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Novel mannose binding natterin-like protein in the skin mucus of Atlantic cod (*Gadus morhua*)

Binoy Rajan, Deepti M. Patel, Yoichiro Kitani, Kiron Viswanath, Monica F. Brinchmann

PII: S1050-4648(17)30433-3

DOI: 10.1016/j.fsi.2017.07.039

Reference: YFSIM 4721

To appear in: *Fish and Shellfish Immunology*

Received Date: 8 March 2017

Revised Date: 29 June 2017

Accepted Date: 21 July 2017

Please cite this article as: Rajan B, Patel DM, Kitani Y, Viswanath K, Brinchmann MF, Novel mannose binding natterin-like protein in the skin mucus of Atlantic cod (*Gadus morhua*), *Fish and Shellfish Immunology* (2017), doi: 10.1016/j.fsi.2017.07.039.

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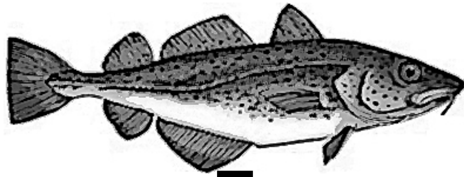
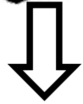
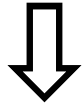



Atlantic cod skin mucus



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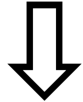

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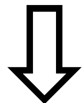

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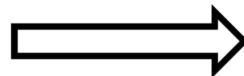
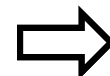
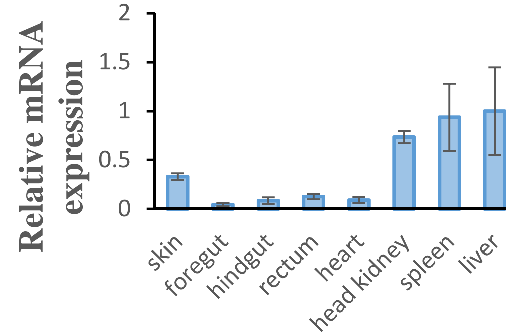


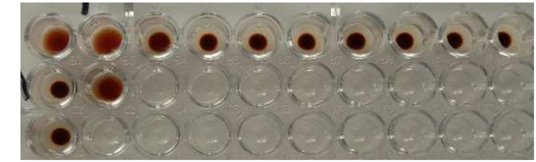
SDS-PAGE
and LC-MSMS



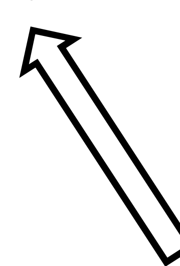
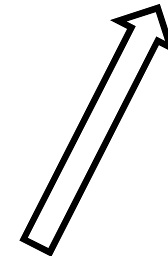
Natterin-like protein identified



Cloning and sequencing and primer design.
Tissue distribution of *nlp* transcripts analyzed



Nlp gives Ca^{++} depended and mannose inhibitable hemagglutination

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1 **Title:** Novel mannose binding natterin-like protein in the skin mucus of Atlantic cod
2 (*Gadus morhua*).

3 Authors: Binoy Rajan, Deepti M. Patel, Yoichiro Kitani, Kiron Viswanath and Monica F.
4 Brinchmann*

5 Affiliation:

6 Faculty of Bioscience and Aquaculture, Nord University, 8049 Bodø, Norway.

7 ***Corresponding Author:***

8 *Monica Fengsrud Brinchmann

9 Faculty of Bioscience and Aquaculture

10 Nord University, 8049 Bodø, Norway

11 E-mail address: Monica.F.Brinchmann@Nord.no

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26 **Abstract**

27 This study presents the first report of purification of natterin-like protein (Nlp) in a
28 non-venomous fish. The peptide identities of purified cod Nlp were confirmed
29 through LC-MSMS and matched to a cod expressed sequence tag (EST). A partial
30 cod *nlp* nucleotide sequence was amplified and sequenced based on this EST.
31 Multiple sequence alignment of cod Nlp showed considerable homology with other
32 teleost Nlps and the presence of an N-terminal jacalin-like lectin domain coupled with
33 a C-terminal toxin domain. *nlp* expression was higher in skin, head kidney, liver and
34 spleen than in other tissues studied. Hemagglutination of horse red blood cells by Nlp
35 was calcium dependent and inhibited by mannose. A *Vibrio anguillarum* bath
36 challenge however, did not alter the expression of cod *nlp* transcripts in the skin and
37 gills. Further functional characterization is required to establish the significance of
38 this unique protein in Atlantic cod and other teleosts.

39

40

41 **Keywords**

42 Lectin; isolation; natterin-like; innate immunology; hemagglutination; tissue
43 distribution; qPCR; Atlantic cod; mucosal immunology; gill; skin; *Vibrio*
44 *anguillarum.*

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46

47 **Highlights**

48 - A mannose binding lectin was isolated from Atlantic cod skin
49 - The lectin was identified as natterin-like protein with a jacaline-like lectin
50 domain
51 - Hemagglutination by Nlp was inhibited by mannose.
52 - The expression of cod *nlp* did not change during a *Vibrio anguillarum* bath
53 challenge

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54 **1. Introduction**

55 Lectins are proteins which possess carbohydrate-binding characteristics, and are non-
56 enzymatic in nature [1]. Although initially purified and characterized from plants, a
57 wide array of lectins from microorganisms to vertebrates, including those from
58 teleosts and other aquatic animals, have been reported [2,3]. Lectin families are
59 defined based on their carbohydrate ligands and divalent cation requirement for
60 binding. Several types of lectins, including different forms of mannose-binding
61 lectins (MBL), have been identified in fish skin mucus [4]. MBLs belong to the
62 collectin family of C-type lectins that binds to mannose moieties present in microbial
63 pathogens and opsonize them, thereby activating phagocytosis and/or the complement
64 pathway [5]. Other functions include direct or indirect roles in agglutinating
65 microorganisms, in scavenging cellular debris, in cell-cell communication, as
66 inflammatory mediators and mitogens of immune cells [6].

