The fish pathogen *Aliivibrio salmonicida* LFI1238 can degrade and metabolize chitin despite major gene loss in the chitinolytic pathway

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Supplementary material

Supplementary figures

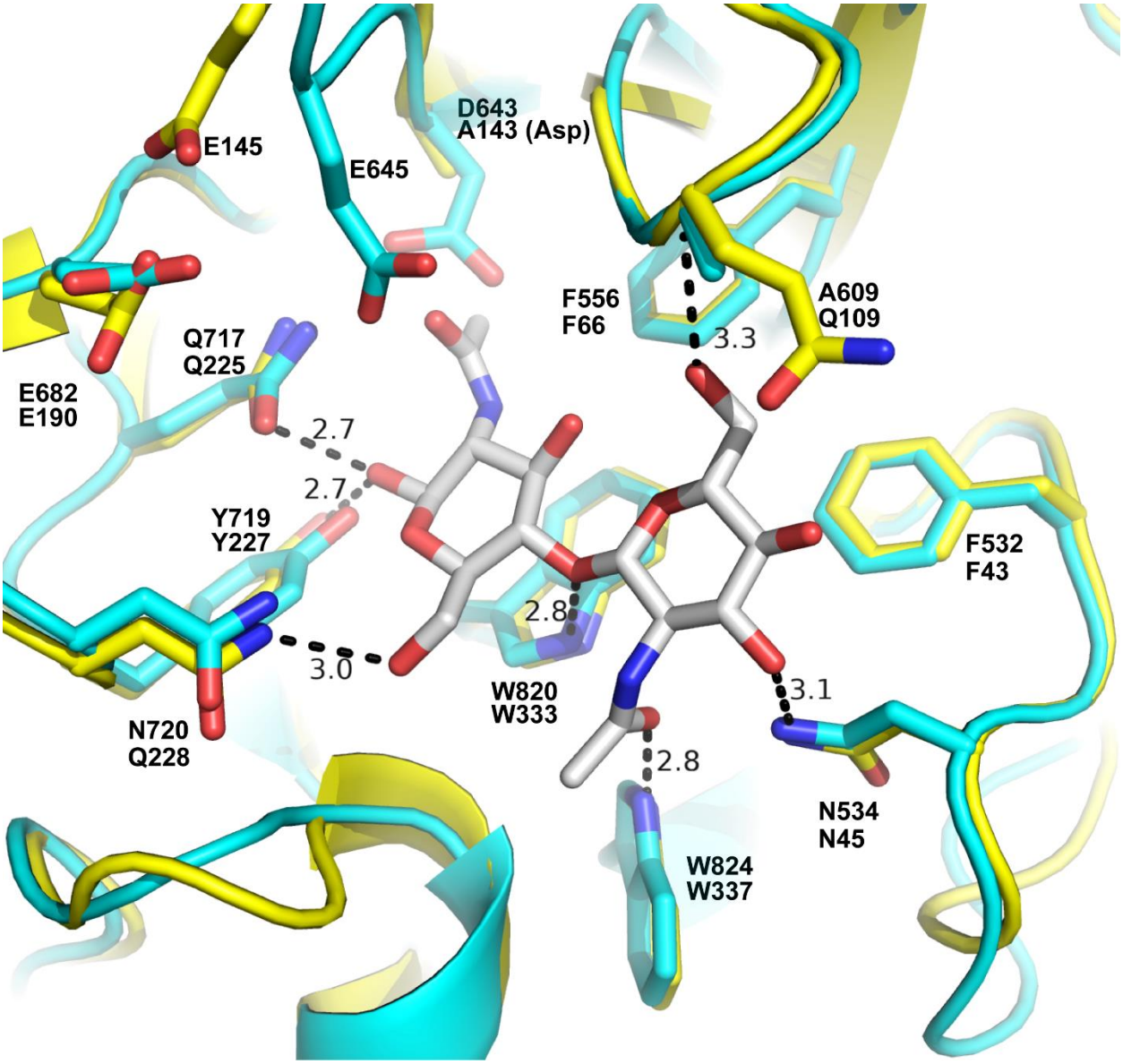
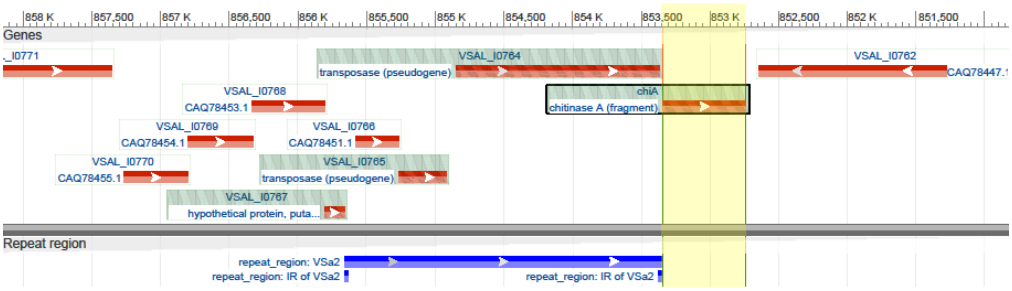


Figure S1. Active site of the *As*Chi18A homology model superimposed on ChiNCTU2 (D143A variant). Proteins are shown as cartoon representation with side chains shown in stick representation (*As*Chi18A colored cyan, ChiNCTU2 D143A [PDB id: 3N13] colored yellow). Side chains are labeled showing *As*Chi18A amino acid numbers above the ChiNCTU2 numbers. The chitobiose ligand bounbd in the -1 and -2 subsite of the ChiNCTU2 active is shown in stick representation with gray colored carbon atoms. Hydrogen bonds are illustrated by dashed lines and distances (Å) are indicated. It should be noted that the positioning of the ChiNCTU2 catalytic acid, E145, deviates from the position observed in the ChiNCTU2 wild type structure (which is more similar to the positioning of AsChi18A E645) due to the absence of an Asp at position 143.

VSAL_I0763



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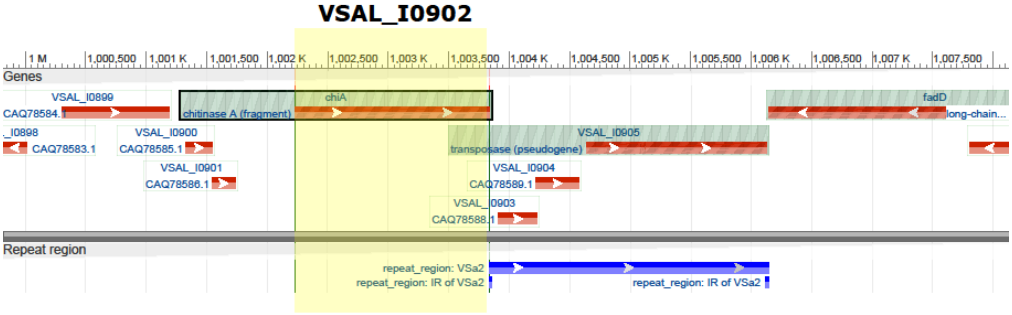
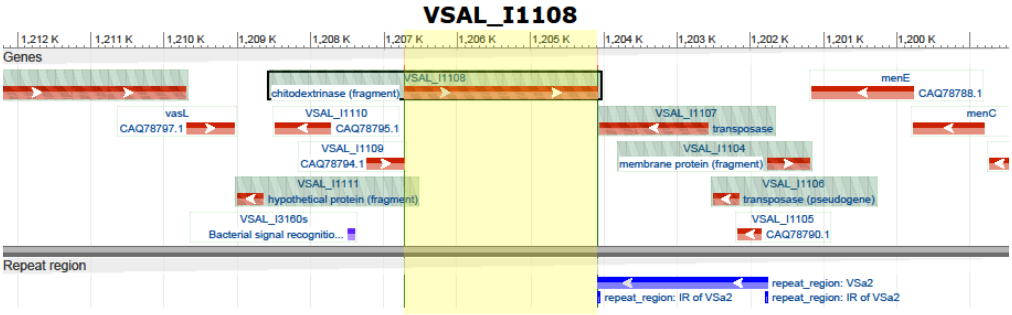
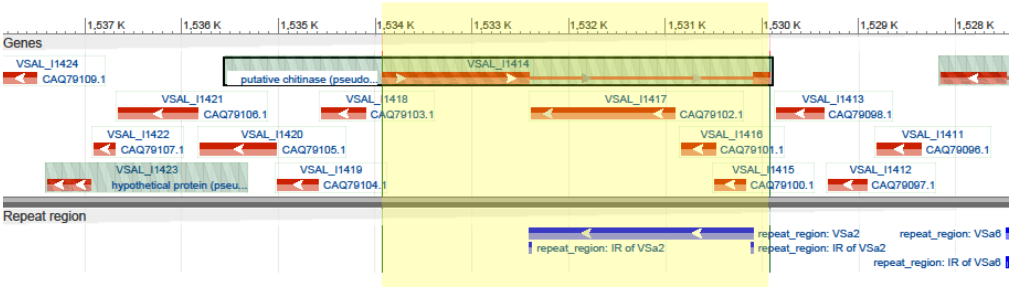


