



Supplemental Material to:

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Biofilm-degrading enzymes from *Lysobacter gummosus*

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α -Lytic proteases

Lgum1 1 MSVSKSRSRSFARASVFCVAVAVAALTSGAALAAAGEVSPALKFAMORDLGHFPCCQLAQQY
 Lgum2 1 --MSLSQSARRACRRSLFLALASAASLVSGAAFAADSIDPRLQLAMORDLGHSAKQLPQY
 Lgum3 1 -----MRKFSLSLTATVAMTVSGAALAAADDLAPGLKAAAMORDLGHSGTOVTFEY
 Lgum4 1 -----MRKFSMSVLAATVAMTVSGAAFAADDLSPALKTAMORDLGHSSSTQLSOY
 Lgum5 1 --MQSTGFSSRRSGAHVICALSLLCMVAFDAAAATDLQVSPTLHQAAQHDLGHSSAQVDQL
 Lenz 1 -MYVSNHRSRRVARVSVSCLVAALAAAMSCGAALAADQVDPQLKFAMORDLGHFPCCQLPQY
 LspA 1 -MSVSKSNARGFARASVFCVAVAATLSSGAFAADIVSPELKFAMORDLGHFPCCQLAQQY
 LspB 1 -----MSVSKSNLRSRASLVAVAALASGSAMATDGNPTLKLAMORDLGHSSAQVAQM

Lgum1 61 LQTEKLAREQAASIEREFGGNFAGSWIERKDDGSFRVVAATAG--ARKSSTLGGVEVRNVR
 Lgum2 59 LKTQSLSLRQASAKRALGSNFAGSWIERKADGSFGFVATSCTCKAARSLGGVEVRSVR
 Lgum3 50 LKAERLAAQOEKAVEKQLGRNFAGAWLERKADGSFGFVAATT-SIKAAKAAAGIETROAR
 Lgum4 50 LKVERLAEQOEKIVQAQLGRNFAGAWVERKADGSFGFVAAST-SIKNAKAAAGVETROAR
 Lgum5 59 LQVQRDAPAEALARRRLGAHFVGGAWVERASDGRYRFVVGSSD-PRGPKLIDGVQIRQVR
 Lenz 60 LQTEKLARTQAAAIEREFGGNFAGSWIERNEDGSFRLVAATSG--ARKSSTLGGVEVRNVR
 LspA 60 LQTEKLARTQAAAIERDLGSLFAGSWIERNADGSFRLVAATAG--ARKASALGGVEVRSVR
 LspB 55 VQADRILATTQEAALRRRLGSGFAGSWIERNDDGSFRVVAATSG--AQKSVAAAGVEVRHVR

Lgum1 120 HSLKRLQOSMDLLDAGAHARVKGVSKPLAGVQIHWYVDVRSNIVVVKVDEGATDAGIDFVA
 Lgum2 119 HSLGQLENAFAALESQVRSRVAGVSKPLGGVHSWRIDPVINSVVVSLAPGATEDGVDFVA
 Lgum3 109 HSLSALN----AAKQLDSQLARNGKAPKGVYSWAVDLPSNSVIVGVAPGAEDTAVDFVA
 Lgum4 109 HSLAALN----SAKAQLDSLARSAPKGVYSWVVDLPSNSVVVGVAPGAEEACVDFVA
 Lgum5 118 YSLSELEAAKARLDRSVRARVSGISRPIIDGVHSWYVDPGSNSVIVSVAPDAMERATDLAA
 Lenz 119 YSLKQLQNSMAQLDAGANARVKGVSKPLDGVQSWYVDPNSNAVVKVDDGATEAVDFVA
 LspA 119 HSLKQLQNSMAQLDAASNARVKGVSKPLDGVQSWYVDPVSNNAVVRVDQGAERAVDFVA
 LspB 114 HSLKQLQNSMAALDRDARRRVPGLSKLRSVQSWYVDPITNSVVVSVAPGADEEAVDFVA

Lgum1 180 LSGADSGAVRIESAPGQLQTTANIVGGIEYSINN----TNLCSLGFVSVTRGATKGFVTAG
 Lgum2 179 LSGADAGAVRFETDEGTPQLLANVIGGDQYLWEVTEG--YYPCSVGFVSVTKGETKGFVTAG
 Lgum3 165 RSGLDITAVRFETMAEAPQRRIAIQGGRGYLRNPGDGYLYACSVGFVTKSTTPGFATAG
 Lgum4 165 LSGLDARVRFETLNEAPQRRIAVQGGRGMLRDPGDGYLYACSVGFIVTKGSTTPGFATAG
 Lgum5 178 VSGADSGLLRFQQTTPGVPQPTSSVYAGRSYKNG----ALSCSVGFVAVMQGATKGFVTAG
 Lenz 179 LSGADSAQVRIEESPGKLQTTANIVGGIEYSINN----ASLCSVGFVSVTRGATKGFVTAG
 LspA 179 VSGADAGAVRIEEVPGTLQTSVNVLGGIEYSINN----ATLCSVGFVSVTRGATKGFVTAG
 LspB 174 VSGADISSIRVEEAVGTPQTTATVQGGIEYRMLPDLGRVGLCSVGFVTKGTIKGFATAG

▼ ▼
 Lgum1 236 HCGTVNSIARIG-----GAQVGTFAGRVFPG-----NDRAWVSLTSAQTLLLRVWNG
 Lgum2 238 HCGQVGRVVLGTG--DVEALVPLGTFTAVNHPG-----TDMAWVIVNPEHTLLGQVKDY
 Lgum3 225 HCCNAGEVVYNEDSQWVPGVRLGAFAASTMPD--GNETGPDRCVWQVDSHTLSASVYGY
 Lgum4 225 HCGTVGEIVYQEVSQWNPVVRVGTFSASTMETPSGSQTGPDRCVWVVDITHTLSPSVYGY
 Lgum5 234 HCGTAGETIGLD-----GAPAGYFAASEFPG-----TDRAWVALNANHELFPPLITNY
 Lenz 235 HCGTVNATARIG-----GAVVGTFAARVFPG-----NDRAWVSLTSAQTLLLRVWANG
 LspA 235 HCGGVGAIVRIG-----GTQVGSFAARVFPG-----NDRAWVSVGSAHTLQGAVSNY
 LspB 234 HCAKAGQSVQIS-----GVNVGTFTTASHFPN-----TDRAWVITGAAHTLLGSVITNY

