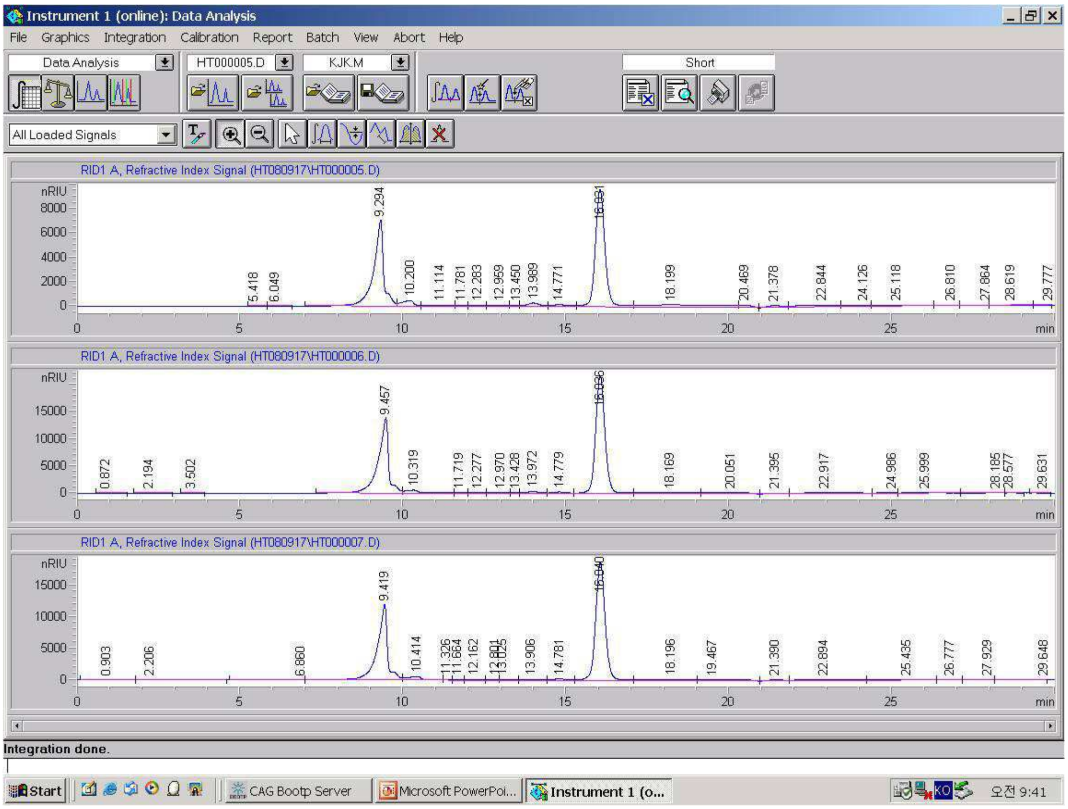
Sample	rX
Sample Info	
Barcode	
Operator	hee taek kim

#	Time	Area	Height	Width	Area%	Symmetry
1	0.857	1090.9	16	0.8743	0.065	0.633
2	2.82	3131.3	38	0.9882	0.188	1.072
3	4.335	4412.7	50.3	1.0632	0.265	0.817
4	5.701	5435.7	60.2	1.0972	0.326	0.346
5	9.623	636377.3	23922.6	0.3536	38.169	2.857
6	10.18	15576.5	706.5	0.2961	0.934	0.231