VSAL_I1108



VSAL_I1414



VSAL_I1942

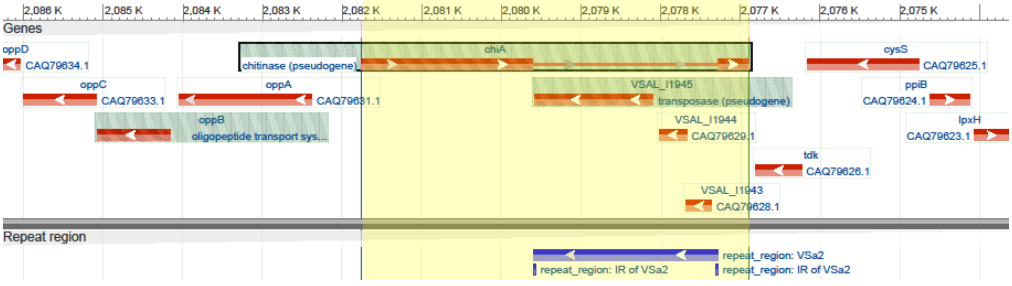


Figure S2. Genomic arrangement of pseudogenes related to chitin catabolism. The genomic region related to pseudogenes VSAL_I0763, VSAL_I0902 (*As*Chi18Bp), VSAL_I1108 (*As*Chi18Dp), VSAL_I1414 (*As*Chi19p) and VSAL_I1942 (*As*Chi18Cp) are shown, the yellow highlighting indicating the region represented by the pseudogene. Potentially coding sequences are shown in red bars with white arrows indicating direction. The insertion sequence repeat region is indicated by blue bars. The illustrations were made using the NCBI Graphical Sequence Viewer (Version 3.42.0) by analysis of the *Al. salmonicida* genome sequence (Genbank identifier: FM178379.1). Further information on the occurrence and function of IS elements in *Al. salmonicida* has been published by A. Kashulin et al. (1) and can be consulted for further information on this topic.

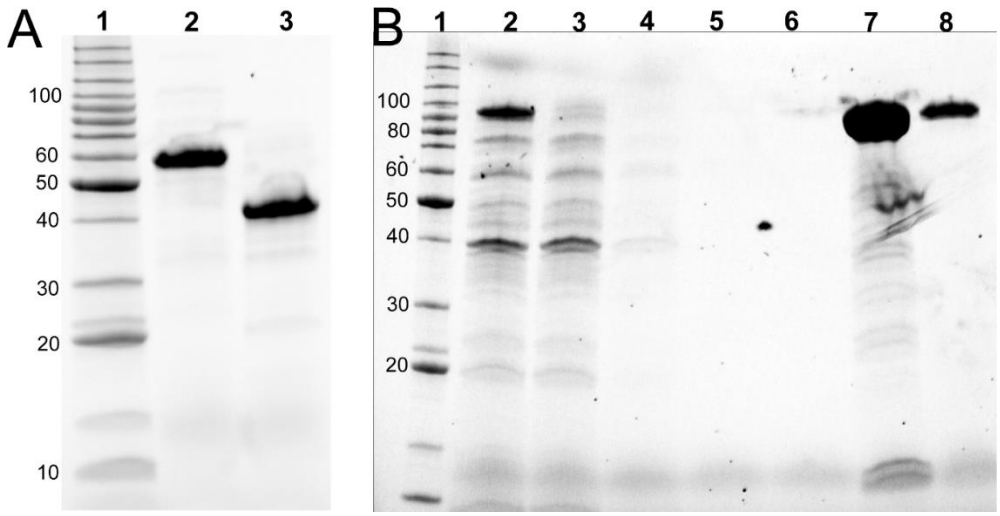


Figure S3. Analysis of protein purity. SDS-PAGE was used to determine protein purity. Panel A shows an SDS-PAGE gel with lanes displaying protein benchmark ladder (Invitrogen) in lane 1, *As*LPMO10A in lane 2 and *As*LPMO10B in lane 3. The SDS-PAGE gel in panel B displays the stages of protein purification for *As*Chi18A, showing the protein benchmark ladder in lane 1, cell free extract from an induced culture in lane 2, flow through in lane 3, wash fraction in lane 4-6 and the eluted protein in lanes 7 and 8. Only fractions containing highly pure protein were used in biochemical assays. All proteins used in biochemical assays were estimated to be >95% pure.

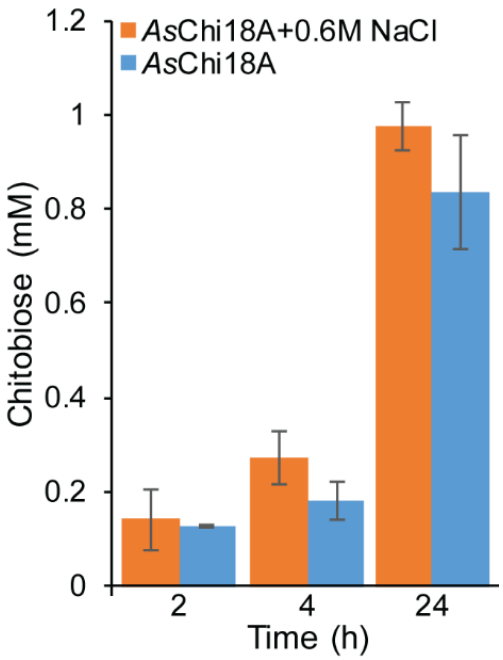


Figure S4. Influence of NaCl on *As*Chi18A activity. The amount of $(GlcNAc)_2$ (chitobiose) released from 10 mg/mL β-chitin in Tris-HCl pH 7.5 by 1.0 μM *As*Chi18A in the presence and absence of 0.6 M NaCl was evaluated at three time points (n=3). Reactions were incubated at 30°C.