Lgum1 283 SS-YVTVRGSAAEAAVGAAVCRSGRTTGYRCGTITAKNVTANYAEG-----TVRGLTIAN
 Lgum2 289 ADGLLSVKGSVEAPVGAALCRSGRTTGYKCGTIRAKNVSVRVCFDR-----YVGMETETN
 Lgum3 283 GSGDVTVHGSTEAAVGGALCRSGRTSGWRCCGAIRTKNVTVSYVDDTGQPDGTVTGLTQTT
 Lgum4 285 GNGDVTVVRGSTEAAVGAALCRSGRTSGWHCCGAIRSKNNTVSYVDDAGNPDGTVTGLTRTS
 Lgum5 281 AGCFFAVRGSVEAGYSAVVCRSGWKTGYQCGLITANQNVTVNSTRG-----VVFGLTQSN
 Lenz 282 SS-FVTVRGSAAEAAVGAAVCRSGRTTGYRCGTITAKNVTANYAEG-----AVRGLTQGN
 LspA 282 SGGTIAIRGSAAEAAVGAAVCRSGRTTGYRCGNITAKNVTANYAEG-----AVRGLTQGN
 LspB 281 TCGSVAVKGSVEAAVGAAVCRSGRTTGYKCGTITAKNVTVNYCTLG-----TVRGLTIAN

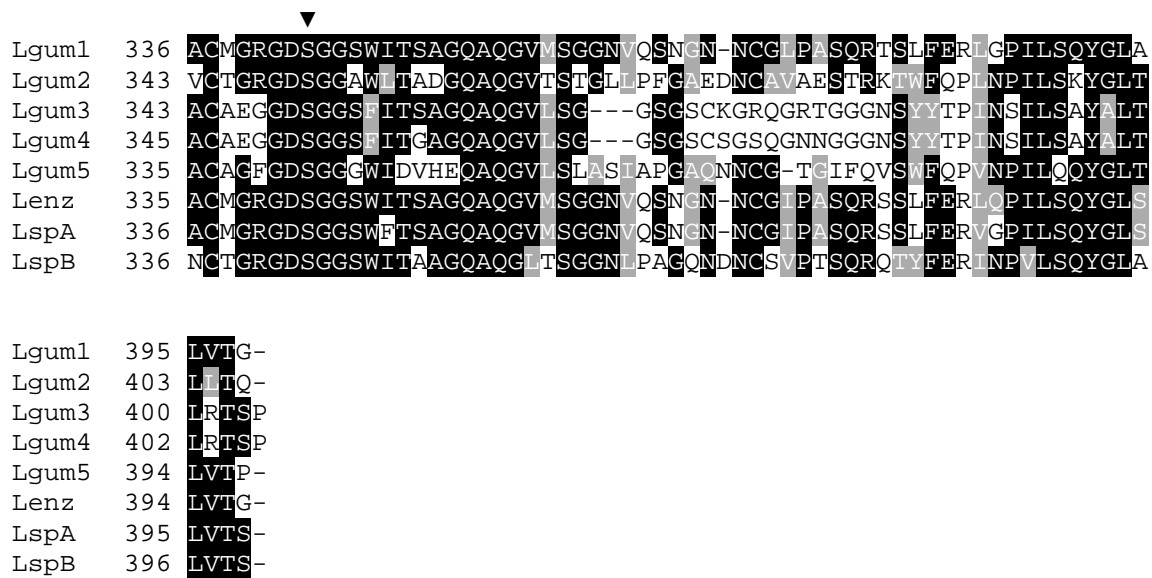


Figure S1. Sequence alignment of α -lytic proteases.

Predicted signal peptides are shown in red. Prodomains reported in published work are shown in orange. The residues constituting the catalytic triad are marked with arrowheads. Signal peptides were predicted with SignalP (<http://www.cbs.dtu.dk/services/SignalP/>). The alignment was generated with ClustalW (<http://www.ch.embnet.org/software/ClustalW.html>) and edited with BoxShade (http://www.ch.embnet.org/software/BOX_form.html).

- Lgum1, *L. gummosus* isoform 1 (GenBank accession no. KF738067);
- Lgum2, *L. gummosus* isoform 2 (GenBank accession no. KF738068);
- Lgum3, *L. gummosus* isoform 3 (GenBank accession no. KF738069);
- Lgum4, *L. gummosus* isoform 4 (GenBank accession no. KF738070);
- Lgum5, *L. gummosus* isoform 5 (GenBank accession no. KF738071);
- Lenz, *L. enzymogenes* (GenBank accession no. AAA74111);
- LspA, *Lysobacter* sp. XL1 (GenBank accession no. ACZ72924);
- LspB, *Lysobacter* sp. XL1 (GenBank accession no. ACZ72925).

