Table P1. Primers used for mutant construction.

Name	5' Composition 3'	Sequence position	orf
Primers to construct the in-frame deletion mutant AH-3Δ2.1			
2.1-A	ACGCGTCGACCGTCTATGGCTACTCCAAGC (*Sal*I site)	1016	1.1
2.1-B	CCCATCCACTAAACTTAAACAACCACGTCGGGTCAATTCGT	1690	2.1
2.1-C	TGTTTAAGTTTAGTGGATGGGGCCATTTTCCCTCGTACCGA	3289	2.1
2.1-D	ACGCGTCGACGGCTCTGGGAGAGATAAAGC (*Sal*I site)	3953	3.1
Primers to construct the in-frame deletion mutant AH-3Δ3.1			
3.1-A	ACGCGTCGACGCTATCAGGACAACCTGACG (*Sal*I site)	2850	2.1
3.1-B	CCCATCCACTAAACTTAAACATGTGGTGGTTTTATCGAGCA	3409	3.1
3.1-C	TGTTTAAGTTTAGTGGATGGGTTTCTTGGCCTGCCGCTC	4382	3.1
3.1-D	ACGCGTCGACAGATATCTGGCAAGCCGATA(*Sal*I site)	5004	4.1
Primers to construct the in-frame deletion mutant AH-3Δ4.1			
4.1-A	GGAAGATCTGTGGAAATACGTTGCGGTAG (*Bgl*II site)	4021	3.1
4.1-B	CCCATCCACTAAACTTAAACACATCTGCTGTACAACCATCG	4645	4.1
4.1-C	TGTTTAAGTTTAGTGGATGGGCCTTCCGATTATCTGACTGGT	5111	4.1
4.1-D	GGAAGATCTGGTGGCCTTTTCGTTGAACA (*Bgl*II site)	5810	5.1
Primers to construct the in-frame deletion mutant AH-3Δ5.1			
5.1-A	GGAAGATCTGTTTTGGTGAGATTGGCTGT (*Bgl*II site)	4764	4.1
5.1-B	CCCATCCACTAAACTTAAACACACGTTTGCCACTTCTGTT	5429	5.1
5.1-C	TGTTTAAGTTTAGTGGATGGGGACAACCTTTCCGCTCTTTC	6294	5.1
5.1-D	GGAAGATCTACTCCGAGTCGATTCAACAG (*Bgl*II site)	6931	6.1
Primers to construct the defined insertion mutant AH-3Δ7.1			
7.1-F	CGCTACATGAAAGACCTGC	8331	7.1
7.1-R	CTGATCAAACACGCTTCCC	7792	7.1
Primers to construct the in-frame deletion mutant AH-3Δ2.2			
2.2-A	ACGCGTCGACGCACCCATTCTGACTGGAC (*Sal*I site)	3184	3.2
2.2-B	CCCATCCACTAAACTTAAACAGAGGCTCTGGGACATCACC	2643	2.2
2.2-C	TGTTTAAGTTTAGTGGATGGGCCGGAGCAGGTGATAGAG	1715	2.2
2.2-D	ACGCGTCGACGGGCATTGATGATGGTCAC (*Sal*I site)	1126	1.2
Primers to construct the defined insertion mutant AH-3Δ4.2			
4.2-F	AGTTTTGCCATGCTCAAGC	4777	4.2
4.2-R	CAGTATGCACTGCAACTCG	4178	4.2

Table P2. Primers used for mutant complementation with different plasmid vectors.