67 Lectins have a single carbohydrate recognition domain (CRD) or a repeat of CRDs.
68 Lectins with multiple carbohydrate ligands and/ or with unique molecular architecture
69 include among others, the ubiquitous collectin and selectin groups within the C-type
70 lectin family [6]. There are several such lectin types identified in fish as well as in
71 aquatic invertebrates. For example, intelectins with a fibrinogen related domain
72 (FReD) identified from the skin mucus of *Silurus asotus* (Amur catfish) [7]. A novel
73 lectin with domain features identical to mammalian plasma kallikrein was isolated
74 from skin mucus of flathead (*Platycephalus indicus*) [8]. An unique lectin was
75 identified in periwinkle (*Littorina littorea*), which has an N-terminal F-type lectin
76 domain followed by several EGF (epidermal growth factor) domains [9]. Orthologues
77 of mammalian lectin type siglecs have also been reported in fish genomes. They are
78 sialic acid-binding lectins with an Ig (Immunoglobulin) fold [10].

79 Carbohydrate affinity chromatography has been the preferred method for purification
80 of lectins that can bind to specific carbohydrate ligands and/or matrix [11]. We have
81 reported the presence of galectin-1 in Atlantic cod skin mucus, purified it using
82 lactose affinity chromatography, and shown that the molecule gave hemagglutination
83 and agglutinate pathogenic bacteria [12]. We have also identified a 13 kDa mannose-
84 binding lectin in Atlantic cod skin mucus based on proteomic studies [13]. In this

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85 study, we report the purification of a novel lectin with a unique primary structure
86 from the skin mucus of Atlantic cod.

87 **2. Materials and methods**

88 *2.1 Fish and mucus collection*

89 Adult Atlantic cod weighing 2-3 kg, obtained from Cod Juveniles AS (Bodø,
90 Norway), were used in this study. The fish were anesthetized with MS-222 (70 mg L^{-1})
91 and killed with a blow to the head. Skin mucus samples were collected from 50 fish
92 using sterile glass microscope slides. The mucus samples were pooled together,
93 transferred to sterile 50 ml tubes and immediately stored at -80°C for later use. Skin
94 tissue samples for RNA extraction for cloning and tissue distribution work were
95 collected and snap frozen in liquid nitrogen from fish reared at the indoor facilities of
96 University of Nordland (now renamed Nord University), Norway. All animal
97 handling procedures were performed according to guidelines set by Animal Research
98 Authority in Norway.

99 *2.2 Mannose affinity chromatography and gel filtration*

100 Prior to analysis, skin mucus samples were thawed on ice, diluted 1:10 with binding
101 buffer (20mM Tris-HCl, pH 7.5, 0.5M NaCl, 10 mM $CaCl_2$, 10 mM MgCl2) and
102 homogenized with a VDI 12 hand-held homogenizer (VWR, Norway). The diluted
103 mucus was centrifuged twice at 3000 *g* to remove cell debris and was batch bound to
104 10 mL mannose coupled sepharose (GALAB Technologies, Germany) at 4°C for 1 h.
105 Batch bound slurry was washed 3 times with binding buffer and loaded on to the
106 chromatography column (Bio-Rad, CA, USA) and manually eluted using elution
107 buffer (one step elution; binding buffer + 0.5 M mannose). The protein fractions (1
108 mL each) were monitored (absorbance, 280 nm) and collected using Biologic LP
109 fraction collector (Bio-Rad). Affinity purified, pooled and concentrated fractions were
110 further separated by gel filtration on Sephacryl S-200 HR (GE Healthcare, UK)
111 column. Flow rate in this column was maintained at 0.5 mL min^{-1} and 2 mL fractions
112 were collected. The eluted fractions corresponding to a single peak in the chart were
113 pooled and concentrated using nanosep 3 kDa cutoff columns (Pall corporation, WA,
114 USA). Isolated protein was mixed with Laemmli sample buffer and was loaded on to
115 12 % SDS-PAGE gels. Gels were stained with colloidal Coomassie Blue stain (0.08%

116 Coomassie Blue G-250, 1.6% ortho-phosphoric acid and 8% ammonium sulphate in
117 buffered 20% methanol) and images were captured using the ChemiDOC™ XRS
118 imaging system (Bio-Rad). The protein band (corresponding to ≈ 35 kDa) was
119 analysed by LC-MSMS (Liquid chromatography-mass spectrometry) as described
120 elsewhere [13]. The pkl files obtained after LC-MSMS were analysed using
121 MASCOT (http://www.matrixscience.com/) with the following settings: enzyme:
122 trypsin with one missed cleavage, fixed modification: carbamidomethyl of cysteine
123 and variable modification: oxidation of methionine, peptide charge: 2+ and 3+,
124 peptide tolerance: 100 ppm and MS/MS ion tolerance: 0.1 Da. The taxonomic class
125 searched was *Actinopterygii* (ray finned fishes).