β-Lytic metalloendopeptidases

Lgum	1	-----MGLAALLTCCVLAATISGGAGAAERGLSCQ-----
Lenz	1	-----
Lsp	1	-----MKKISKAGLGLALMCALATIGGNASAQGHGLSSE-----
Alyt	1	-----MKKISKAGLGLALVCALATIGGNAARR-ATAQRR-----
Paer	1	MQHKRSRAMASPRSPFLFVLLALAVGGTANAHHDDGLPFRYSAELLGQLQLPSVALPLND
Lgum	29	DLVYSYDEMFDIDAYLAKNAPHLSRHAESISHWAGYSGISPKVLIALMEQQSGAITRR
Lenz	1	-----
Lsp	35	DLVYSYDEMFDIDAYLAKHAPHLRKHSEESHWAGYSGISPKVLIALMEQQSGANS--
Alyt	34	GSGVFDYDEMFDIDAHHLAKHAPHLRKHSEESHWAGYSGISRSVDRADGAAERAVTP--
Paer	61	DLFLYGRDAEAFDLEAYLAVNAPALNDKSEYLEHWSGYYSINPKVLIITLMVMQSGPLG--
Lgum	89	HAAADAARPFGCALAQAKDFNGQTRVVAQALREALYENDGPDAGCAVT-----VAR
Lenz	1	-----
Lsp	93	--AKRATNRPFGLARADGFGQTRVVALALRESLYERFPDGAAGPVT-----LAR
Alyt	92	--SARRIVRSASWRAPTASARRPARSRWCASRCTSAIPTRQAGDAG-----PRQ
Paer	119	APDERALAAPLGRLSAKRGFDHQVRIVLQQLSRRYYGFREYQLHQAAARKAVGEDGLNAA
Lgum	140	ANPLQALFERAGASQASAKLSGDGEFQLVYGRLEFNEPRQAQAPSAR--FAKAGPDVQPLIS
Lenz	1	-----S
Lsp	142	ANPLQALFERSQDNPEAANLRGDGEFQLVYGRLEFNEPRQKAASDR--FAKAGPDVQPLIS
Alyt	141	SAAGAVRAFRRQRAGGRARRRRRVPAGLRPPVQRTAPGQNGFG--P--LRQRRPGRAAIS
Paer	179	SAALLLILLREGAKASRVQGNPLGVAQTRQLRFGTAAEELLQPRNRVARQLQAKAALAP
Lgum	198	PNGLLQFPFRGARWHVGGHAHTNTGSGNYPMSSLDMSLGGGWGSNQSNWVSASANG--SF
Lenz	2	PNGLLQFPFRGASWHVGGHAHTNTGSGNYPMSSLDMSRGG--GSNQNGNWVSASAAGGSF
Lsp	200	PNGLLQFPFRGASWHVGGHAHTNTGSGNYPMSSLDMSRGGGWGSNQNGNWVSASAAG--SF
Alyt	197	PNGLLQFPFRGASWHVGGHAHTNTGSGNYPMSSLDMSRGGGWGSNQNGNWVSASAAG--SF
Paer	239	PSNLMQLPWRQGYSWQPNGAHSNTGSG-YPYSSFDASVDWPRWGSATYSVVAAHAG--TV
Lgum	257	KRHSSCFAEIVHSGGWSTTYHLMNIRYNTGANVGSNTAIANPANTRAQALCNGGSSSTGP
Lenz	60	KRHSSCFAEIVHTGGWSTTYHLMNIQYNTGANVSMNTAIANAPNTQAQALCNGGQSTGP
Lsp	259	KRHSSCFAEIVHTGGWSTTYHLMNIQYNTGANVSMNTAIANPANTQAQALCNGGQSTGP
Alyt	256	KRHSSCFAEIVHTGGWSTTYHLMNIQYNTGANVSMNTAIANPANTQAQALCNGGQSTGP
Paer	296	RVLSRCQVRVTHPSGWATNYHHMDQIQVSNQQVVSADTKLGVYAGNINTALCEGGSSSTGP
▼ ▼		
Lgum	317	HEHWSLKLNGSFYHLNGAYLSGYRITATGNSYDTNCSRFLYAKN--GQNYCSG--WFTNPG
Lenz	120	HQHWSLKLQNGSFYHLNGTYLSGYRITATGSSYDTNCSRFLYTKN--GQNYCYG--YYVNPG
Lsp	319	HEHWSLKLQNGSFYHLNGTYLSGYRITATGSSYDTNCSRFLYTKN--GQNYCYG--YYVNPG
Alyt	316	HEHWSLKLQNGSFYHLNGTYLSGYRITATGSSYDTNCSRFLYTKN--GQNYCYG--YYVNPG
Paer	356	HLHFSLLYNGAFVSLQGASFGPYRINVGTSNYDNDCRRYFYNYCSAGTTHCAFRPLYNPG
Lgum	374	H--
Lenz	177	PN-
Lsp	376	PN-
Alyt	373	PN-
Paer	416	LAL

Figure S2. (previous page) Sequence alignment of β -lytic metalloendopeptidases.

Predicted signal peptides are shown in red. Prodomains reported in published work are shown in orange. SignalP did not identify a single peptide in the *L. gummosus* sequence. The residues binding the catalytic zinc ion are marked with an arrowhead.

Lgum, *L. gummosus* (GenBank accession no. KF738072);

Lenz, *L. enzymogenes* (GenBank accession no. P00801);

Lsp, *Lysobacter* sp. IB-9374 (GenBank accession no. BAB86844);

Alyt, *Achromobacte lyticus* (GenBank accession no. AAA21906);

Paer, *Pseudomonas aeruginosa* staphylolysin/LasA (GenBank accession no. AAC12656).

For the β -lytic metalloendopeptidase from *L. enzymogenes*, only the sequence of the mature enzyme determined by direct protein sequencing is available.