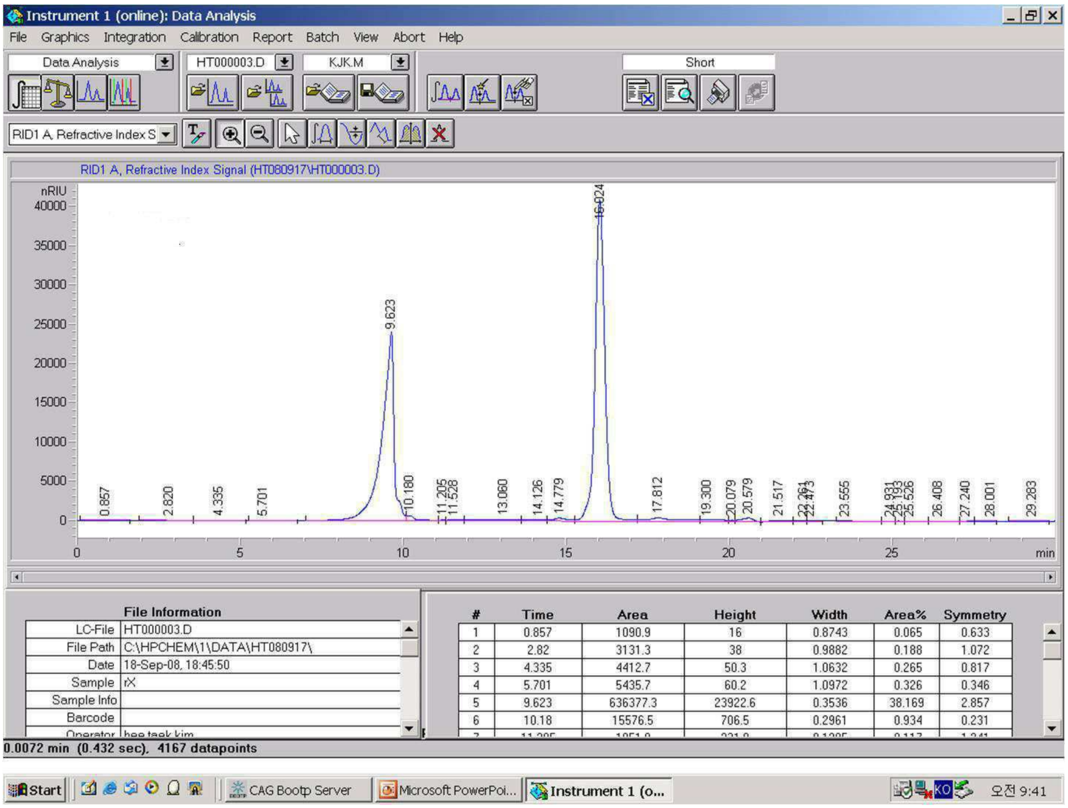
0.0072 min (0.432 sec), 4167 datapoints

4a



Integration done.

4b



File Information

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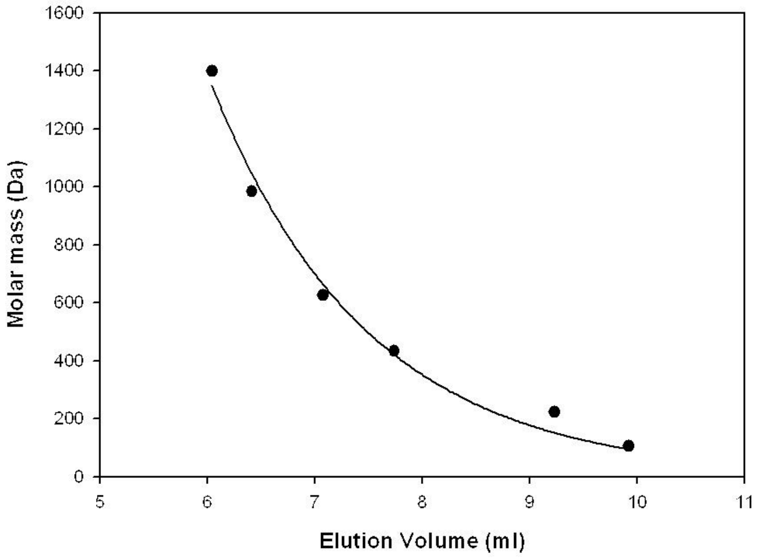
Date	18-Sep-08, 18:45:50
Sample	rX
Sample Info	
Barcode	
Operator	hee taek kim

#	Time	Area	Height	Width	Area%	Symmetry
1	0.857	1090.9	16	0.8743	0.065	0.633
2	2.82	3131.3	38	0.9882	0.188	1.072
3	4.335	4412.7	50.3	1.0632	0.265	0.817
4	5.701	5435.7	60.2	1.0972	0.326	0.346
5	9.623	636377.3	23922.6	0.3536	38.169	2.857
6	10.18	15576.5	706.5	0.2961	0.934	0.231