126 *2.3 Bioinformatic analysis and cloning of natterin*

127 Based on the peptide matches identified through LC-MSMS, nucleotide sequences
128 corresponding to the peptide sequences flanking the matched EST were used to
129 design primers, perform PCR and amplify the putative partial nucleotide sequence of
130 the identified protein. Briefly, cDNA for the PCR was prepared from the total RNA
131 extracted from the skin, as described elsewhere [14]. The amplicon was then cloned in
132 to the pCR4-TOPO vector using TA TOPO cloning kit (ThermoFisher Scientific,
133 MA, USA) and the DNA was extracted and sequenced. The partial nucleotide
134 sequence was translated and was used as the query for the BLASTp algorithm at
135 NCBI database to retrieve similar protein sequences, which were aligned using
136 MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/). The domain features of cod and
137 other teleost proteins were predicted using SMART (http://smart.embl-heidelberg.de)
138 or NCBI CDD (conserved domain database). The primers used for cloning the partial
139 *cod nlp* cDNA were codNatF (CGCACATGACTGAATGGC) and codNatR
140 (GAGTAGGGGAGGTCAATGACT)

141 *2.4 Hemagglutination assay.*

142 The hemagglutination assay was done with defibrinated horse erythrocytes.
143 Erythrocytes (ThermoFisher Scientific, MA, USA) were trypsinated, fixed in
144 glutaraldehyde, and diluted to 4 % as reported earlier [12] with some modification. In
145 short, erythrocytes were washed three times in TBS I buffer (Tris buffered saline; 20
146 mM Tris-HCl, 150 mM NaCl, pH 7.6), treated with 0.1 % trypsin EDTA in TBS-I for
147 1 h at room temperature, and extensively washed in TBS-I, and fixed in 1 %

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148 glutaraldehyde in TBS-I for 1 h. Fixed erythrocytes were washed twice in 0.1 M
149 glycine in TBS-I, washed extensively in TBS-I, and diluted to 4 % erythrocyte
150 concentration.

151 The hemagglutination assay was performed in U bottomed microtiter plates (VWR,
152 Norway). In each test well 20 µl of protein sample (Nlp), 20 µl of 4% erythrocyte
153 suspension, 20 µl of 0.5 % BSA-TBS I and 20 µl TBS II (TBS I + 10 mM CaCl2, pH-
154 7.6) were added. To find the hemagglutination titre, 2-fold serial dilutions of the
155 protein sample (undiluted 401 µg/ml) were used. The final concentrations of Nlp was
156 hence 100 µg/ml in the first well. In control well, the protein sample was replaced
157 with 20 µl TBS II. To check mannose dependency, TBS I was replaced with 20 µl of
158 0.5 M mannose in TBS-I, and to check calcium dependency 10 mM EDTA in TBS I
159 replaced TBS-II.

160 For visualization of agglutination in light microscopy erythrocytes was mixed with
161 TBS-I (control), TBS-I and Nlp or TBS-I with EDTA and Nlp, respectively, and
162 inspected within 5 minutes.

163 *2.5 Vibrio anguillarum bath challenge*

164 Juvenile Atlantic cod of average size 90 g procured from a commercial hatchery and
165 held at the Pathogen-challenge facilities of the Institute of Marine Research, Bergen,
166 Norway were used for the experiment. This study, conducted in cooperation with the
167 University of Nordland, was approved by the Norwegian Animal Research Authority
168 (http://www.FDU.no). Twenty-four fish each were introduced in to two 500 L
169 experimental tanks and were fed on laboratory prepared fishmeal-based feed. A
170 suspension of *V. anguillarum* (strain H610) at 1.6x 10^7 cfu mL^{-1} was inoculated into
171 one tank, after lowering the water level. The control tank was mock-challenged with
172 culture media (tryptic soy broth with 1.5% NaCl). The water level was brought back
173 to the normal level in both tanks after 1 h exposure. Gill and skin samples from 6 fish
174 each for initial (0h), 4 h, 24 h and 48 h time points were collected and snap frozen in
175 liquid nitrogen before being stored at -80°C.

176 *2.6 qPCR and statistical analysis*

177 Natterin mRNA transcript levels were evaluated using quantitative realtime PCR. For
178 the challenge experiment total RNA extraction was carried out as described before

179 [15]. Total RNA (500 ng per reaction) was reverse transcribed using Quantitect RT
180 kit (Qiagen, Venlo, The Netherlands). Quantitative PCR was carried out using a
181 LightCycer 96 (Roche) and FastStart Universal SYBR Green Master mix (Roche).
182 Natterin primers used for q-PCR were codNat_qF2
183 (GGCTCCGACATGGACTGTAT) and codNat_qR2
184 (TTTGTTTACCTGGGGTGTATAC). These primer sets have been designed around
185 intron-exon boundaries. Three reference genes namely, ubiquitin (*ubi*) [16],
186 elongation factor 1 alpha (*ef1a*) [17] and cyclophilin A (*cyca*) [18] were used for
187 calculating the normalization factor. No-template and non-reverse transcribed controls
188 were included for each primer set. The thermal profile for qPCR was 95°C for 10
189 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. The specificity of PCR
190 amplifications was determined by melting curve analysis and further confirmed by
191 Sanger sequencing.