Lysyl endopeptidases

Lgum_Lep1 1 ----MKRLYGSLALLSLSIGAAIACPTILPAAFDPHPNVSPIIDKIALRTMPAVDVEKLLKAQ
 Lgum_Lep2 1 ----MKRICGSLLLLGLSISAALAAPASRPAAFDHNNLSSVDKIAVRMPAVDVAKLKAE
 Lgum_Lep3 1 ----MKRFTLTLGLLALSIGTAIAGPALRPAAFDNARTSRIDRIALRSMPAVDVAKLRAE
 Alyt_API 1 ----MKRICGSLLLLGLSISAALAAPASRPAAFDYANLSSVDKIALRTMPAVDVAKAKAE
 Lsp_LepA 1 ----MKRICGSLLLLGLSISAALAAPASRPAAFDYANLSSVDKIALRTMPAVDVAKAKAE
 Lsp_LepB 1 ----MKRITLTLGLLALSIGSALAAPALRPAAFDHARLARVDRIALRVMPAVDVAKLRAE
 Lenz_27796 1 ----MKRICGSLLLLGLSISAALAAPASRPAAFDHNNLSSVDKIAVRMPAVDVAKLKAE
 Lenz_LysC 1 -----
 Paer_PIV 1 MHKRTYLNACLVLALAAGASQALAAPMASEMAGDVAVLQASPASTGHARFANPNAAISAA

Lgum_Lep1 57 DRLRDKR--GDIPRFAPPMIVDMTPLNSGVWED-LDADHVVWRQVRVSE-----
 Lgum_Lep2 57 DLLRDKR--GDIPRFAPPIKVDMTPLNSGVWED-LDADNVVWRQVRVSE-----
 Lgum_Lep3 57 DVQRAAR--REVPRFATALPLNIDTLTSGTWED-LDADHVVWRTRVLESK-----
 Alyt_API 57 DLQRDKR--GDIPRFALADVDMTPQNSGAWWEYTDNQFANWRQVRVSE-----
 Lsp_LepA 57 DLQRDKR--GDIPRFALADVDMTPQNSGAWWEYTDNQFANWRQVRVSE-----
 Lsp_LepB 57 DVKRNAR--NEVPRFATALAVDIDTLKDGAWED-LDADTAVWRTRVLESK-----
 Lenz_27796 57 DVLRDKR--GDIPRFAPPIKVDMTPLNSGVWED-LDADNVVWRQVRVSE-----
 Lenz_LysC 1 -----
 Paer_PIV 61 GIHFAAPPARRVARAAPLAPKPGTPLLVGVGLKTATPEIDETTLEWIDTPDGRHTARFPI

Lgum_Lep1 103 KAMSLNFGFTQYYMPAGGRMLIYPATQTKCGDRELIREYTDNRDNNEMSQLWTAIVPGQEA
 Lgum_Lep2 103 KALSlnFGFTQYHMPQGGRLLVYPATQAAGDRNLISEYTDARDNNAQGLWTAIVPGQEA
 Lgum_Lep3 103 NALTLNFHFTQFKLPAGARMLIYPADQTPSSSANFVRSFTSADNADGQLWTPVVIGDEA
 Alyt_API 104 KALSlnFGFTDYMPAGGRMLIYPATQAPAGDRGLISQYDASNNNSARQLWTAIVVPGAEA
 Lsp_LepA 104 KALSlnFGFTDYMPAGGRMLIYPATQAPAGDRGLISQYDASNNNSARQLWTAIVVPGAEA
 Lsp_LepB 103 DALSLNFHFDKFKLPEGARMLIYPADQAPSAAGRVRSEFTSADNNAFGELWTPVVVGEEA
 Lenz_27796 103 KALSlnFGFTQYHMPQGGRLLVYPATQAAGDRNLISEYTDARDNNAQGLWTAIVPGQEA
 Lenz_LysC 1 -----
 Paer_PIV 121 SAAGAASLRAAIRLETHSGSLPDDVLLHFAGAGKEIFEASGKDLVSNRPYVWVPEGDTL

Lgum_Lep1 163 VIEVVVPRASVGLKLRRLTKINHDYVGFGLARRMAQASGEKGTSGICNVDVVCPEGNGR
 Lgum_Lep2 163 VIEVVVPRAKLGELKRLKLGQVGHYDVGFGPLARRMAAASGEKGVSGQCNVDVVCPEGDGR
 Lgum_Lep3 163 VIEVVVPRAKAGELKRLGVEVNDYVGIKRLAR-DAALDQAKGVSGSCNIDVVCPEGNGH
 Alyt_API 164 VIEAVIPRDKVGEFKLRRLTEVNHDYVGFGLARRMAAASGEKGVSGSCNIDVVCPEGDGR
 Lsp_LepA 164 VIEAVIPRDKVGEFKLRRLTEVNHDYVGFGLARRMAAASGEKGVSGSCNIDVVCPEGDGR
 Lsp_LepB 163 VIEVVVPRAKLGELKRLKLGQVGHYDVGFGKLVKRGVQQQAGTKAASGSCNIDVVCPEGNGY
 Lenz_27796 163 VIEVVVPRAKLGELKRLKLGQVGHYDVGFGPLARRMAAASGEKGVSGQCNVDVVCPEGDGR
 Lenz_LysC 1 -----GVSGSCNIDVVCPEGNGH
 Paer_PIV 181 TVEIVIPAN----LQPGDLRLSVPQVSYFADSLYKACYRDGFGASGSCNIDVVCPEGNGT

Lgum_Lep1 223 RDIIRSVGVYS-----KNGSLACTGSLVNNTANNKKMYFLTAHHCGMTTAAANISIV
 Lgum_Lep2 223 RDIIRSVAAYS-----KNGSLACTGSLVNNTANDKKMYFLTANHCGMTTASTAASIV
 Lgum_Lep3 222 RDIIRSVAAYS-----KQGTWVCTGSLVNNSANDKKMYFLTANHCGMTTAAIASSIV
 Alyt_API 224 RDIIRAVGAYS-----KSGTLACTGSLVNNTANDKKMYFLTAHHCGMTTASTAASIV
 Lsp_LepA 224 RDIIRAVGAYS-----KSGTLACTGSLVNNTANDKKMYFLTANHCGMTTASTAASIV
 Lsp_LepB 223 RDIIRAVGAYS-----KQGTWVCTGSLVNNTANDKKMYFLTANHCGMTTASVNNISIV
 Lenz_27796 223 RDIIRSVAAYS-----KSGSLACTGSLVNNTANDKKMYFLTANHCGMTTASTAASIV
 Lenz_LysC 19 RDIIRSVAAYS-----KQGTWVCTGSLVNNSANDKKMYFLTANHCGMTTAAIASSIV
 Paer_PIV 237 RAYDNATAAVAKMVFTSSADGGSYICTGILNNGNSPKRQLFWSAAHC-IEDQATAAIIQ