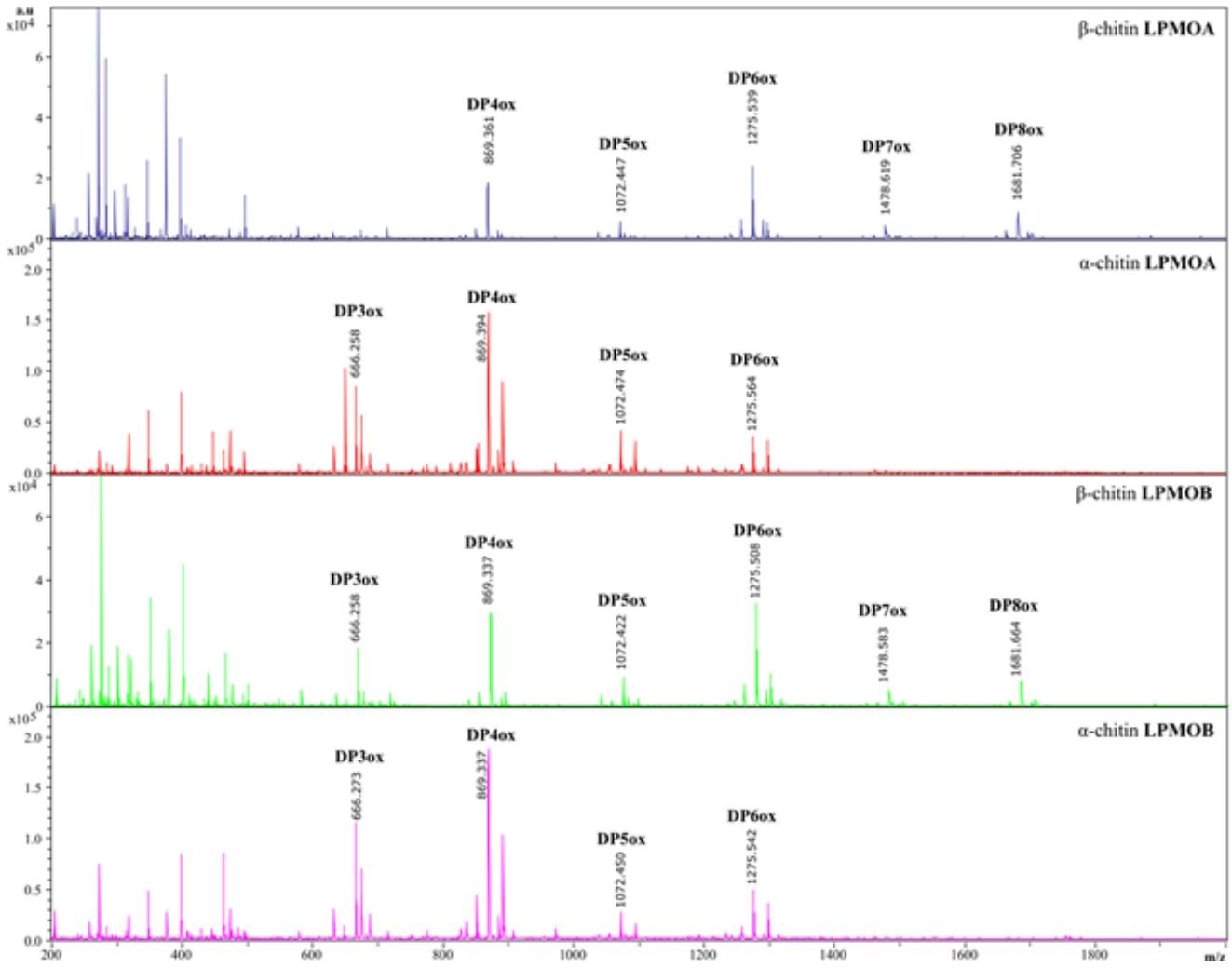


Figure S5. MALDI-TOF MS analysis of oxidized products generated by *As*LPMO10A and -B from *A. salmonicida* on chitin (α and β). The MS spectra show soluble C1 oxidized chito-oligosaccarides, i.e. aldonic acids. The degree of polymerization of each product is indicated by "DPn ox", where n equals the number of monosaccharides in the chain. The main peaks are labelled with the respective masses.

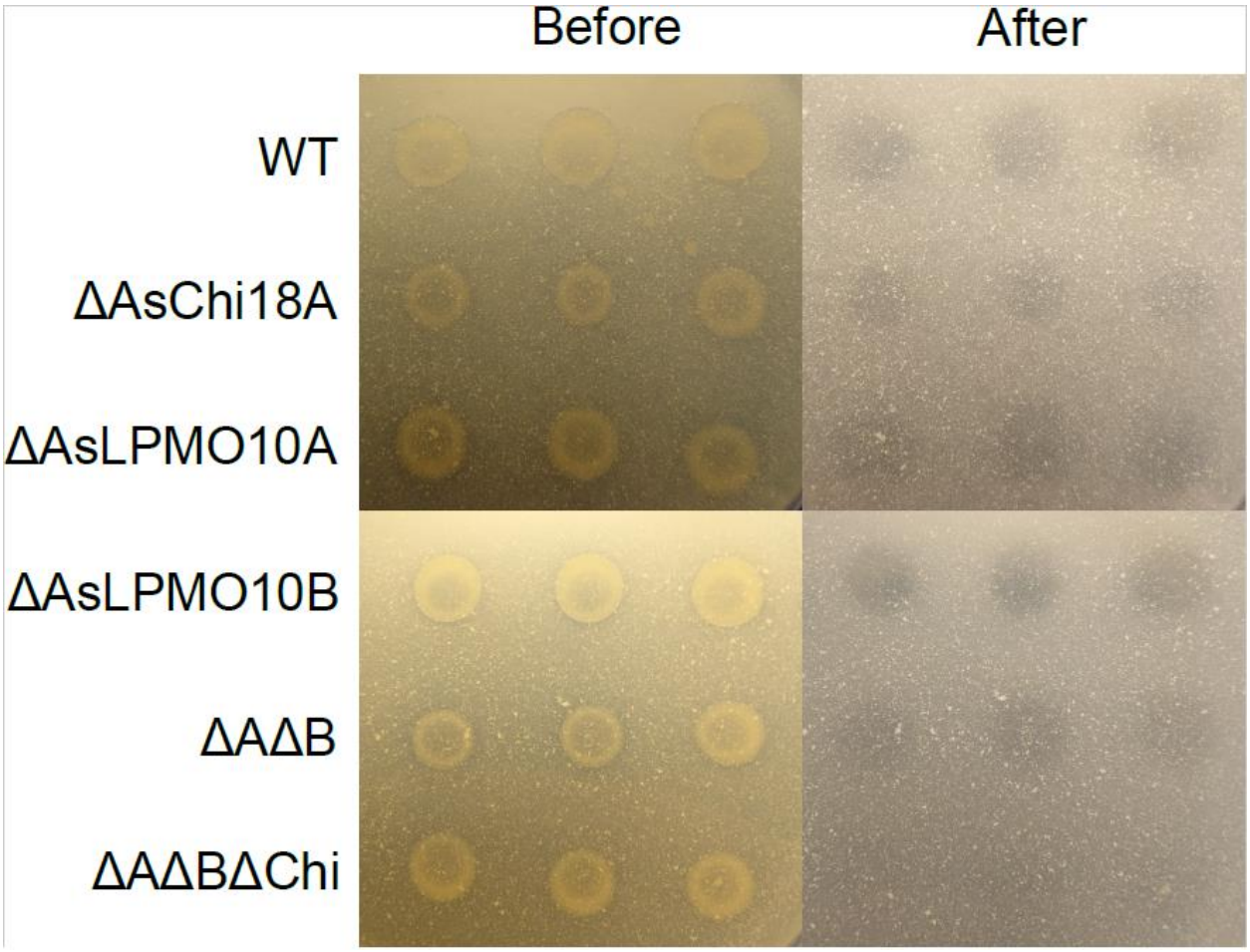


Figure S6. Chitin degradation assay. Images show photographs of agar plates containing LB25 supplemented with 2% colloidal chitin with *Al. salmonicida* variants (indicated on the left side of the image) spotted in triplicate and allowed to grow at 12 °C for 20 days. The photographs show the agar plates before (left) and after (right) the colonies had been removed by gentle washing. Halos indicate chitin degradation.

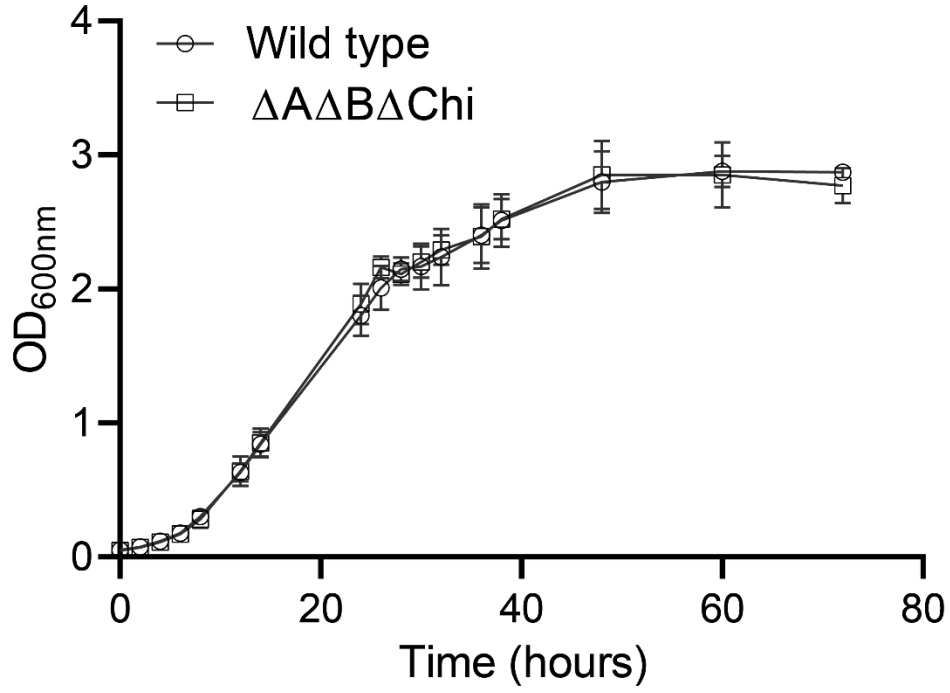


Figure S7. Growth of *Al. salmonicida* LFI1238 variants. Growth of the wild type *Al. salmonicida* LFI1238 strain compared to the triple knock-out strain (ΔAΔBΔChi) in LB25 broth. Standard deviation is indicated by error bars (n=3).