0.0072 min (0.432 sec). 4167 datapoints

5



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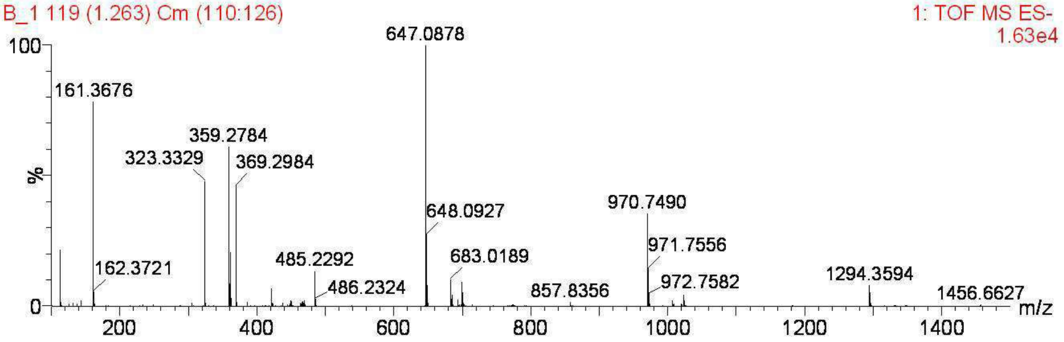

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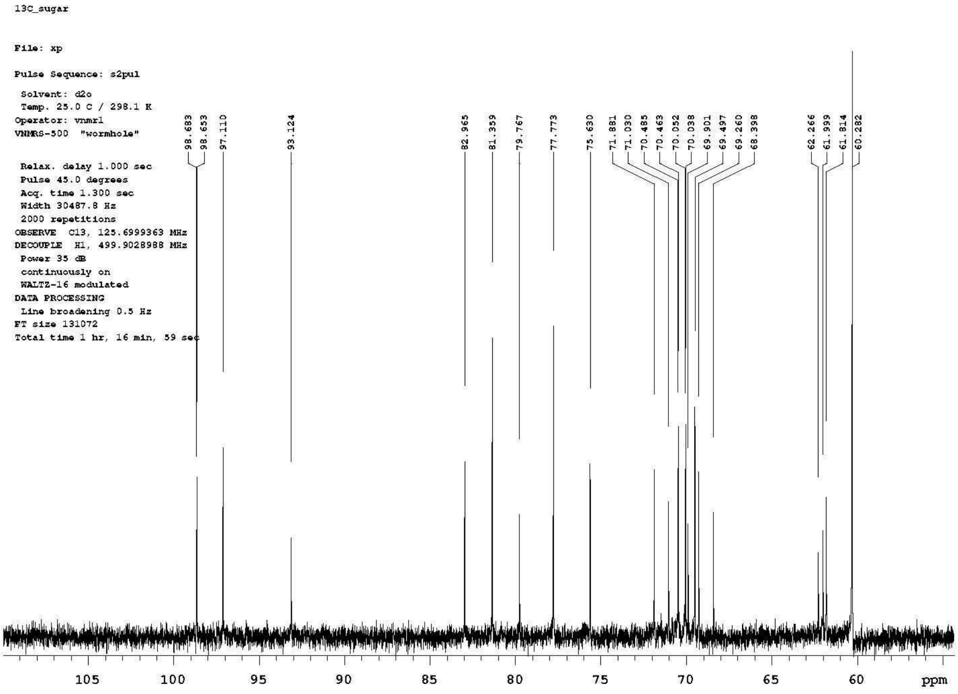

<110> KOREA UNIVERSITY RESEARCH AND BUSINESS FOUNDATION

<120> A NOVEL AGARASE AND AN ENZYMATIC PRODUCTION METHOD OF
AGAROOLIGOSACCHARIDE FROM AGAROSE USING THE SAME

<160> 4

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<212>    PRT
<213>    Artificial Sequence


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<223>    Beta-agarase



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Leu Asn Ala Arg Ala Ser Ile Glu Thr Tyr Thr Gly Ile Asn Gly Glu
             20                  25                  30


Pro Ser Lys Gly Leu Lys Leu Ala Met Gln Ser Lys Gln His Ser Tyr
         35                  40                  45


Thr Gly Leu Ala Ile Val Pro Glu Gln Pro Trp Asp Trp Ser Glu Phe
     50                  55                  60