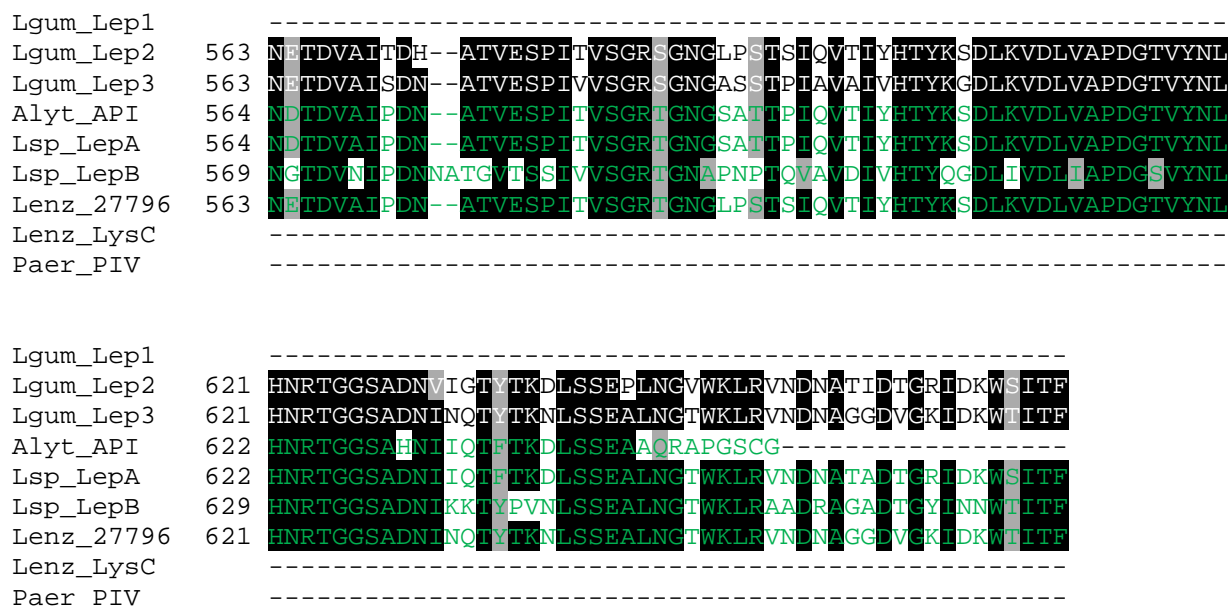


Figure S3. Sequence alignment of lysyl endopeptidases.

Predicted signal peptides are shown in red. Prodomains and C-terminal extension domains reported in published work are shown in orange and green, respectively. The residues constituting the catalytic triad are marked with arrowheads.

Lgum1, *L. gummosus* isoform 1 (GenBank accession no. KF738073);

Lgum2, *L. gummosus* isoform 2 (GenBank accession no. KF738074);

Lgum3, *L. gummosus* isoform 3 (GenBank accession no. KF738075);

Alyt_API, *Achromobacter lyticus* Achromobacter protease I (GenBank accession no. P15636);

Lsp_LepA, *Lysobacter sp. IB-9374* LepA (GenBank accession no. BAB32450);

Lsp_LepB, *Lysobacter sp. IB-9374* LepB (GenBank accession no. BAC22111);

Lenz_27796, *L. enzymogenes* ATCC 27796 (Kuhlman et al. 2009, Ref. 52);

Lenz_LysC, *L. enzymogenes* Lys-C (GenBank accession no. Q7M135);

Paer_PIV, *Pseudomonas aeruginosa* protease IV (GenBank accession no. Q7M135).

For the of lysyl endopeptidases Lys-C from *L. enzymogenes*, only the sequence of the mature enzyme determined by direct protein sequencing is available.


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Lgum  530  DTDFAINDNSTVDSPIITVSGRTGNAPTNAQVSVNI IHTYRGDLKVDLVAPDGS SLYNLSNR
Vcho  515  KGKPIITGLSGSRGGEDFYFTFTVINS GSVVVSISGGITGDADLYVKAGSKPTTSSWDCRPYR
Vvul  514  NNTPVSNLTGNKGSQVFYFTFTVDRNATAVVSISGGSGDADLYLKAGSKPTTSSWDCRPYR
Paer  -----

Lgum  590  AGGSADNLTGTFTFNLSSEPLNGTWKLRVNDNANADVGRIDTWSITF
Vcho  575  SGNAEQCSISAVVGTTYHVMIRGYSNYSGVTLRLD-----
Vvul  574  YGNNEQCSVSAAPGTTYHVMIRGYSNYSGVRLKIQY-----
Paer  -----

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Figure S4. Sequence alignment of M4 family metalloproteinases.

Predicted signal peptides are shown in red. Prodomains and C-terminal extension domains reported in published work are shown in orange and green, respectively. The residues binding the catalytic zinc ion are marked with arrowheads.

Lgum, *L. gummosus* (GenBank accession no. KF738078);

Vcho, *Vibrio cholera* hemagglutinin/protease (GenBank accession no. ZP_06942058);

Vvul, *Vibrio vulnificus* metalloprotease (GenBank accession no. AF102028);

Paer, *Pseudomonas aeruginosa* elastase (GenBank accession no. AFM37281).