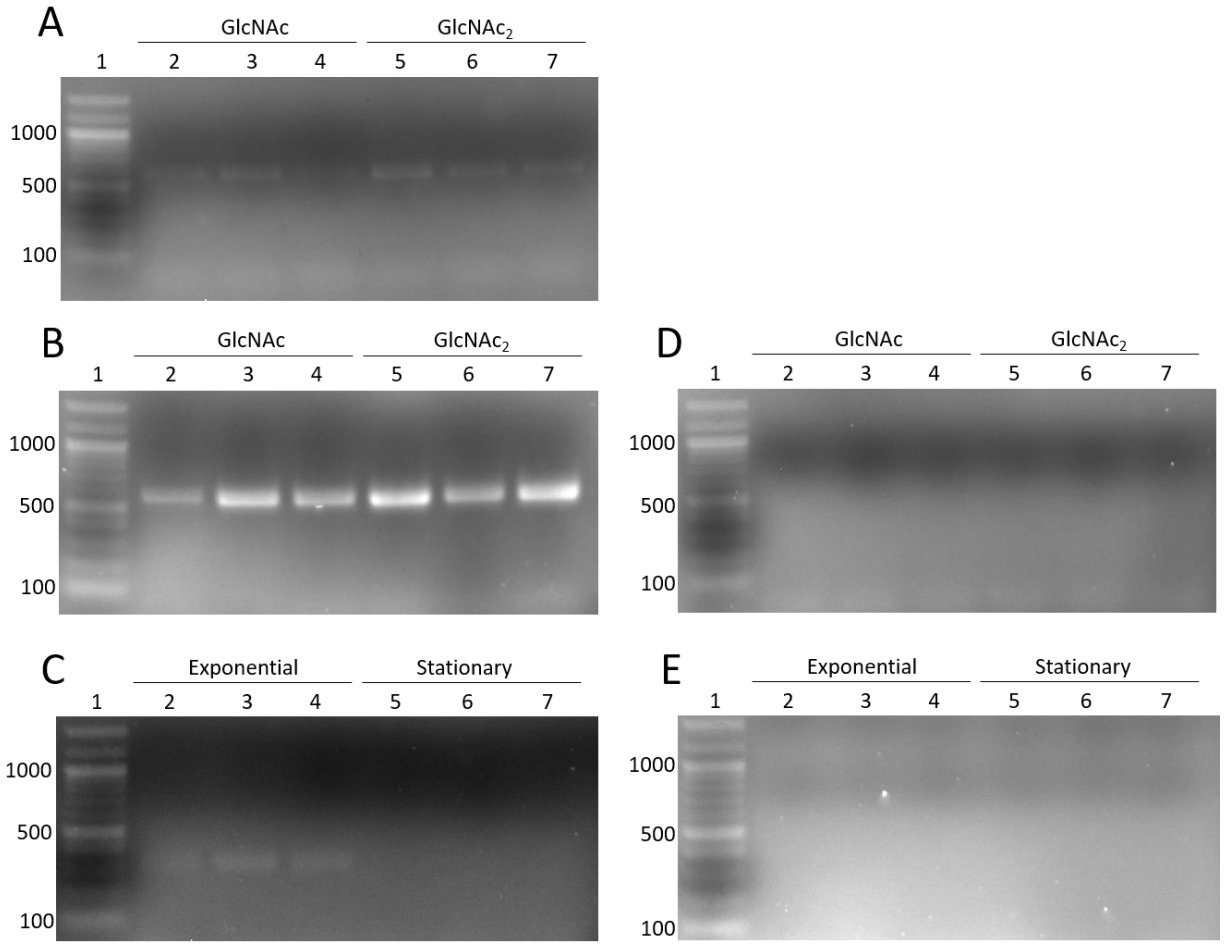


Figure S8. Gene expression analysis by PCR amplification of cDNA. Panel A and B shows the products formed in PCR experiments using cDNA from samples obtained during exponential growth in GlcNAc and $GlcNAc_2$ combined with primer pairs *GH18Expression* and *10AExpression*, respectively. Panel D shows the PCR experiments using -RT controls as template combined with primer pair *10AExpression*. The -RT templates used in Panel D corresponds to the cDNA applied to Panel A and B. Panel C shows the products formed in PCR experiments using cDNA from samples obtained during exponential and stationary growth in glucose combined with primer pair *I0902Expression*. In this case gene expression was evaluated as positive (+) during exponential growth (lane 2-4) and negative (-) during stationary growth (lane 5-7). Panel E shows the products formed in PCR experiments using the -RT samples corresponding to the cDNA template used in Panel C combined with primer pair *I0902Expression*. Lane 1; 100 bp DNA ladder, lanes 2-4 or 5-7; biological replicates within the same condition.