Thr Ser Ala Ser Leu Tyr Phe Asp Ile Val Ser Val Gly Asp His Ser
 65                  70                  75                  80


Thr Gln Phe Tyr Leu Asp Val Thr Asp Gln Asn Gly Ala Val Phe Thr
                 85                  90                  95


Arg Ser Ile Asp Ile Pro Val Gly Lys Met Gln Ser Tyr Tyr Ala Lys
            100                 105                 110


Leu Ser Gly His Asp Leu Glu Val Pro Asp Ser Gly Asp Val Asn Asp
        115                 120                 125


Leu Asn Leu Ala Ser Gly Leu Arg Ser Asn Pro Pro Thr Trp Thr Ser
    130                 135                 140


Asp Asp Arg Gln Phe Val Trp Met Trp Gly Val Lys Asn Leu Asp Leu
145                 150                 155                 160


Ser Gly Ile Ala Lys Ile Ser Leu Ser Val Gln Ser Ala Met His Asp
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                165                 170                 175

Lys Thr Val Ile Ile Asp Asn Ile Arg Ile Gln Pro Asn Pro Pro Gln
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Asp Glu Asn Phe Leu Val Gly Leu Val Asp Glu Phe Gly Gln Asn Ala
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Lys Val Asp Tyr Lys Gly Lys Ile His Ser Leu Glu Glu Leu His Ala
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Ala Arg Asp Val Glu Leu Ala Glu Leu Asp Gly Lys Pro Met Pro Ser
225                 230                 235                 240

Arg Ser Lys Phe Gly Gly Trp Leu Ala Gly Pro Lys Leu Lys Ala Thr
                245                 250                 255

Gly Tyr Phe Arg Thr Glu Lys Ile Asn Gly Lys Trp Met Leu Val Asp
            260                 265                 270

Pro Glu Gly Tyr Pro Tyr Phe Ala Thr Gly Leu Asp Ile Ile Arg Leu
        275                 280                 285

Ser Asn Ser Ser Thr Met Thr Gly Tyr Asp Tyr Asp Gln Ala Thr Val
    290                 295                 300

Ala Gln Arg Ser Ala Asp Asp Val Thr Pro Glu Asp Ser Lys Gly Leu
305                 310                 315                 320

Met Ala Val Ser Glu Lys Ser Phe Ala Thr Arg His Leu Ala Ser Pro
                325                 330                 335

Thr Arg Ala Ala Met Phe Asn Trp Leu Pro Asp Tyr Asp His Pro Leu
            340                 345                 350

Ala Asn His Tyr Asn Tyr Arg Arg Ser Ala His Ser Gly Pro Leu Lys
        355                 360                 365

Arg Gly Glu Ala Tyr Ser Phe Tyr Ser Ala Asn Leu Glu Arg Lys Tyr
    370                 375                 380

Gly Glu Thr Tyr Pro Gly Ser Tyr Leu Asp Lys Trp Arg Glu Val Thr
385                 390                 395                 400
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Val Asp Arg Met Leu Asn Trp Gly Phe Thr Ser Leu Gly Asn Trp Thr
                405                 410                 415

Asp Pro Ala Tyr Tyr Asp Asn Asn Arg Ile Pro Phe Phe Ala Asn Gly
            420                 425                 430

Trp Val Ile Gly Asp Phe Lys Thr Val Ser Ser Gly Ala Asp Phe Trp
        435                 440                 445

Gly Ala Met Pro Asp Val Phe Asp Pro Glu Phe Lys Val Arg Ala Met
    450                 455                 460

Glu Thr Ala Arg Val Val Ser Glu Glu Ile Lys Asn Ser Pro Trp Cys
465                 470                 475                 480

Val Gly Val Phe Ile Asp Asn Glu Lys Ser Phe Gly Arg Pro Asp Ser
                485                 490                 495

Asp Lys Ala Gln Tyr Gly Ile Pro Ile His Thr Leu Gly Arg Pro Ser
            500                 505                 510

Glu Gly Val Pro Thr Arg Gln Ala Phe Ser Lys Leu Leu Lys Ala Lys
        515                 520                 525