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Lgum_Lep2  572  HATVESPITVSGRSGNGLPSTSIQVTTIYHTYKSDLKVDLVAPDGTVYNIHNRTGGSadNV
Lgum_HAP   538  NSTVDSPIITVSGRTGNAPTNAQVSVNI IHTYRGDLKVDLVAPDGS SLYNLSNRAGGSADNL

Lgum_Lep2  632  IGTYTKDLSSEPLNGVWKL RVNDNATIDTGRIDKWSITF
Lgum_HAP   598  TGTFTFNLSSEPLNGTWKLRVNDNANADVGRIDTWSITF

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Figure S5. Comparison of the C-terminal extension domains from *L. gummosus* lysyl endopeptidase isoform 2 (Lgum_Lep2) and the *L. gummosus* hemagglutinin/protease homolog (Lgum_HAP).

OmpA-like proteins

Ecol 1 -MKKTAIAIAVALAGFATVAQAAPKDNTWYTGAKLGWSQYHDTGFIN----N---NGPTH
 Sent 1 -MKKTAIAIAVALAGFATVAQAAPKDNTWYAGAKLGWSQYHDTGFIH----N---DGPTH
 Eaer 1 -MKKTAIAIAVALAGFATVAQAAPKDNTWYAGGKLGWSQHDTGMYNSNLNN---NGPTH
 Smar 1 -MKKTAIALAVALAGFATVAQAAPKDNTWYTGAKLGWSQYHDTGYGNGYQNGIGNGPTH
 Lgum1 1 MNKKLLCAALLGGLSIAQVASAQDFDDRWWYITCAAGMNIQDNDRGTR-----NAPFG
 Lgum2 1 ----MLSTALLAGLAFSQAASAQDFDDRWWYVTGAVGMNIQDNDRGTR-----NAPFG

Ecol 53 ENQLGAGAFGGYQVNPYVGFEMGYDWLGRMPYKGSVENGAYKAQGVQLTAKLGYPITDDL
 Sent 53 ENQLGAGAFGGYQVNPYVGFEMGYDWLGRMPYKGDNINGAYKAQGVQLTAKLGYPITDDL
 Eaer 57 ESQLGAGAFGGYQVNPYLGFEMGYDWLGRMPYKGVKVNAGAFSSQAVQLTAKLGYPITDDL
 Smar 60 KDQLGAGAFGLGYQANQYLGFEIIGYDWLGRMPYKGSVNINGAFKAQGVQLAAKLSTYPIADDL
 Lgum1 53 AIGLGKFLNKNWSLDGELNYQN-----PKFDDNODANWSQYGLSLDLRHRHVTDGRN
 Lgum2 49 AIGLGKFLNKNWSLDGELNYQN-----PKFDDNODANWSQYGLSLDLRHRHVTDGRN

Ecol 113 DVYTRLGGMVWRADTKSNVYG----KNHDTGVSPVFAGGVEYAITPEIATRLEYQWVNN
 Sent 113 DVYTRLGGMVWRADTKSNVPGG-PSTKDHDTGVSPVFAGGTEYAITPEIATRLEYQWVNN
 Eaer 117 DIYTRLGGMVWRADSSNSIAG----DNHDTGVSPVFAGGVEAMTRDIATRLEYQWVNN
 Smar 120 DIYTRLGGMVWRADSKANYGRTGQRLSDHDTGVSPVLAAGVVEYALTKNWATRLIYQVFSN
 Lgum1 105 WNPYILMGLGYQRSEEEYLLPSVLSVSPGNRKDGNLAAKVGVGIQCDLGRVGIIRTELAYRAD
 Lgum2 101 WNPYILMGLGYQRSEEEYLLPSVLSVSPGNRKDGNLAAKVGVGIQCDLGRVGIIRTELAYRAD

Ecol 168 IGDAAHTIGTRPDNGMLSLGVSYRFGQGEAAPVVAPAPAPAPEVQTKHFTLKSD-----
 Sent 172 IGDANTIGTRPDNGILSLVGVSYRFGQGEAAPVVAPAPAPAPEVQTKHFTLKSD-----
 Eaer 172 IGDAGTVGVRPDNGMLSVGVSYRFGQEDNAPVVAPAPAPAPEVITKHTFTLKSD-----
 Smar 180 IGDAGTVGARPDNTMLSLGVSYRFGQDD-VVAPAPAPAPAPEVETKHTFTLKSD-----
 Lgum1 165 FDDTSVAAPQEDWFGDVLASVGIVIPLGPEPVAAVAPAPVVEQGCADKDDDDGQGVNCCDD
 Lgum2 161 FDDTSVAAPQEDWFGDVLASVGIVIPLGPEPVAAVAPAPVVEQGCADKDDDDGQGVNCCDD

Ecol 221 -----VLFTFNKATLKPEGQAALDQLYSQLSNIDPKDGS
 Sent 225 -----VLFNFNKSTLKPPEGQAALDQLYSQLSNIDPKDGS
 Eaer 225 -----VLFNFNKATLKPEGQAALDQLYTSQLSNIDPKDGS
 Smar 232 -----VLFNFNKSTLKAPEGQAALDQLYTSQLSNIDPKDGS
 Lgum1 225 KCPGSQAGQTIGPDGCAVPVTIDLKGVNFDKATLRPDVAVSILNEAAEILKRYP--DLK
 Lgum2 221 KCPGSQAGQTIGPDGCPVPSIDLKGVNFDKATLRPDSVAAILNEAAEILKRNPP--SLR

Ecol 255 VVVLGYTDTRIGSDAYNQALSERRAQSVVDYLIISKGIPADKIS-ARGMGESNPVTGNTCDN
 Sent 259 VVVLGFTDRIGSDAYNQGLSEKRAQSVVDYLIISKGIPSDKIS-ARGMGESNPVTGNTCDN
 Eaer 259 AVVLGYTDTRIGSEQYNQKLSEKRAQSVVDYLVAKGIPANKIS-ARGMGESDPVTGNTCDN
 Smar 266 VVVLGYTDAVGSQYNQKLSEQRAQSVVDYLVMSKGIPSDKIS-ARGMGESADAVTGNTCGY
 Lgum1 283 VEVAGHTDLCKGDIYNQKLSERRAKAAYDYLIISNGVDAGRLTGPVGYGESRPLESTPQTF
 Lgum2 279 VEVAGHTDLCKGDAYNQKLSERRAKAVDYLIISNGVDAGRLTGPVGYGESRPLESTPQTF