Name	5' Composition 3'	Sequence position	orf
COMPLEMENTATION WITH pBAD33			
Primers to complement the mutant AH-3Δ2.1			
2.1-F	TCCCCCGGGGGTTTGATCCCCCTCTGAC (*Sma*I site)	1544	1.1
2.1-R	CTAGTCTAGAATGGTCAATGCAAACAGGG (*Xba*I site)	3429	3.1
Primers to complement the mutant AH-3Δ3.1			
3.1-F	TCCCCCGGGTTTCCCTCGTACCGATGTT (*Sma*I site)	3295	2.1
3.1-R	CTAGTCTAGAACACTTCTTTGGCCACGTC (*Xba*I site)	4748	4.1
Primers to complement the mutant AH-3Δ4.1			
4.1-F	TCCCCCGGGTTTCTGCAGGGGTACTGGT (*Sma*I site)	4428	3.1
4.1-R	CTAGTCTAGACGTTTGCCACTTCTGTTCC (*Xba*I site)	5427	5.1
Primers to complement the mutant AH-3Δ5.1			
5.1-F	TCCCCCGGGGGGATTGGGGTCAGTAAAC (*Sma*I site)	5259	5' of 5.1
5.1-R	CTAGTCTAGACTTGAGTTGCAGGTCGTTG (*Xba*I site)	6440	6.1
Primers to complement the mutant AH-3Δ6.1			
6.1-F	TCCCCCGGGAACCTTTCCGCTCTTTCGC (*Sma*I site)	6294	5.1
6.1-R	CTAGTCTAGACAAGATCATCGCCAATCCG (*Xba*I site)	7654	7.1
Primers to complement the mutant AH-3Δ7.1			
7.1-F2	TCCCCCGGGGATGCTCAACAACGACCTC (*Sma*I site)	8561	5' of 7.1
7.1-R2	CTAGTCTAGAGCGAACAGGACATCAACTC (*Xba*I site)	7411	6.1
Primers to complement the mutant AH-3Δ2.2			
2.2-F	AAAAGTACTGGCATAATCTCGGCCAG (*Sca*I site)	2760	5' of 2.2
2.2-R	CTAGTCTAGAGGGTTCTTCGGCTTGTAGA (*Xba*I site)	1503	1.2
Primers to complement the mutant AH-3Δ3.2			
3.2-F	TCCCCCGGGAATTCGAGCATGGCCCGC (*Sma*I site)	2896	5' of 3.2
3.2-R	CTAGTCTAGAGCGATCACCCGGAAGACA (*Xba*I site)	3888	4.2
Primers to complement the mutant AH-3Δ4.2			
4.2-F2	TCCCCCGGGCTCCTTGCATTGGTGACAG (*Sma*I site)	4874	5' of 4.2
4.2-R2	CTAGTCTAGAGTCAAATACGCCGACCTCT (*Xba*I site)	3670	3.2
Primers to complement the mutant AH-3Δ1.3			
1.3-F	AAAAGTACTCATCCATTTTGCCACCATT (*Sca*I site)	1505	5' of 1.3
1.3-R	CTAGTCTAGAGGTAGAGGCCAGCAGGTTA (*Xba*I site)	366	3' of 1.3
COMPLEMENTATION WITH pGEMT-easy			
Primers to complement *E. coli* CJB26			
2.3-F	GTGACAACAATCCCCGATG	1405	1.3
2.3-R	ATCAGCGCCAGATCAAACT	2503	3' of 2.3
1.2-F	ACGCGTCGACCCGATCGTGCTGCAAGTG (*Sal*I site)	1658	2.2
1.2-R	ACGCGTCGACCACGACCTTCAGCGACTC (*Sal*I site)	160	3' of 1.2

Figure A. Charge deconvoluted electrospray ionization mass spectra of the core oligosaccharides released by mild acid hydrolysis from the LPSs of *A. hydrophila* mutants. Figure 6D shows a higher-molecular-mass part of the spectrum; a lower-molecular-mass part contains ion peaks at *m/z* 958.30 (major) and 1150.38 (minor). Schematic structures and the calculated molecular masses (M_r) of the most representative compounds are shown in the insets.

Figure B. Charge deconvoluted electrospray ionization mass spectrum of the whole LPS of *A. hydrophila waaC* mutant. Schematic structure of the major compound and the calculated molecular mass (M_r) are shown in the inset. 12:0, lauric acid; 3HO14:0, 3-hydroxymyristic acid.