192 For *nlp* tissue distribution analysis cDNA prepared from mRNA isolated from skin,
193 gills, foregut (middle of first half of gut), hindgut (middle of second half of gut),
194 rectum (rectal chamber), muscle, liver, spleen and head kidney were used. The
195 samples were the same as used previously [13]. PCR was run with preincubation
196 (95˚C for 10 min), two step amplification (95˚C for 10s, 60˚C for 30s), and melting
197 (95˚C for 10s, 65˚C for 60s, 95˚C for 1s). The total number of cycles was 45.
198 Bestkeeper [15] was used to assess the stability of the housekeeping genes *ubi*, *ef1a*
199 and *cyca* (table 1 and 2). The geometric mean of all the genes was used for relative
200 expression of natterin among tissues. Delta Ct method was used for analysis and one-
201 way analysis of variance (ANOVA) was done followed by post-hoc Tukey HSD
202 (Honestly Significant Difference) test.

203 **3. Results and discussion**

204 The mannose affinity chromatography of Atlantic cod skin mucus (in the presence of
205 divalent cations) and subsequent SDS-PAGE analysis showed 3 protein bands; a ~13
206 kDa band, a 35 kDa band and a heavy molecular weight band. We were primarily
207 interested in the major 35 kDa protein, since we thought it could be cod intelectin.
208 However, mass spectrometric analysis of the ≈ 35 kDa band (Fig. 1 C and D) obtained
209 after mannose elution (Fig 1. A) and gel filtration (Fig 1 B) indicated that the protein
210 belonged to a group of natterin-like proteins (Nlp) (NCBI dbEST Accession:

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211 GW854606). Primers designed based on the matched cod EST (Fig 2) amplified the
212 target 523 bp nucleotide sequence which was shown to be Nlp using BLAST
213 searches. The partial cod *nlp* sequence was submitted to GenBank (GenBank :
214 KP242020). Like other teleost Nlps, cod Nlp although a partial sequence, showed the
215 presence of N-terminal jacalin-like domain and C-terminal aerolysin/ *Clostridum*
216 *perfringens* like toxin domain (Fig. 2). Natterins were earlier identified as a novel
217 family of proteins from the venom of *Thalassophyrne nattereri* [19], a member of the
218 venomous stone fish family Batrachoididae. Further bioassays have indicated that
219 natterins have kininogenase activity and can cause nociception and edema in mice,
220 confirming their role as stone fish toxins [19]. Nlp (PL-toxin I and II) exhibiting
221 similar activity were also purified from the skin mucus of Oriental stinging catfish
222 (*Plotosus lineatus*) [20]. A natterin-like gene cloned from lamprey (*Lampetra*
223 *japonica*) blood was found to have pore-forming aerolysin-like domain in addition to
224 an N-terminal lectin domain [21].

225 Jacalins originally belong to a group of galactose-binding lectins found within the
226 plant family Moracea, but several mannose-binding lectins that share the jacalin-like
227 domain have also been identified [22]. Jacalins have been reported to specifically
228 interact with T cell subsets [23] as well as being associated with plant stress [22].

229 Atlantic cod *nlp* is expressed in all tissues analysed (Fig. 3); the expression was high
230 in skin, head kidney, liver and spleen. Isolated natterin-like protein gave
231 hemagglutination (Fig 4) in the presence of calcium (Fig. 4B and 4D). The
232 hemagglutination was inhibited with mannose and partly inhibited with EDTA (Fig
233 4C and 4D), indicating that natterin binds to mannose on red blood cells in a calcium
234 dependent manner. We have previously used a lactose affinity column to isolate
235 galectin-1 from Atlantic cod [12], natterin-like protein was not found, suggesting that
236 Nlp cannot bind lactose.

237 We were interested to understand the modulation of the cod *nlp* in the event of a
238 bacterial infection. A *V. anguillarum* bath challenge however, failed to identify a
239 significant differential expression on the mucosal surfaces (skin and gills; Fig 5) of
240 cod during the early phase of infection. The infection in the challenge experiment was
241 ascertained by confirmation of high levels of *il1b* transcripts in the gills 48 h post
242 challenge [14]. The results suggest that the lectin might be constitutively expressed,

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243 rather than upregulated during infection. The mechanism of Nlp secretion in to the
244 mucosal mileu is not known, there is no evidence of a signal peptide in the available
245 fish Nlp sequences but nonclassical secretion of protein with jacalin-like domains are
246 observed in some instances [24], and could explain the presence of Nlp in cod skin
247 mucus.

248 This is the first report on purification of an Nlp using mannose affinity
249 chromatography, showing the mannose-binding activity of cod Nlp and possibly other
250 fish Nlps as well. The mannose-binding activity, coupled with the pore-forming
251 domain of the natterin-like proteins [25], suggests that cod Nlp is a lectin with
252 potential toxin function. There are examples of proteins with an architecture involving
253 the combination of a lectin domain with a pore forming toxin domain like the toxic
254 perivitellin protein Pc PV2 from aquatic apple snail *Pomacea canaliculata* [26]. The
255 presence of Nlp in cod skin mucus is interesting because Atlantic cod is not
256 associated with any venom apparatus or known toxic effects. Interestingly, the
257 presence of an aerolysin domain is reported in a wide range of eukaryotic organisms
258 in combination with other functional domains. Their occurrence could be due to
259 horizontal gene transfer events from bacteria to eukaryotes and their persistence could
260 indicate functional significance to the host [27], in eg. protection against parasites.
261 Further functional analysis of the purified cod Nlp is important to identify the role of
262 these proteins in cod innate immune defense, especially on the mucosal surfaces.

263

264 **Acknowledgements**

265 This study was supported by the Research Council of Norway project ‘Mucosal
266 immune system of Atlantic cod-Creating a knowledge base’ (184703). The FUGE
267 (Functional Genomics) platform (and Jack-Ansgar Bruun) in Tromsø, Norway is
268 thanked for generating the MS data. The authors thank Hari Rudra, Ann Cathrine
269 Einen and Rolf Hetelid Olsen, Institute of Marine Research for their technical support
270 in conducting the bacterial challenge experiment.