Ecol 314 --VKQRAALIDCLAPDRRVEIEVKGIKDVVTQPQA
 Sent 318 --VKPRAALIDCLAPDRRVEIEVKGVKDVVTQPQA
 Eaer 318 --VKARAALIDCLAPDRRVAIEVKGKDVVTQPQA
 Smar 325 KSGRATKAQIAACLAPDRRVEIEVKGIKDVVTQPQA
 Lgum1 343 PACKS-----EKNRRTEINVAQN-----
 Lgum2 339 PACKS-----EKNRRTEINAQN-----

Figure S6. (previous page) Sequence alignment of OmpA-like proteins.

Predicted signal peptides are shown in red.

Ecol, *Escherichia coli* (GenBank accession no. ABJ00366);

Sent, *Salmonella enterica* serovar Typhimurium (GenBank accession no. ABJ00366);

Eaer, *Enterobacter aerogenes* (GenBank accession no. P09146);

Smar, *Serratia marcescens* (GenBank accession no. AGE17540);

Lgum1, *L. gummosus* isoform 1 (GenBank accession no. KF738076);

Lgum2, *L. gummosus* isoform 2 (GenBank accession no. KF738077).

Violet bars indicate the transmembrane β -strands of the *E. coli* OmpA determined by X-ray diffraction (Pautsch and Schulz, 1998). Blue bars show the putative transmembrane β -strands of the *L. gummosus* proteins predicted with TMBETA-NET (<http://psfs.cbrc.jp/tmbeta-net/>).

α -Lytic protease

MSVSKSRRRSFARASVFCVAVAVAALTSGAALAAAGEVSPALKFAMQRDLGIFPGQIAQYLQTEKLAREQAASIE
REFGGNFAGSWIERKDDGSFRVVAATAGARKSSTLGGVEVRNVRHSLKRLQQSMDLLDAGAHARVKGVSKPLAGV
QTWYVDVRSNTVVVKVDEGATDAGIDFVALSGADSGAVRIESAPGQLQTTANIIIGGIEYSINNTNLCSIGFSVTR
GATKGFVTAGHCGTVNSIARIIGGAQVGTFAGRVFPGNDRWVSLTSAQTLPRVVNGSSYVTVRGSAAEAVGAAV
CRSGRTTGYRCGTITAKNVTANYAEGTVRGLTNANACMGRGDSGGSWITSAGQAQGVMSGGNVQSNGNCCGLPAS
QRTSLFERLGPILSQYGLALVTG

β -Lytic metalloendopeptidase

MGLALLTCCVLAAISGGAGAAERGLSGQDLVYSYDEMDFDFDIDAYLAKNAPHLRHAESISHWAGYSGISPKVLI
ALMEQQSGAITRRHAAADAARKPFGALAQAKDFNGQTRVQAQALREALYENDGPDAGAVTVARANPLQALFERA
GASQASAKLSGDGEFQLVYGRFLFNEPRQAQAPSARFAKAGPDVQPLSPNGLLQFPFPRGARWHVGAHTNTGSGN
YPMSSLDMSLGGGWSNQSNVTWVSASANGSFKRHSSCFAEIVHSGGWSTTYHLMNIRYNTGANVGSNTAIANPA
NTRAQALCNGGSSTGPEHWSLKLNGSFYHLNGAYLSGYRITATGNSYDTNCSRFLYLAKNGQNYCSGWFTNPGH

Lysyl endopeptidase 1

MKRLYGLALLLSLSIGAAIAGPTTLPAAFDHPNVSPIDKIALRTMPAVDVEKLLKAQDRLRDKRGDIPRFAFPMTV
DMTPLNSGVWEDLDADHVVRQRVRSDKAMSLNFGFTQYYMPAGGRMLIYPATQTKGGDRELIREYTDNRNEMS
QLWTAIVPGQEAVIEVVVPRASVQQLKRLTKINHDYVGFGLARRMAQASGEKGTSGICNVDVVCPDGNRRDI
IRSVGVYSKNGSLACTGSLVNNTANNKKMYFLTAHHCGMTTAAAANSIVVYWNYQNSTCRPPNTAINGTDGDGLL
NQSQSGSVVRATSANSDFTLLELRTAANPAFNLFWAGWDRRNQNFPSAIGIHPNVAEKRISLSVSPTSFFVAWGG
GAGTTHLNVQWQPTGGVTEPGSSGSPLYSPEKRVIGQLHGGPSSCSATGNSRSDQYGRVFTSWTGGGTAATRLSN
WLDPGATGATFINGIN

Lysyl endopeptidase 2

MKRICGSLLLLGLSISAALAAPASRPAAFDHNNISSVDKIAVRAMPVAVAKLKAEDLLRDKRGDIPRFAFPKIV
DMTPLNSGVWEDLDADNVIWRQRVRSEKALSLNFGFTQYHMPQGGRLLVYPATQAAAGDRNLISEYARDNNAQG
QLWTAIVPGQEAVIEVVVPRAKLGELKLLKLGQVGHYVGFGLARRLAAASGEKGVSGQCNVDVCPDGDGRRDI
IRSVAAYSKNGSLACTGSLVNNTANDKKMYFLTANHCGMTTASTAASIVVYWNYQNSTCRAPNTPASGANGDGM
SQNQSGSTVKASYADSDFTLLELNNTAANPAFNLYWAGWDRRDQNFSSAIAIHPNVAEKRISLSTSPTSFFVAWGG
GAGTTHLNVQWQPTGGVTEPGSSGSPLYSPEKRVIGQLHGGPSSCSATGTNRSDQYGRVFTSWTGGGTASTRLSN
WLDAGNTGAQFIDGLDSSGGTPTNTPPVANFTSTTSGLTATFTDTSTDSGDTIASRSWNFGDGTSTATNPTKTY
AAGTYTTLTVDNNGATHTKTGSVTVSGTPGSQTYTNETDVAITDHATVESPI TVSGRSGNGLPSTSIQVTIYH
TYKSDLKVDLVAPDGTVYNLHNRTGGSADNVI GTYTKDLSSEPLNGVWKL RVNDNATIDTGRIDKWSITF