Figure A

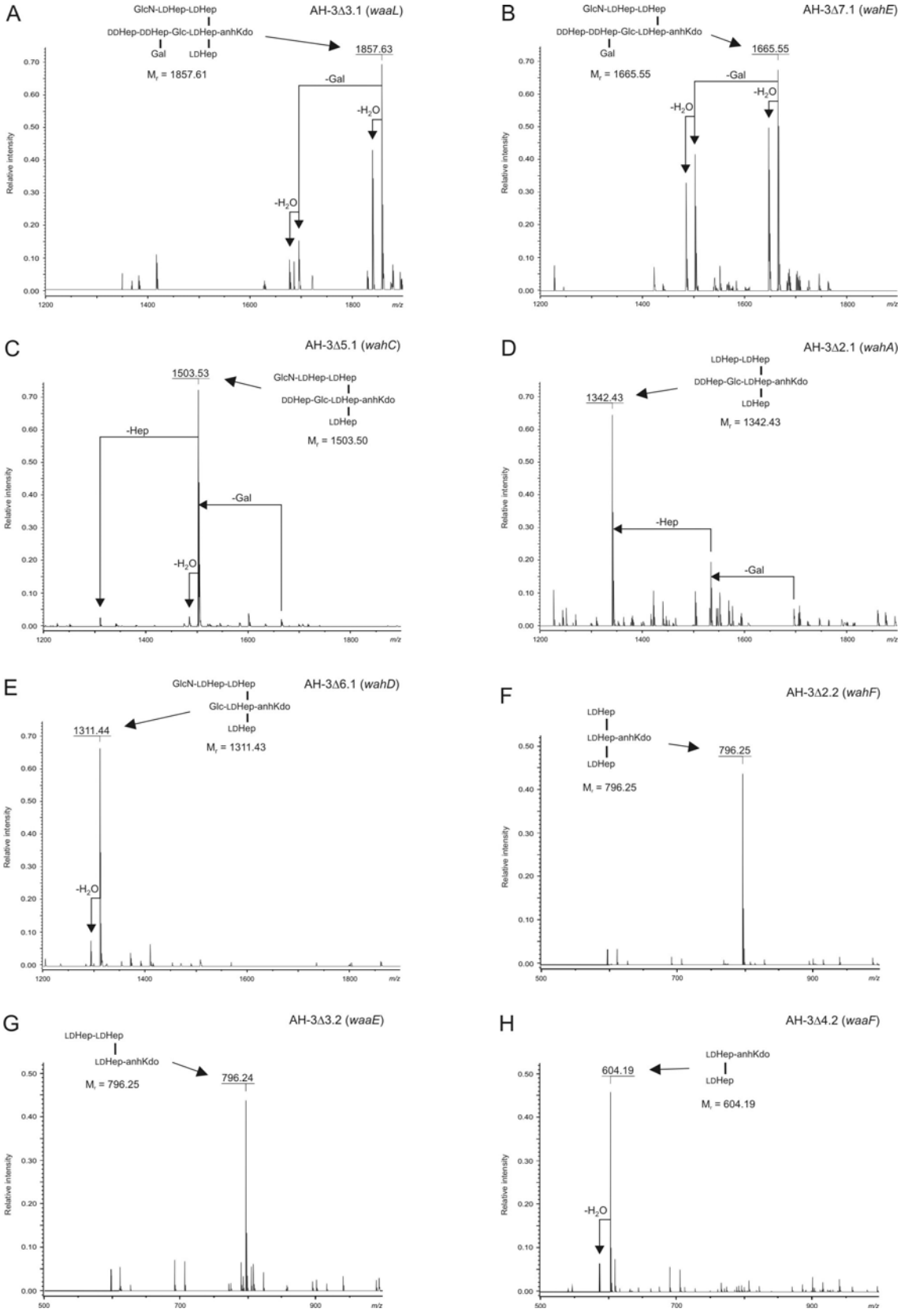
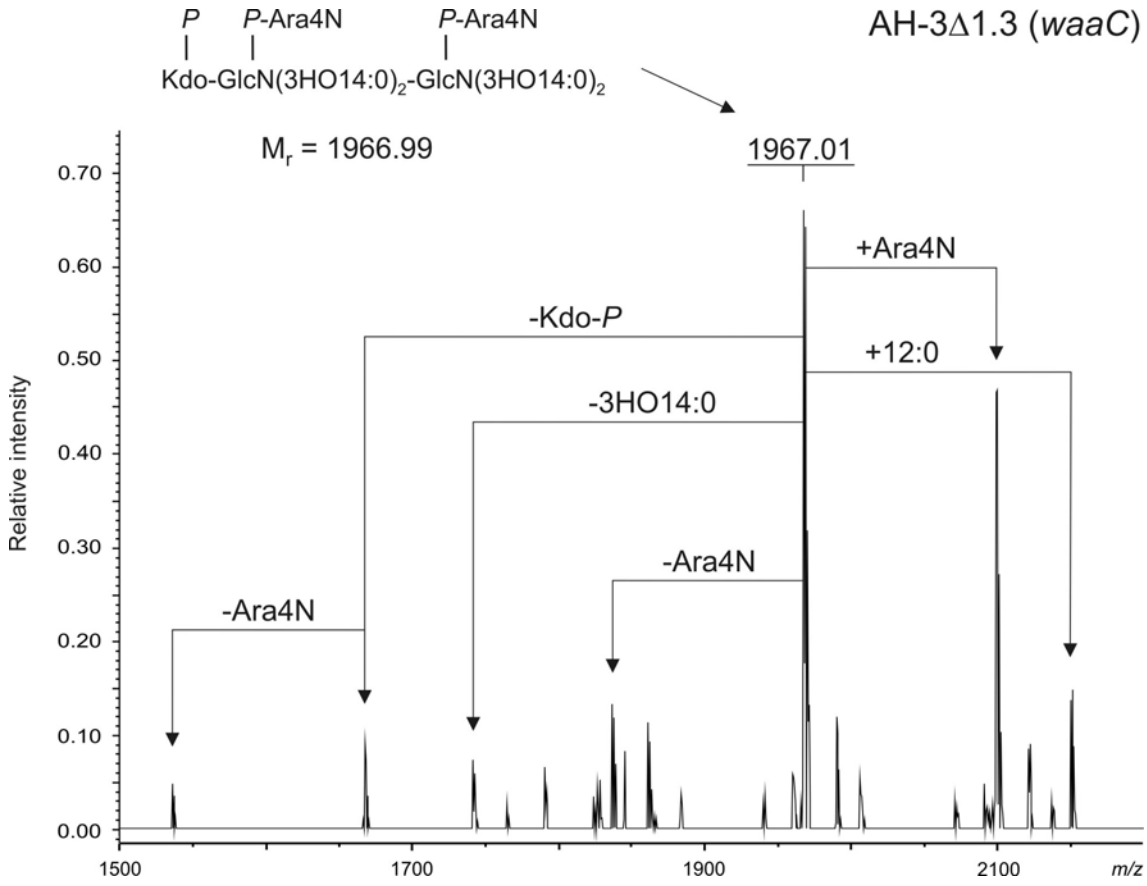