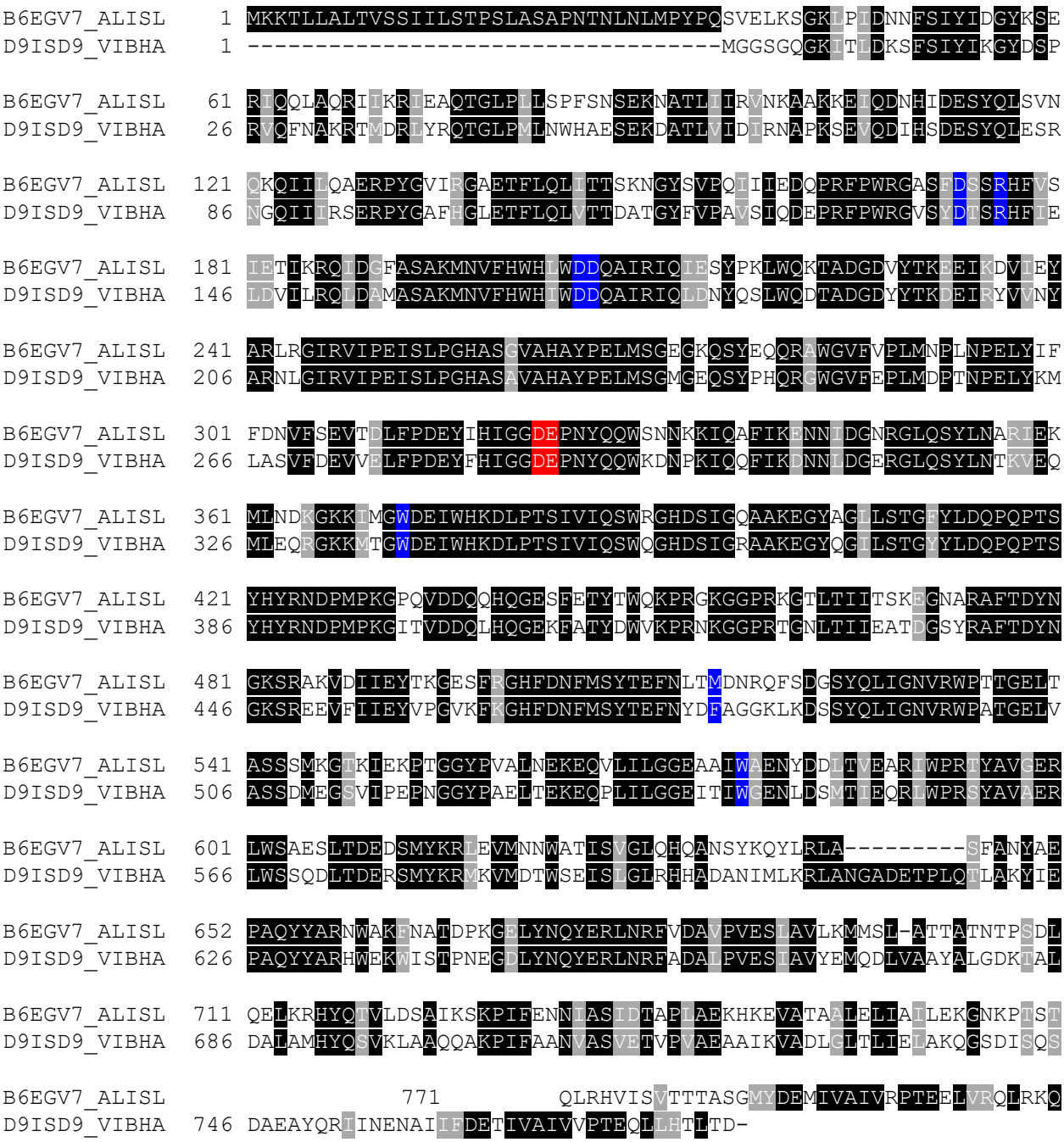


Figure S9. Pairwise sequence alignment of the *Al. salmonicida* GH20 (UniProt ID B6EGV7) and *Vibrio harvey* GH20 *Vh*NAG1 (UniProt ID D9ISD9). The alignment was made using the EMBOSS pairwise sequence alignment tool using default parameters. The Alignment was formatted using BoxShade. Based on data from W. Suginta et al. (2), catalytic amino acids are shown in red shading and amino acids involved in substrate binding are shown in blue shading.

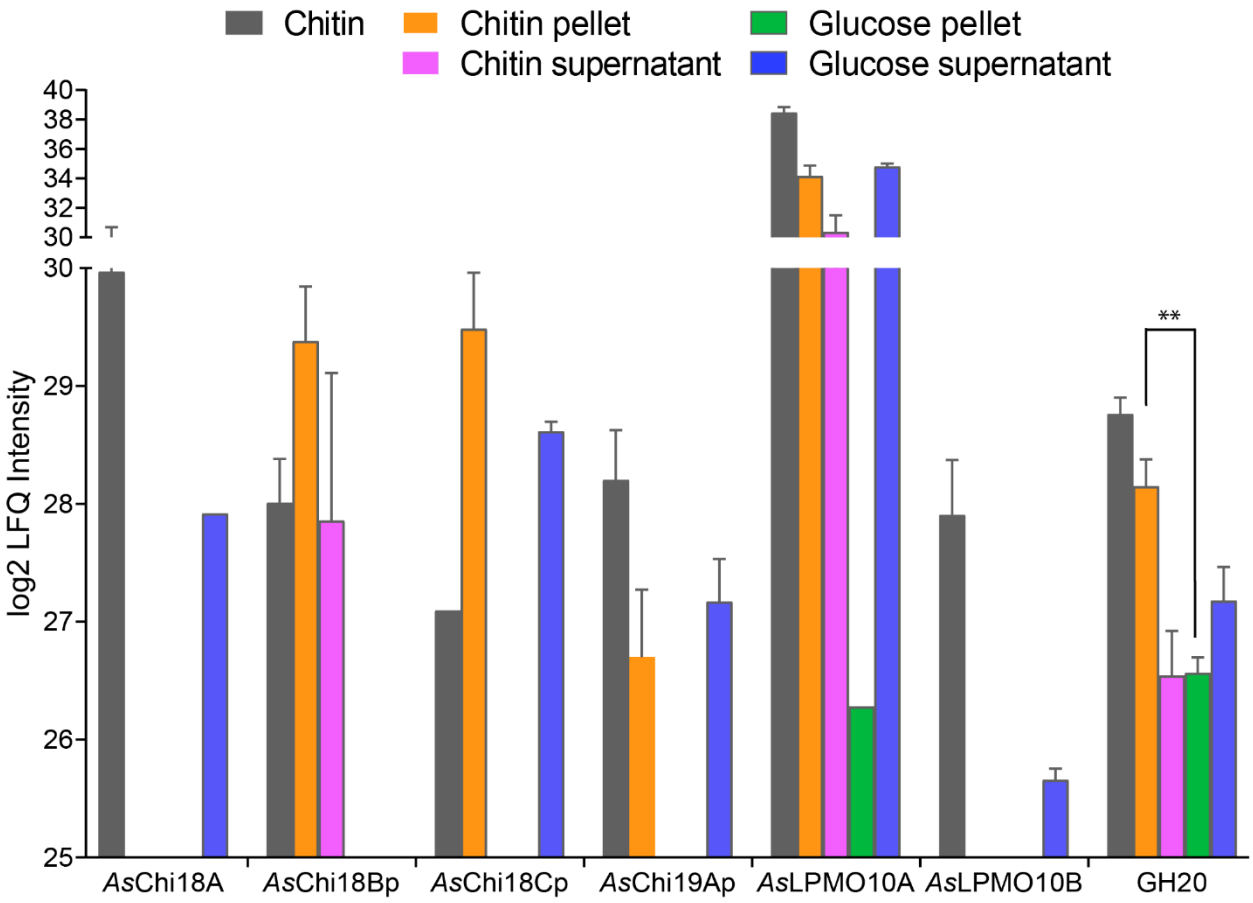


Figure S10. ***Al. salmonicida* protein abundances.** The abundance of selected *Al. salmonicida* proteins related to chitin catabolism displayed in bar chart format as a supplement to Figure 9. Log2 LFQ values shown represent the average values obtained from the label free proteomics data (Supplementary Dataset 1). Error bars are shown for proteins that were detected in two or three biological replicates. The GH20 β-*N*-acetylhexosaminidase was calculated to have a statistically significant 1.58 log2 fold higher abundance during growth on chitin compared to glucose (p=0.0082; paired two-tailed t test).

```
OCH20886.1         1 MALLTNKMKLNTIALALLGAGFSVQSHANDMTNPEGGIVVGYWHNWCDGR     50

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1        51 GYQAGNAPCMTLKETNPMYNVVDISFMKVYDTAEGRIPTFRLDPTVGLSE    100

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       101 AEFIEQIKELNAQGRSVVIALGGADAHIELKRGDETAFADEIIRLVEVYG    150

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       151 FDGLDIDLEQAAVTAADNQWVIPEALKMVKDHYRTQGKNFLITMAPEFPY    200

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       201 LKAGDKYIPYLERLEGYYDWINPQFYNQGGDGVWVDEENAWITQNSDAMK    250

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       251 EKFIYYISDSLINGTRGFHKIPHDKLVFGIPTNSDAAATGFVKEPQDLYN    300

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       301 AFDALKQQGQPLRGVMTWSINWDVGTDAAGTPYNSSFINDYGPYVHGQTP    350

VSAL_I0763         1 --------------------------------------------------       0

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VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       401 NTQVLGDNVLTYSVTDSDGNETKQGRNVEVYSALPELTGVTNTTIKIDSA    450

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       451 FDPLTGVKATDAEDGDLTSQINVEGSVDTTLAGKYSLTYSVKDSANQTAT    500

VSAL_I0763         1 --------------------------------------------------       0

OCH20886.1       501 ATRTVTVNDGSEVCVTPWKADQVYLTDETTSHNGKTWQAGWWTQGDEPGT    550
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OCH20886.1       551 TGEWGVWKLIGDTDCGGVIPDPEAELSAKITGLSNEYSVIDSAATITFTI    600
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VSAL_I0763        20 TGEWGVWKLIGDTDCGGVIPDPEAELSAKITGLSNEYSVIDSAATSTFTI     69

OCH20886.1       601 TTNEMASTTVDIIDRMGNSVTSYSEQVTGTQAISLPLTNVEEGYYSMRLL    650
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I0763        70 TTNEMASTTVDIIDRMGNSVTSYSEQVTGTQAISLPLTNVEEGYYSMRLL    119

OCH20886.1       651 ASNDDNSVEERYSFNLVSEDTTTPPTTDVPAYEAGKTYAEGDQVLATDGN    700
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I0763       120 ASNDDNSVEERYSFNLVSEDTTTPPTTDVPAYEAGKTYAEGDQVLATDGN    169

OCH20886.1       701 VYQCKAWPYTPWCSSSAYAPAESQLWANAWDKK    733
                     |||||||||||||||:||||||||||||||||
VSAL_I0763       170 VYQCKAWPYTPWCSSSSYAPAESQLWANAWDKK    202
```

Figure S11. Pairwise sequence alignment of VSAL_I0763 (family GH18 chitinase fragment) and a *Aliivibrio logei* family GH18 chitinase (genebank accession OCH20886.1). The family GH18

catalytic chitinase sequence motif is indicated by yellow highlighting. The sequence alignment was made using EMBOSS Needle with default parameters.