Tyr Lys Thr Ile Ala Ala Leu Asn Asn Ala Trp Gly Leu Lys Leu Ser
    530                 535                 540

Ser Trp Ala Glu Phe Asp Leu Gly Val Asp Val Lys Ala Leu Pro Val
545                 550                 555                 560

Thr Asp Thr Leu Arg Ala Asp Tyr Ser Met Leu Leu Ser Ala Tyr Ala
                565                 570                 575

Asp Gln Tyr Phe Lys Val Val His Gly Ala Val Glu His Tyr Met Pro
            580                 585                 590

Asn His Leu Tyr Leu Gly Ala Arg Phe Pro Asp Trp Gly Met Pro Met
        595                 600                 605

Glu Val Val Lys Ala Ala Ala Lys Tyr Ala Asp Val Val Ser Tyr Asn
    610                 615                 620

Ser Tyr Lys Glu Gly Leu Pro Lys Gln Lys Trp Ala Phe Leu Ala Glu
625                 630                 635                 640
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Leu Asp Lys Pro Ser Ile Ile Gly Glu Phe His Ile Gly Ala Met Asp
                645                 650                 655


His Gly Ser Tyr His Pro Gly Leu Ile His Ala Ala Ser Gln Ala Asp
            660                 665                 670


Arg Gly Glu Met Tyr Lys Asp Tyr Met Gln Ser Val Ile Asp Asn Pro
        675                 680                 685


Tyr Phe Val Gly Ala His Trp Phe Gln Tyr Met Asp Ser Pro Leu Thr
    690                 695                 700


Gly Arg Ala Tyr Asp Gly Glu Asn Tyr Asn Val Gly Phe Val Asp Val
705                 710                 715                 720


Thr Asp Thr Pro Tyr Gln Glu Met Val Asp Ala Ala Lys Glu Val Asn
                725                 730                 735


Ala Lys Ile Tyr Thr Glu Arg Leu Gly Ser Lys
            740                 745




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<212>    DNA
<213>    Artificial Sequence


<220>
<223>    Gene of beta-agarase



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gcaagtatag aaacctatac cggtataaat ggcgagccga gtaaagggtt aaagttggcg        120


atgcagtcca agcagcacag ttatactggc cttgccattg tgccagagca gccttgggat        180


tggagcgagt ttacctctgc tagcttgtat ttcgatatag tcagtgttgg cgatcattcc        240


acacaatttt atttagatgt taccgaccaa aatggcgccg tgtttacccg cagtattgat        300
```

attccagtgg gtaaaatgca atcgtactac gccaagttaa gcggtcacga tttagaagtg 360

cccgatagtg gagacgttaa cgatttaaac ctcgcctctg gcttgcgttc taacccgcct 420

acatggacat ctgacgatag gcagtttgtt tggatgtggg gagtgaaaaa tttagatttg 480

tcgggcattg ctaaaatatc gctaagtgtg caaagcgcaa tgcacgataa aacagttatt 540

atcgataata ttcgtattca acccaacccg ccgcaagatg aaaacttcct tgtcggtttg 600

gtagacgagt ttggccaaaa cgccaaagtt gattacaagg gtaaaatcca tagtttagaa 660

gaattgcatg cagcgcgcga tgtggaactg gccgagcttg atggcaagcc aatgcctagt 720

cgctctaagt ttggcggttg gttggccggc cccaagctaa aagctacagg gtactttcgc 780

acagaaaaaa ttaacggtaa atggatgcta gtagacccag aagggtaccc ttactttgct 840

acgggtttag acattattcg cctatctaat tcatctacca tgactggtta cgattacgat 900

caagctactg ttgctcagcg ctctgccgac gatgtaacac ctgaagactc aaaaggttta 960

atggcagtga gcgaaaaatc atttgctacg cgccacctag catcgccaac acgagcggca 1020

atgtttaact ggttgccaga ttacgatcac cctctcgcaa atcattataa ctaccgtcgc 1080

tctgcgcatt ccggcccact gaaacgcggt gaagcctaca gcttctacag tgccaacctt 1140

gagcgtaaat acggtgaaac ttaccccggt tcttacttgg ataagtggcg cgaagtaacg 1200

gtagacagaa tgctaaactg gggctttacc tcgctaggca actggactga cccagcatat 1260

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