271

272 **Figure legends**

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274 **Fig 1** Purification of cod natterin-like protein. A) Crude, ten-fold diluted skin mucus
275 was batch bound to mannose-sepharose in the presence of Ca^{2+}/ Mg^{2+} and one-step
276 eluted with 0.5M mannose. B) The fraction corresponding to single largest elution
277 peak after mannose elution was passed through a gel filtration column (sephacryl S-
278 200HR) at a flow rate of 0.5 mL min^{-1} C) SDS-PAGE gel of representative fractions
279 corresponding to the single largest peak during mannose elution D) SDS-PAGE gel
280 corresponding to representative fraction of the single largest peak in gel filtration. M
281 stands for marker. Black arrow indicates the band corresponding to cod Nlp.

282 **Fig 2** Alignment of partial cod Nlp amino acid sequences with the sequences of
283 teleost Nlps. *G. morhua* (GenBank: AKO698112), *Salmo salar* (GenBank:
284 NP_001134309), *Oncorhynchus mykiss* (GenBank: CDQ92460), *Esox lucius*
285 (GenBank: XP_010866454), *Danio rerio* (GenBank: XP_ 005166416), *Lethenteron*
286 *camtschaticum* (GenBank: AFX60113). LC-MSMS generated peptides that matched
287 cod Nlp are indicated in red. The forward and reverse primers on the cod Nlp
288 sequence are in bold red and underlined. Dashed line on the top of the alignment
289 indicates partial span of the jacalin-like lectin domain and solid line indicates the
290 toxin like domain (Epsilon-toxin domain/ aerolysin). Domain predictions are based on
291 NCBI's Conserved Domain Database (CDD). Symbols below the alignment: * single,
292 fully conserved residue. : (colon) groups of strongly similar properties - scoring > 0.5
293 in the Gonnet PAM 250 matrix. . (period) groups of weakly similar properties -
294 scoring =< 0.5 in the Gonnet PAM 250 matrix (as explained in the ClustalW /
295 MUSCLE documentation.

296 **Fig 3** Relative levels of cod *nlp* transcripts in tissues of Atlantic cod expressed
297 relative to liver. Quantification was done by q-PCR and values are expressed as mean
298 ± SEM (n=3). Bars with no common letter are significantly different ($p<0.05$) by one-
299 way ANOVA and post-hoc Tukey HSD (Honestly Significant Difference) test.

300 **Fig 4.** Hemagglutination assay with natterin-like protein. (A). Red blood cells in TBS-
301 I with $CaCl_2$ (control), (B). Red blood cells in TBS-I with $CaCl_2$ with Nlp, (C). Red
302 blood cells with Nlp and EDTA. (D). For the plate analysis of agglutination red blood
303 cells in the presence of TBS-I with $CaCl_2$ and BSA were used. Final concentration of
304 Nlp was 100 µg/ml in A1, wells A2 till A10 are two-fold serially diluted. B1 is
305 negative control without Nlp. B2 is 100 µg/ml Nlp with EDTA. C1 is 100 µg/ml Nlp
306 with EDTA.

307 **Fig 5** Relative levels of cod *nlp* transcripts in the skin (A) and gill (B) tissues of cod.
308 Quantification was done by q-PCR and values are expressed as mean ± SEM (n = 6).
309 Initial samples (black bars) refer to zero hour samples.

310

311 **References**

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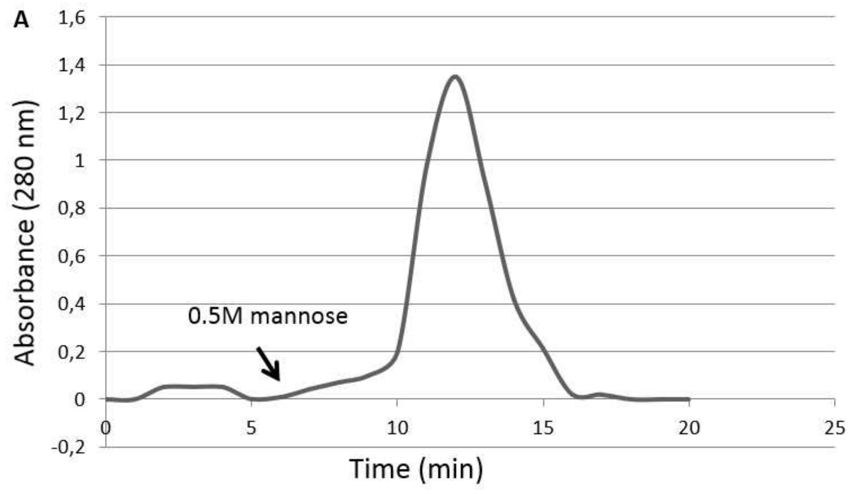
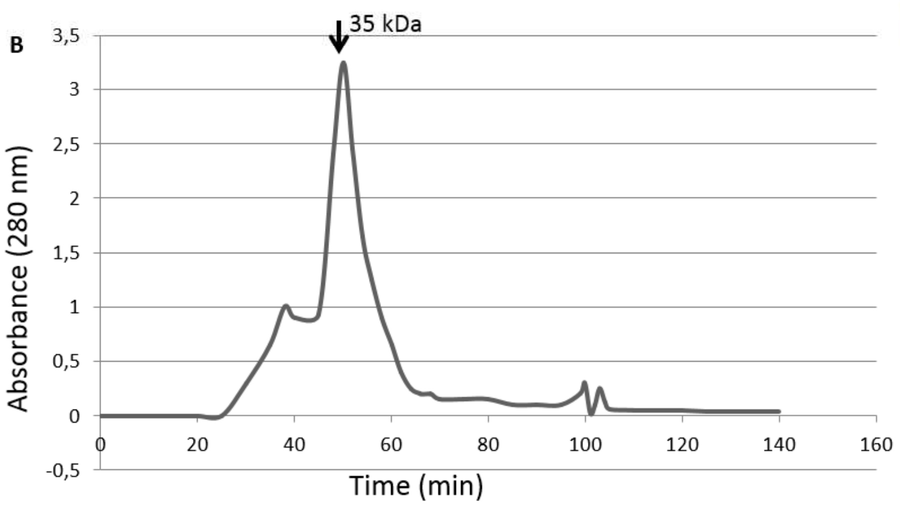
Table 1 PCR efficiency of the primers