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OCH20886.1         1 MALLTNKMKLNTIALALLGAGFSVQSHANDMTNPEGGIVVGYWHNWCDGR     50
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I0902         1 MALLTNKMKLNTIALALLGAGFSVQSHANDMTNPEGGIVVGYWHNWCDGR     50

OCH20886.1        51 GYQAGNAPCMTLKETNPMYNVVDISFMKVYDTAEGRIPTFRLDPTVGLSE    100
                     ||||||||.||||||||||||||||||||||||||||||||||||||||||
VSAL_I0902        51 GYQAGNAPYMTLKETNPMYNVVDISFMKVYDTAEGRIPTFRLDPTVGLSE    100

OCH20886.1       101 AEFIEQIKELNAQGRSVVIALGGADAHIELKRGDETAFADEIIRLVEVYG    150
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I0902       101 AEFIEQIKELNAQGRSVVIALGGADAHIELKRGDETAFADEIIRLVEVYG    150

OCH20886.1       151 FDGLDIDLEQAAVTAADNQWVIPEALKMVKDHYRTQGKNFLITMAPEFPY    200
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VSAL_I0902       151 FDGLDIDLEQAAVTAADNQWVIPEALKMVKDHYRTQGKNFLITMAPEFPY    200

OCH20886.1       201 LKAGDKYIPYLERLEGYYDWINPQFYNQGGDGVWVDEENAWITQNSDAMK    250
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I0902       201 LKAGDKYIPYLERLEGYYDWINPQFYNQGGDGVWVDEENAWITQNSDAMK    250

OCH20886.1       251 EKFIYYISDSLINGTRGFHKIPHDKLVFGIPTNSDAAATGFVKEPQDLYN    300
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
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VSAL_I0902       401 NTQVLGDNVLTYSVTDSDGNETKQGRNVEVYSALPELTGVTNTTIKIDST    450

OCH20886.1       451 FDPLTGVKATDAEDGDLTSQINVEGSVDTTLAGKYSLTYSVKDSANQTAT    500
                     |||||.||||||||||||.||||||||||||||||||||||||||||||||
VSAL_I0902       451 FDPLAGVKATDAEDGDLTPQINVEGSVDTTLAGKYSLTYSVKDSANQTAT    500

OCH20886.1       501 ATRTVTVNDGSEVCVTPWKADQVYLTDETTSHNGKTWQAGWWTQGDEPGT    550
                     ||||||||||||||:||||||||||||||||||
VSAL_I0902       501 ATRTVTVNDGSEVCVSPWKADQVYLTDETTSHN----------------    533

OCH20886.1       551 TGEWGVWKLIGDTDCGGVIPDPEAELSAKITGLSNEYSVIDSAATITFTI    600

VSAL_I0902       534 --------------------------------------------------    533

OCH20886.1       601 TTNEMASTTVDIIDRMGNSVTSYSEQVTGTQAISLPLTNVEEGYYSMRLL    650

VSAL_I0902       534 --------------------------------------------------    533

OCH20886.1       651 ASNDDNSVEERYSFNLVSEDTTTPPTTDVPAYEAGKTYAEGDQVLATDGN    700

VSAL_I0902       534 --------------------------------------------------    533

OCH20886.1       701 VYQCKAWPYTPWCSSSAYAPAESQLWANAWDKK    733

VSAL_I0902       534 --------------------------------    533
```

Figure S12. Pairwise sequence alignment of VSAL_I0902 (family GH18 chitinase fragment) and a *Aliivibrio logei* family GH18 chitinase (genebank accession OCH20886.1). The family GH18

catalytic chitinase sequence motif is indicated by yellow highlighting. The sequence alignment was made using EMBOSS Needle with default parameters.

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WP_065610756.      1 MEHSNLKRKHNYKFTLSTLTISCLMAFNAQAAIDCGPLNTWSGDTVYNGG     50

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                       ||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1108         1 --VKQGNNAYKAKYWTQNNDPATAGEWGAWQDLGSCSGDLVNIAPTVDLT     48

WP_065610756.    101 SPSSTDNIAIGDIVTLTASAADSDGSVVRVDFSVDGSVIASSTTSPYSAP    150
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VSAL_I1108        49 SPSSTDNIAIGDIVTLTASAADSDGSVVRVDFSVDGSVIASSETSPYSTS     98

WP_065610756.    151 WTALEGSHTFSAQSYDDKGAVSTESSVVVAVTGTPTDNIAPTASLNLSAS    200
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VSAL_I1108        99 WTALEGSHTFSAQSYDDKGAVSTESSVVVAVTGTPTDNIAPMASLNLSAS    148

WP_065610756.    201 SVELGAIVALTADATDSDGTIDKVDFYVNNTLIGTRATAPYTLQYKTTAA    250
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VSAL_I1108       149 SVELGATVALTADATDSDGTIDKVDFYVNNTLIGTTATAPYTLQYKTTSA    198

WP_065610756.    251 GSLSVYAKATDNLGASTNSSPSTLTVTSSLPIADNCRPDGMYQTEGVNVP    300
                     |||||||||||||||||| ||||||||||||||||||||||||||||||
VSAL_I1108       199 GSLSVYAKATDNLGASTNSS-STLTVTSSLPIADNCRPDGMYQTEGVNVP    247

WP_065610756.    301 YCTVYDKEGRELMGADHPRRVIGYFTSWRDGGDDQNSYLVNDIPWEQLTH    350
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VSAL_I1108       248 YCTVYDKEGRELMGADHPRRVIGYFTSWRDGGDDQNSYLVNDIPWEQLTH    297

WP_065610756.    351 INYAFVSIGSDGNVNVGDVTDPNNAATGKEWAGVEIDPTLGFKGHFGALA    400
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VSAL_I1108       298 INYAFVSIGSDGNVNVGDVTDPNNAATGKEWAGVEIDPTLGFKGHFGALA    347

WP_065610756.    401 TAKAKHGVKTLISIGGWAETGGHFGTDGNRVADGGFYTMTTNADGSINQA    450
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VSAL_I1108       348 TAKAKHDVKTLISIGGWAETGGHFGTDGNRVADGGFYTMTTNADGSINQA    397

WP_065610756.    451 AIEKFAISAVEMMRKYKFDGLDIDYEYPTSMAGAGNPDDKTFSESRRPYL    500
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WP_065610756.    501 MKSYHELMRVLREKLDVASSEDNIHYMLTIAAPSSAYLLRGMETMAVTKY    550
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VSAL_I1108       498 LDYVNIMSYDLHGAWNDHVGHNAALFDTGKDSELAQWSVYDTEAYGGIGY    547

WP_065610756.    601 LNTDWAFHYFRGSMPAGRINIGVPYYTRGWQGVTGGENGLWGRAPLPDQA    650
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WP_065610756.    651 QCDAGTGEGEKNNCGYGALGIDNMWHDKNSYGQEMGAGSNPMWHAKNLQE    700
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WP_065610756.    751 EDKASINVKADYVIDKEIGGIMFWELAGDYNCYVLDANGKRTSVDATEAA    800
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WP_065610756.    801 CQTGNGEYHMGNTMTKAIYDKFAAATPYGNTVATGALPTETVDIAISIDG    850
                     ||||||||||||||||||||||||||||||||||||.||||||||||||
VSAL_I1108       748 CQTGNGEYHMGNTMTKAIYDKFAAATPYGNTVATGALPAETVDIAISIDG    797

WP_065610756.    851 FKVGDQNYPINPKISFTNNTGQDIPGGTEFQFDIPVSAPDNAKDQSGGGL    900
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1108       798 FKVGDQNYPINPKISFTNNTGQDIPGGTEFQFDIPVSAPDNAKDQSGGGL    847

WP_065610756.    901 QVIASGHTRADNIGGLDGTMHRVAFTLPAWKALPAGGVYELDMVYYLPIS    950
                     ||||||||||||||||||||||:||||||||||
VSAL_I1108       848 QVIASGHTRADNIGGLDGTMHRIAFTLPAWKAL-----------------    880

WP_065610756.    951 GPANYSVKINNIEYAFTFEQPDLPVADLSTGGGDNGGIPDAGCDATGLVT   1000

VSAL_I1108       881 --------------------------------------------------    880

WP_065610756.   1001 YPDLPQTDVAGNPSHANTGDKIVHNNVIYQANWWTSATPGSDGSWTKVCN   1050

VSAL_I1108       881 --------------------------------------------------    880

WP_065610756.   1051 L   1051

VSAL_I1108       881 -    880
```