Hemagglutinin/proteinase

VNRNVSPTALS FALS LVLASGAAGAAQRVDLHGQDI AKLNQQYKAAVAKSGAAAKANVRHAELISLDADSSLTQI
KSSQDANGTRNYRYQQSFRGVPVFGHEVVVSEDAQGNVRTLFRGSRVAGLAAELPATAPKLSAQALNLAKSAAALG
KRLGALQVRNNESSKQMIYIDDNGRAHMAVYVNYFADAPKGGEPTRPFVILDANS GKVLKQWEGLNHALVGTGPGG
NTKTGQYEGTDFGFNDVAQSGSTCTMNNTNVKTVNLNHGTSGTTAFSYTCPRNTVKAINGAFSPLNDAHVFYFKV
VFDMYSYVGVAPLTFQLSMRVHYSNSYENAFWDGSAMTFGDGATRFYPLVSLDVSASHEVSHGFTEQQSGLVYSG
QSGGINEAYS DIAGEAAEFYMN GTNDFLVGAQIFKASGALRYMANPPQDGRSIGHASNYTSGMDVHYSSGVYNKA
FYTLATKAGWDTVKAFKVFVAKANKDYWTPSTDFNNGACGVETSATDLGFAKADVTAASFVVGVTCP TGPFGPGPT
KYTNDTDFAINDNSTVDSPI TVSGRTGNAPTNAQVSVNI IHTYRGDLKVDLVAPDGSGLYINISNRAGGSADNLTGT
FTFNLSSEPLNGTWKLRVNDNANADVGRIDTWSITF

OmpA-like protein 1

MNKKLLCAALLGGLSIAQVASAQDFDDRWYITGAAGMNIQDNDRGTRNAPFGAIGLGKFLNKNWSLDGELNYQNP
KFDDNQDANWSQYGISLDLRRHFVTDGRNWNPIILMGLGYQRSEEEYLLPSVLSPGNRKDGNLAAKVGVIQGD
LGRVIRTELAYRADFDSDTSVAAPQEDWFGDVLASVGI VIPLGPEPVAAPVVEQGCADKDDDDGDGVNCCDDK
CPGSAQQTIGPDGCAVPVTIDLKGVNFDKATLRPDAVSI LNEAAEILKRYPDLKVEVAGHTDLCKGKDTYNQK
LSERRAKAAYDYLTSNGVDAGRLTGPVGYGESRPLESTPQTFFPACKSEKNRRTELNVQN

OmpA-like protein 2

MLSTALLAGLAFSQAASAQDFDDRWYVTGAVGMNIQDNDRGTRNAPFGAIGLGKFLNKNWSLDGELNYQNPKFDD
NQDANWSQYGISLDLRRHFVTDGRNWNPIILMGLGYQRSEEEYLLPSVLSPGNRKDGNLAAKVGVIQGD
LGRVIRTELAYRADFDSDTSVAAPQEDWFGDVLASVGI VIPLGPEPVAAPVVEQGCADKDDDDGDGVNCCDDK
CPGSAQQTIGPDGCPVPSIDLKGVNFDKATLRPDSVAI LNEAAEILKRNPSLRVEVAGHTDLCKGDAYNQKLSER
RAKAVYDFLTSNGVDAGRLAGPIGYGESRPLESTPQTFFPACKSEKNRRTELNAQN

Figure S7. (previous page) Domain architecture of *L. gummosus* proteins that co-purified with biofilm-degrading activity. Predicted signal peptides, prodomains and C-terminal extension domains are marked in red, orange and green, respectively. Sequences confirmed by peptide mass fingerprinting are underlined.

Table S1. *L. gummosus* proteins identified by peptide mass fingerprinting^a

Predicted protein	Accession no.	Band 1	Band 2	Band 3	Fract. 8	Fract. 17	Best blast hit (tblastn)	Best blast hit (blastp)
Alpha-lytic protease	KF738067	nd ^b	832	596	2292	598	DQ888610	ABI54136
Beta-lytic metallo-endopeptidase	KF738072	nd	245	51	1147	291	AB079667	BAB86844
Lysyl endopeptidase 1	KF738073	nd	nd	nd	389	60	J05128	P15636
Lysyl endopeptidase 2	KF738074	32	nd	nd	229	253	AB045676	BAB32450
Hemagglutinin/proteinase	KF738078	545	51	nd	43	479	EU333168	ACA34431
OmpA-like protein 1	KF738076	93	88	586	87	20	HQ399464	WP_008399696
OmpA-like protein 2	KF738077	93	88	419	88	20	HQ399464	WP_008399696
Metallo-peptidase, family M72	KF738080	nd	nd	nd	189	nd	CP002986	WP_017907847
Glycoside hydrolase, family 16	KF738079	nd	nd	nd	128	nd	CP002047	YP_004968129
Serine peptidase, family S1	KF738081	nd	nd	nd	93	nd	CP000155	WP_018693635
Serine peptidase, family S8	KF738082	nd	nd	nd	89	nd	AB299027	BAF49606
Serine peptidase, family S8	KF738083	nd	nd	nd	66	nd	AC182716	YP_003376995

^a Mascot scores are indicated for the proteins identified in the bands 1, 2 and 3 digested in-gel, as well as ProPac SAX-10 fractions 8 and 17 digested in solution, according to Fig 2. All proteins identified with Mascot scores >40 in at least one of the samples are listed.

^b nd, not detected