Gene name	PCR efficiency	R^2
cycA	89.24 %	0.9999
elfalfa1	90.25%	0.9989
ubiquitin	89.91%	0.9991
natterin	87.60%	0.9976

Table 2 Results of Bestkeeper analysis of reference genes

	cycA	*Elfalfa 1*	*ubi*
coefficient of correlation [r]	0,928	0,963	0,974
coefficient of determination [r^2]	0,861	0,927	0,949
intercept [crossing point]	-2,093	0,429	1,482
slope [crossing point]	1,201	0,891	0,926
SE [crossing point]	±0.427	±0.221	±0.19
p-value	0,001	0,001	0,001
Power [x-fold]	2,30	1,85	1,90





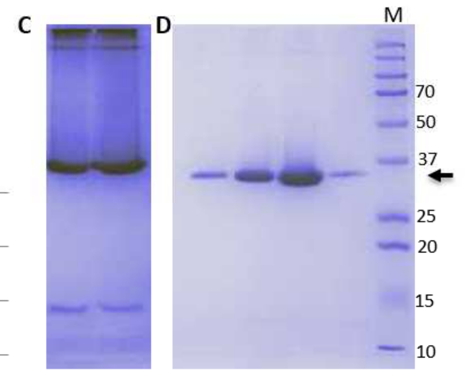


Fig 1

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G morhua          HMTEWPLKIEYSIDVGSGVCLGLEGRSGSDMDCMGFLFINAIKSSVLTDMTYPSLAMYTP
S. salar          KMTSWPLKTEYTIDVGSGICLGLQGRSGSDIDSMGFLFINTIKSSVLTNMEYPTLSLFKP
O. mykiss         KMTSWGLKTEYTIDVGSGICLGLQGRSGSDIDCMGFLFIKTIKSSVLTDMEYPTLSLFKP
E. Lucius         HMNSWPLKTEYSIDVGSGVCLGLQGNCGSDIDCMGFLFISPIRTSVLTDMHYPNLAMFTP
D. rerio          KMTSWGLKTEYPMDVGSGYCLGIKGRSGSDIDCMGFMFLNAVQSAVLTNVNYPTINQLIP
                  KMTDWGLKTEYKIDVGSGICLGVQGRGGSDIDSMGFIFINAIKSSVIQDMKYPTMHQILP
L. camtschaticum  :*..* ** ** :***** ***::*. ***:*.***:*:..:.::*: :: **.:    *

G morhua          QVNKEYVKSVSYHNGSTAAQEHKCAYSRSVTKSTTWSTTTKIESTISLTVKAGIPDLVEV
S. salar          QVTPEYVKSLSHHNDTSLVQEESITYSKTLTKTSSWSVSNKIESTLNVSVKAGIPDLVEV
O. mykiss         QVTPEYVKSVSHQNDTPLVLEKSITYSKTLTKTSSWSVSNKIEFTLNVSVKARIPDLVEL
E. Lucius         QVRKEYIKSVSYHNNTTAPQDQTIQYSRTVTKKSSWTTTNKIESTLSVSVQAGIPDLAEV
D. rerio          KVATEEIKSVSFENKTSVKQEQKVETSKKVIKTSSWSMTKSFSSTFSMEVKAGIPKIAEV
                  NVQMEEIKEMEYKNDTSIVQSYTFESSKKIIKKSSWSTTNKIESTFSLSVKAGIPEVMEV
L. camtschaticum  :*  * :*.:.. * :.   . .   *..: *.::*: :..:. *:.: *:* **.: *:

G morhua          SGGFSVTVGAAQTTSMTSSETITESDEVKVTVPAGKTMTVEATVGRAVIDLPYS
S. salar          TSGFSLTVGVEQSTSLQKTETITESDTINVKIPPGKTLDVEITVGKATIDLDYR
O. mykiss         SSGFSLTVGVEQSTSLQKTETITESGTINVKIPPGKTMDVEITMGKANIDLDYR
E. Lucius         STGWSLTVGHEQSSSMSNEETITEADNATVKIPPGKTVTVEMSVGRAVIDLAYS
D. rerio          STGFSVTFGNESTYSLEQSDERNETLTTTVKVSPKKKVDVHITIGRASFDLPYT
                  ETGFSFTVGSESTHAVEESEEKTETLTFPVTVPTHKTVTVVANIGRADIDLPYT
L. camtschaticum  *:*.*.*  .: :: . :  .*:    *.:.. *.: *  .:*.* :** *
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Fig 2