Figure S13. Pairwise sequence alignment of VSAL_I1108 (family GH18 chitinase fragment) and a *Aliivibrio logei* family GH18 chitinase (genebank accession WP_065610756.1). The family GH18 catalytic chitinase sequence motif is indicated by yellow highlighting. The sequence alignment was made using EMBOSS Needle with default parameters.

```
WP_023603329.      1 MFKLALLPTLLACSFAANSITMTPQTDPLNPTGYVVSKAEIKAAEDAKTL     50
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1414         1 MFKLALLPTLLACSFAANSITMTPQTDPLNPTGYVVSKAEIKAAEDAKTL     50

WP_023603329.     51 DPMYDVWAKALETRPNTVVDLIDVGSATNPENVKRVERVFPASEWFFLTQ    100
                     ||||||||||||||||||||||||||||||||||||||||.|||||||||
VSAL_I1414        51 DPMYDVWAKALETRPNTVVDLIDVGSATNPENVKRVERVFPDSEWFFLTQ    100

WP_023603329.    101 MAAPEYTYTRFLRAIGKFPAFCGEYTDGRDSDAICKKSIVTAFAHFSQET    150
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1414       101 MAAPEYTYTRFLRAIGKFPAFCGEYTDGRDSDAICKKSIVTAFAHFSQET    150

WP_023603329.    151 GGHIAVDNISDNPLALEEWQQALVHVREMGWSEGQEGYTTGCGQNDWQNK    200
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1414       151 GGHIAVDNISDNPLALEEWQQALVHVREMGWSEGQEGYTTGCGQNDWQNK    200

WP_023603329.    201 RWPCAAGQGYFGRGAKQLSYHFNYGAFSEVMYDGDATVLLDNPGIVADSW    250
                     ||||||||||||||||||||||||||||||||||||||||||||||:|||||
VSAL_I1414       201 RWPCAAGQGYFGRGAKQLSYHFNYGAFSEVMYDGDATVLLDNPGVVADSW    250

WP_023603329.    251 LNLASAIWFFLTPQAPKPAMLHVIDRTWSPSQRETDAGIGYGFGTTINVI    300
                     ||||||||||||||||||||||||||||:||||||||||||||||||||||
VSAL_I1414       251 LNLASAIWFFLTPQAPKPAMLHVIDRTWNPSQRETDAGIGYGFGTTINVI    300

WP_023603329.    301 NGGIECGEQNKDKGQPVNRIRYWEGLAKHYQIPVEADETNTCWQQTPYGS    350
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1414       301 NGGIECGEQNKDKGQPVNRIRYWEGLAKHYQIPVEADETNTCWQQTPYGS    350

WP_023603329.    351 LNLNGATDVLYTNWDGNWKYYPDRPEGASFECELVGFQTAYSALVPGDYE    400
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1414       351 LNLNGATDVLYTNWDGNWKYYPDRPEGASFECELVGFQTAYSALVPGDYE    400

WP_023603329.    401 KCVTNFYESHANWPVTRVVETLPTDPTDPGTPGDPNNTWDTNAVYNTGDQ    450
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1414       401 KCVTNFYESHANWPVTRVVETLPTDPTDPGTPGDPNNTWDTNAVYNTGDQ    450

WP_023603329.    451 VVVDGVTYQAQWWNQGDNPATSTTGVWLAVNAAVTPPAEPTPPAPIDPTP    500
                     ||||||||||||||||||||||||||||||||||||.|||||||||||||
VSAL_I1414       451 VVVDGVTYQAQWWNQGDNPATSTTGVWLAVNAAVTPPVEPTPPAPIDPTP    500

WP_023603329.    501 VNPMPPTEPTDPNSTWTAVGTYNTGDQVTVNGVIYQAQWWTQGNNPETSG    550
                     ||||||
VSAL_I1414       501 VNPMPP----------------------------------------------    506

WP_023603329.    551 DWGVWKKV    558

VSAL_I1414       507 --------    506
```

Figure S14. Pairwise sequence alignment of VSAL_I1414 (family GH19 chitinase fragment) and a *Aliivibrio logei* family GH19 chitinase (genebank accession WP_065610756.1). The sequence alignment was made using EMBOSS Needle with default parameters.

```
WP_065612067.      1 MFKTKLGFCTAAITLALSAPTYAAVPGQAIISWMETDFSIIDVDQAATSY     50
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942         1 MFKTKLGFCTAAITLALSAPTYAAVPGQAIISWMETDFSIIDVDQAATSY     50

WP_065612067.     51 KNLVTVKEFAEVPVTWDRWSGESAETWKVLLNGQIVHEESISATASQKAS    100
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942        51 KNLVTVKEFAEVPVTWDRWSGESAETWKVLLNGQIVHEESISATASQKAS    100

WP_065612067.    101 TVLQVRQGGQYSMTVQLCNGTGAVEECSTSAPKDIVVADTDGSHLDPLPM    150
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       101 TVLQVRQGGQYSMTVQLCNGTGAVEECSTSAPKDIVVADTDGSHLDPLPM    150

WP_065612067.    151 NIDPANGNYTTPEGMVSGAYFVEWGVYGRKFAVDQIPAQNLTHILYGFIP    200
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       151 NIDPANGNYTTPEGMVSGAYFVEWGVYGRKFAVDQIPAQNLTHILYGFIP    200

WP_065612067.    201 ICGPNPSLGEIENGNSLAALNRACAGTPDYEVVIHDPWAAVQMPQPQSGH    250
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       201 ICGPNPSLGEIENGNSLAALNRACAGTPDYEVVIHDPWAAVQMPQPQSGH    250

WP_065612067.    251 VHSTPYKGTYGQMMALKQRYPDLKIVPSIGGWTLSDPFYDFVDKSKRDIF    300
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       251 VHSTPYKGTYGQMMALKQRYPDLKIVPSIGGWTLSDPFYDFVDKSKRDIF    300

WP_065612067.    301 VTSVKKFLKTWKFYDGVDIDWEFPGGDGASSTGGDPVNDGPAYVALMQEL    350
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       301 VTSVKKFLKTWKFYDGVDIDWEFPGGDGASSTGGDPVNDGPAYVALMQEL    350

WP_065612067.    351 RAMLDELSAETGKTYELTSAIGAGYDKIEDVDYAAASQYMDYIFAMTYDF    400
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       351 RAMLDELSAETGKTYELTSAIGAGYDKIEDVDYAAASQYMDYIFAMTYDF    400

WP_065612067.    401 FGGWNNVVGHQTAVYCGSHMSQGECDGTGLDDKGEPRKGPAYTISNAIDL    450
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       401 FGGWNNVVGHQTAVYCGSHMSQGECDGTGLDDKGEPRKGPAYTISNAIDL    450

WP_065612067.    451 LLAQGVDAKKLVVGAGMYARGWTGVTRESMTDPTNPMTGVGNGKVAGSWE    500
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       451 LLAQGVDAKKLVVGAGMYARGWTGVTRESMTDPTNPMTGVGNGKVAGSWE    500

WP_065612067.    501 AGVIDYKDVVTNYVNKAGVEVGYDDAAQAAFAYDPSNGDLITYDNKQSVL    550
                     |||||||||||||||||||||||||.|||||||||||||||||||||||||
VSAL_I1942       501 AGVIDYKDVVTNYVNKAGVEVGYDDVAQAAFAYDPSNGDLITYDNKQSVL    550

WP_065612067.    551 AKGEYVRSLGLGGLFAWEIDADNGDILNAMQEGLAGGTVTPPANKKPIAN    600
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I1942       551 AKGEYVRSLGLGGLFAWEIDADNGDILNAMQEGLAGGTVTPPANKKPIAN    600

WP_065612067.    601 AGVDIAVTTPATAQLDGSLSSDSDGTIASYAWTQISGPAVALSNHNTVNA    650
                     ||||||||.||||||||||||||||.|||||:|||||.|||.|||||
VSAL_I1942       601 AGVDIAVTAPATAQLDGSLSSDSDGTITSYAWTQVSGPAVVLSNQNTVNA    650

WP_065612067.    651 SFVTDGFIQSETLQFTLTVTDDKGATASDSVSVVVTVKGTEPVNTPPVAV    700
                     |||||||||||||||||||||||||||||||||||||||:|||||||||
VSAL_I1942       651 SFVTDGFIQSETLQFTLTVTDDKGATASDSVSVVVTVKGTDPVNTPPVAV    700

WP_065612067.    701 IIAPSSVNKGDLVTLDASSSTDAESDPLTFTWAVPSGIDATVTGSTVTFI    750
                     ||||||||||:||||||||
VSAL_I1942       701 IIAPSSVNKGDIVTLDASSS------------------------------    720
```

```
WP_065612067.    751 ADSYTVDTPLSFSVTANDGQASNTATVSVTVLKDAGDPPVTCDNAWDASV    800

VSAL_I1942       721 --------------------------------------------------    720

WP_065612067.    801 VYNGGDQVSNSGKVWEAKWWTQGDDPSTSGDWGVWKEVGISACN    844

VSAL_I1942       721 ---------------------------------------------    720
```