A
AH-3Δ3.1 (waaL)
GlcN-LDHep-LDHep
DDHep-DDHep-Glc-LDHep-anhKdo
Gal
LDHep
1857.63
Mr = 1857.61
-Gal
-H2O
-H2O
B
AH-3Δ7.1 (wahE)
GlcN-LDHep-LDHep
DDHep-DDHep-Glc-LDHep-anhKdo
Gal
1665.55
Mr = 1665.55
-Gal
-H2O
-H2O
C
AH-3Δ5.1 (wahC)
1503.53
GlcN-LDHep-LDHep
DDHep-Glc-LDHep-anhKdo
LDHep
Mr = 1503.50
-Hep
-Gal
-H2O
D
AH-3Δ2.1 (wahA)
LDHep-LDHep
DDHep-Glc-LDHep-anhKdo
LDHep
1342.43
Mr = 1342.43
-Hep
-Gal
E
AH-3Δ6.1 (wahD)
GlcN-LDHep-LDHep
Glc-LDHep-anhKdo
LDHep
1311.44
Mr = 1311.43
-H2O
F
AH-3Δ2.2 (wahF)
LDHep
LDHep-anhKdo
LDHep
796.25
Mr = 796.25
G
AH-3Δ3.2 (waaE)
LDHep-LDHep
LDHep-anhKdo
Mr = 796.25
796.24
H
AH-3Δ4.2 (waaF)
LDHep-anhKdo
LDHep
604.19
Mr = 604.19
-H2O
Relative intensity
m/z

Figure B


P
P-Ara4N
P-Ara4N
Kdo-GlcN(3HO14:0)2-GlcN(3HO14:0)2
AH-3Δ1.3 (waaC)
Mr = 1966.99
1967.01
+Ara4N
-Kdo-P
+12:0
-3HO14:0
-Ara4N
-Ara4N
Relative intensity
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
1500
1700
1900
2100
m/z