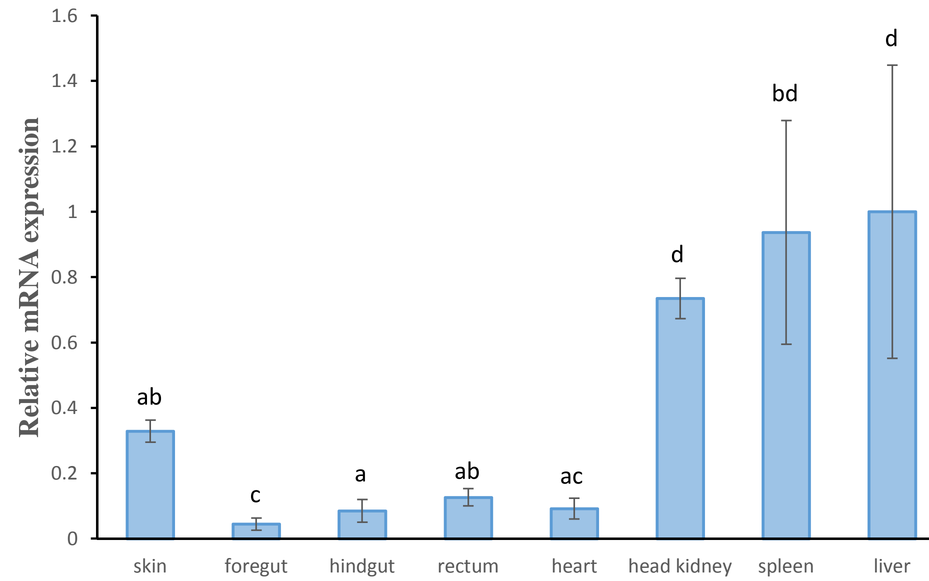
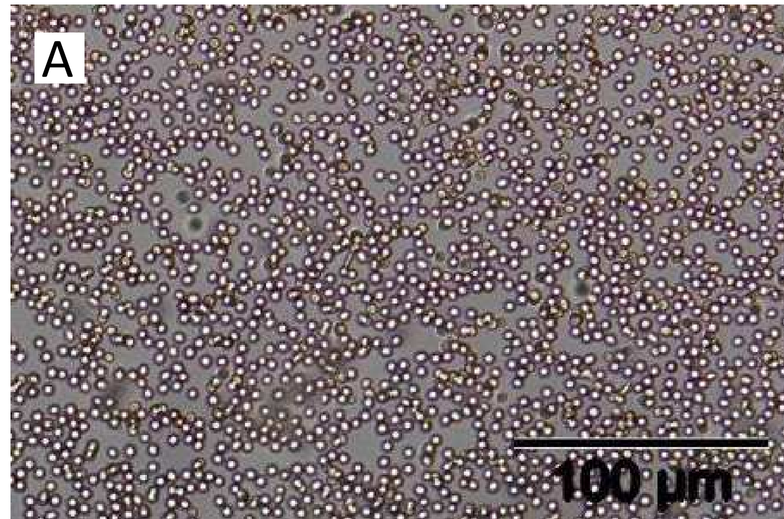
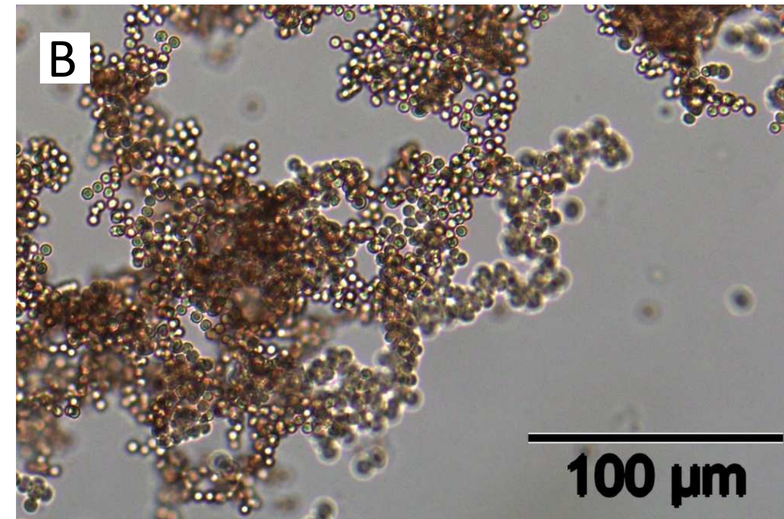
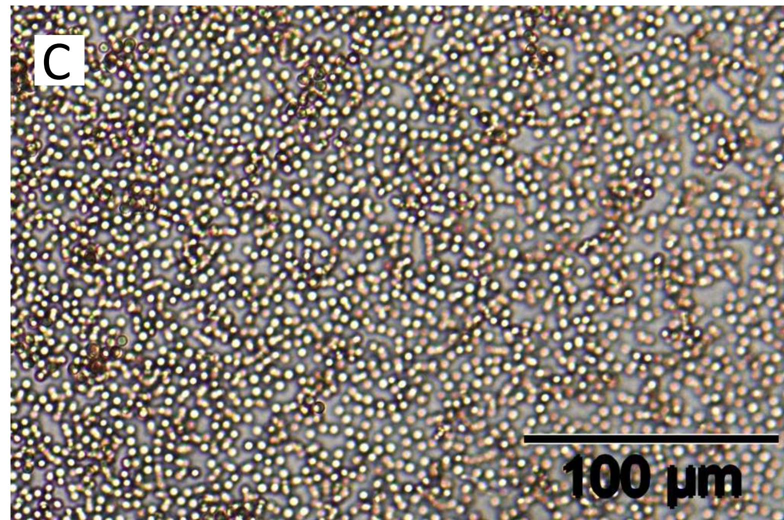
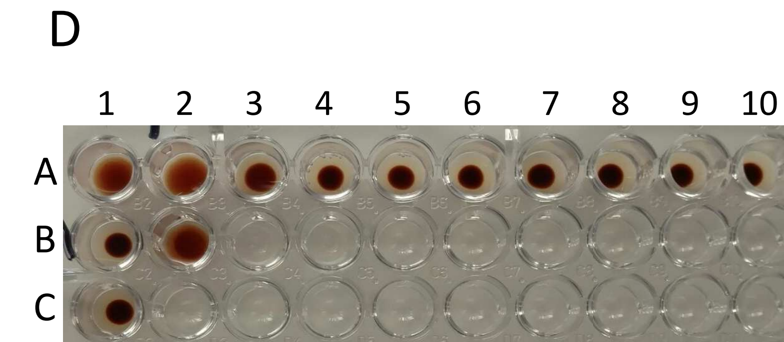