Figure S15. Pairwise sequence alignment of VSAL_I1942 (family GH18 chitinase fragment) and a *Aliivibrio logei* family GH18 chitinase (genebank accession WP_065612067.1). The family GH18 catalytic chitinase sequence motif is indicated by yellow highlighting. The sequence alignment was made using EMBOSS Needle with default parameters.

```
WP_017021178.      1 MENKFFKTTLLGAAIALASSGVTAKEVGINSDFNVEVYGVAAISLVNYNT     50
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I2352         1 MENKFFKTTLLGAAIALASSGVTAKEVGINSDFNVEVYGVAAISLVNYNT     50

WP_017021178.     51 TDNNDASSGYVVENESRIGFRAHKEMFENVLITMQIESGYVDSTDWPHGG    100
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I2352        51 TDNNDASSGYVVENESRIGFRAHKEMFENVLITMQIESGYVDSTDWPHGG    100

WP_017021178.    101 VSGGTLGFRDTFIGASGDWGNVRVGRVLTPLYELVDWPFSNPGLGSVFDW    150
                     ||||||||||||||||.||||.||||||||||||||||||||||||||||
VSAL_I2352       101 VSGGTLGFRDTFIGASGYWGNVCVGRVLTPLYELVDWPFSNPGLGSVFDW    150

WP_017021178.    151 GGIAGHYDRQSNQVRYDSPKFGGFSFATSVGRD--------DNDNGGGAA    192
                     |||||||||||||||||||||||||||||||||        |||||||||
VSAL_I2352       151 GGIAGHYDRQSNQVRYDSPKFGGFSFATSVGRDDNDNDNDNDNDNGGGAA    200

WP_017021178.    193 TRDSSFASANAKYSFEKVTLMGAVEAGSDFNGVAGQDNQSYLVGFEASLP    242
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
VSAL_I2352       201 TRDSSFASANAKYSFEKVTLMGAVEAGSDFNGVAGQDNQSYLVGFEASLP    250

WP_017021178.    243 AGFGIAAAYKVESLDNQAN-----KAGVSQSGVNVEQGSYSIIGQYWNGP    287
                     ||||||||||||||||||.     ||.:.
VSAL_I2352       251 AGFGIAAAYKVESLDNQATRLVFLKAVLM----------------------    279

WP_017021178.    288 IGFKLGYAANLESETNSKTDKDSDSNTISGQLMAVHNGFVPYLRVAGRTV    337

VSAL_I2352       280 --------------------------------------------------    279

WP_017021178.    338 GDADTDIVTRVGLEYGF    354

VSAL_I2352       280 -----------------    279
```

Figure S16. Pairwise sequence alignment of VSAL_I2352 (porin fragment) and a *Aliivibrio logei* family porin (genebank accession WP_017021178.1). The sequence alignment was made using EMBOSS Needle with default parameters.

Supplementary tables

Table S1. Growth rate measurements and max cell density of *Al. salmonicida* cultivated in glucose, GlcNAc and $GlcNAc_2$

Carbon source	Rate constant μ ($hours^{-1}$)	Generation time (hours)	Max cell density (OD_{600})
Glucose	0.065 ± 0.025	5.36 ± 2.17	2.63 ± 0.094
GlcNAc	0.069 ± 0.029	5.16 ± 2.00	1.31 ± 0.022
$GlcNAc_2$	0.055 ± 0.021	4.95 ± 0.89	1.58 ± 0.145

Mean ± SD of three biological replicates.

Table S2. Identified CAZymes sorted by their putative biological processes, according to gene ontology (GO) annotations.

CAZy	Uniprot	Biological process
		Carbohydrate metabolic process [GO:0005975]
AA10	B6EQJ6	*As*LPMO10B
CBM73;GH18	B6EH15	*As*Chi18A
GH13_19	B6EGT4	MalS (Alpha-amylase)
GH20	B6EGV7	VSAL_I2989 (Putative beta-*N*-acetylhexosaminidase)
GT35	B6EQ29	MalP (Alpha-1,4 glucan phosphorylase)
GH77	B6EQ30	MalQ (4-alpha-glucanotransferase)
GH3	B6ERJ6	NagZ (Beta-hexosaminidase)*
		Chitin binding [GO:0008061]
AA10;CBM73	B6EQB6	*As*LPMO10A
		Cell cycle, cell division, protein import [GO:0007049/ 0051301/ 0017038]
PL22	B6EGK3	TolB (Tol-Pal system protein TolB)
		Formaldehyde catabolic process [GO:0046294]
CE1	B6EH03	YeiG (S-formylglutathione hydrolase)
		Glycogen biosynthetic process [GO:0005978]
GT5	B6EQL7	GlgA (Glycogen synthase)
		Lipid A biosynthetic process [GO:0009245]
CE11	B6ELH0	LpxC (UDP-3-O-acyl-N-acetylglucosamine deacetylase)
GT19	B6EJW7	LpxB (Lipid-A-disaccharide synthase)
		Lipopolysaccharide biosynthetic process [GO:0009103]
GT9	B6EPB8	RfaF (ADP-heptose-LPS heptosyltransferase II)
		Peptidoglycan metabolic process [GO:0000270]
GH23	B6EJV5	MltD (Membrane-bound lytic murein transglycosylase D)
GH23	B6EGC8	Slt (Soluble lytic murein transglycosylase)
		Trehalose catabolic process [GO:0005993]
GH13_29	B6ERJ9	TreA (Trehalose-6-phosphate hydrolase)
		Not assigned
GH103	B6EIW0	VSAL_I1069 (Putative exported protein)
GT2	B6EKR9	VSAL_I1407 (Putative glycosyl transferase)
GT51	B6EM36	MrcA (Penicillin-binding protein 1A)

*Also, Cell cycle [GO:0007049];cell division [GO:0051301];cell wall organization [GO:0071555];peptidoglycan biosynthetic process [GO:0009252];peptidoglycan turnover [GO:0009254];regulation of cell shape [GO:0008360]

Supplementary references

1. Kashulin A, Sørum H, Hjerde E, Willassen NP. 2015. IS elements in *Aliivibrio salmonicida* LFI1238: Occurrence, variability and impact on adaptability. Gene 554:40-49.
2. Suginta W, Chuenark D, Mizuhara M, Fukamizo T. 2010. Novel beta-*N*-acetylglucosaminidases from *Vibrio harveyi* 650: cloning, expression, enzymatic properties, and subsite identification. BMC Biochem 11:40.