Figure 3


A
100 µm


B
100 µm


C
100 µm


D
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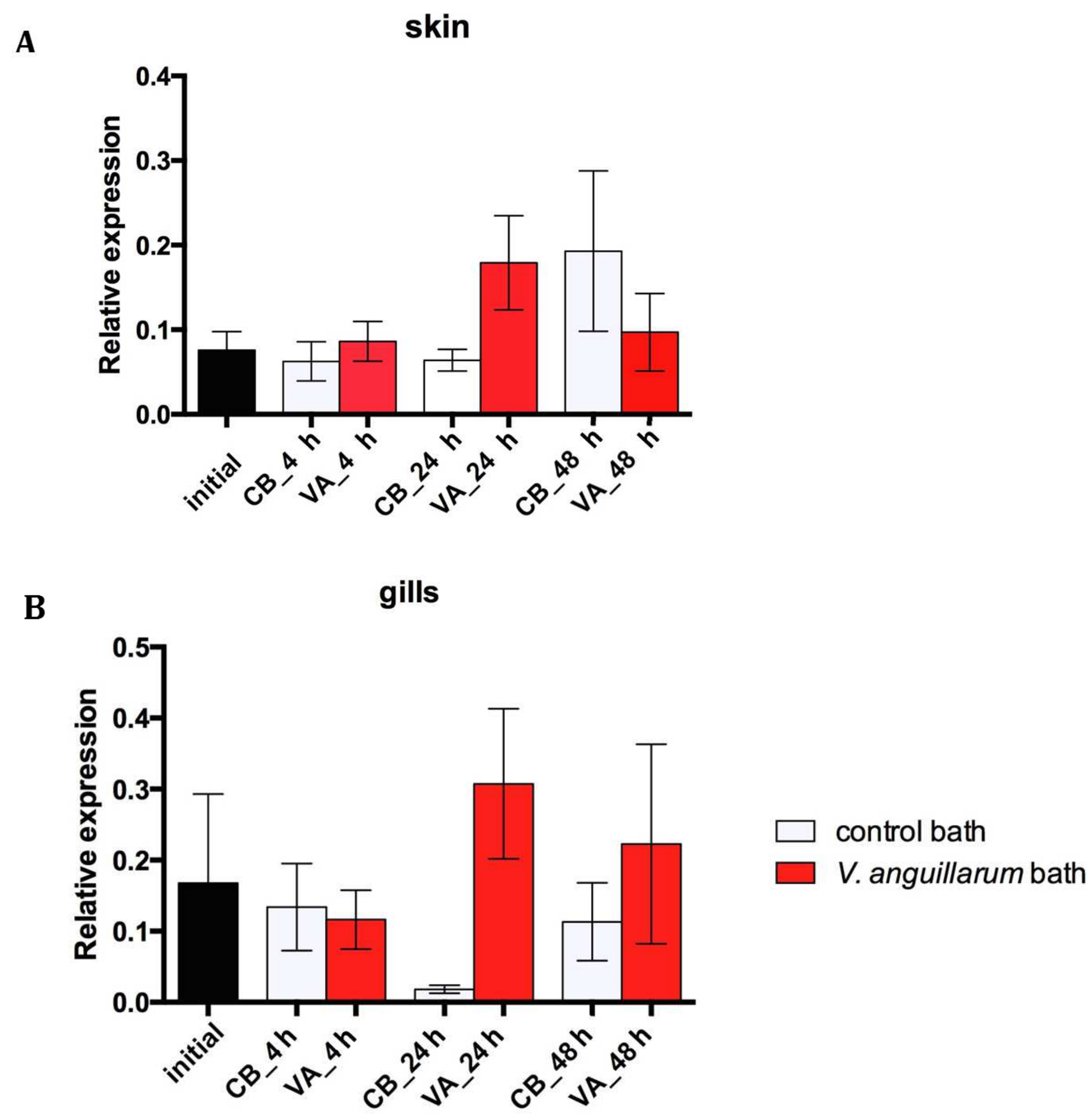

Figure 5

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Highlights

- A mannose binding lectin was isolated from Atlantic cod skin
- The lectin was identified as natterin-like protein with a jacaline-like lectin domain
- Hemagglutination by Nlp was inhibited by mannose and partly with EDTA.
- The expression of cod nlp did not change during a Vibrio anguillarum bath challenge

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