Lysinibacillus fusiformis protein BFZC1_10652

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          axYS22-A1    1    GENLSYNFVEGDYV   14
                            G N+SYNF+ GDYV
protein BFZC1_10652    32   GINMSYNFI-GDYV   44
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Cytophaga hutchinsonii protein BFZC1_10652

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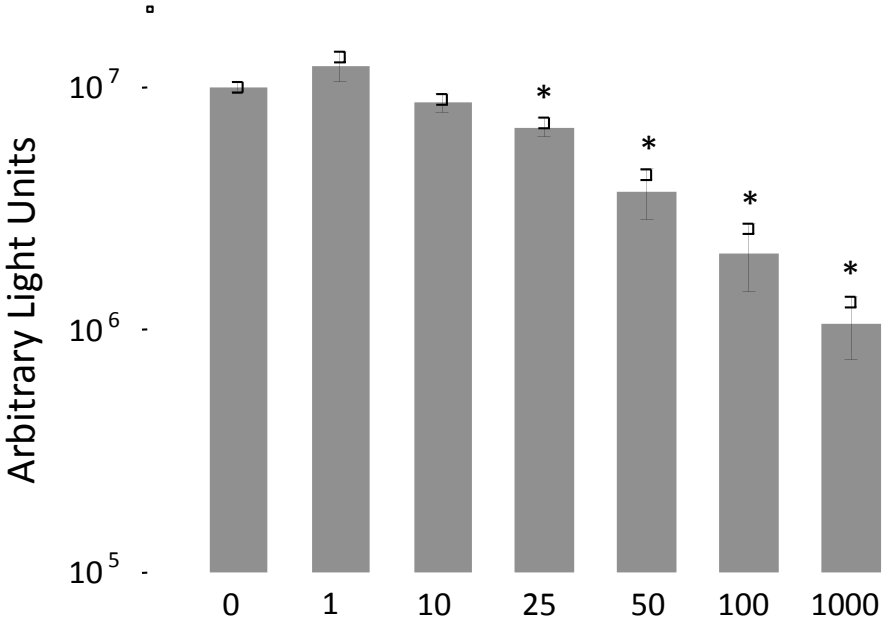
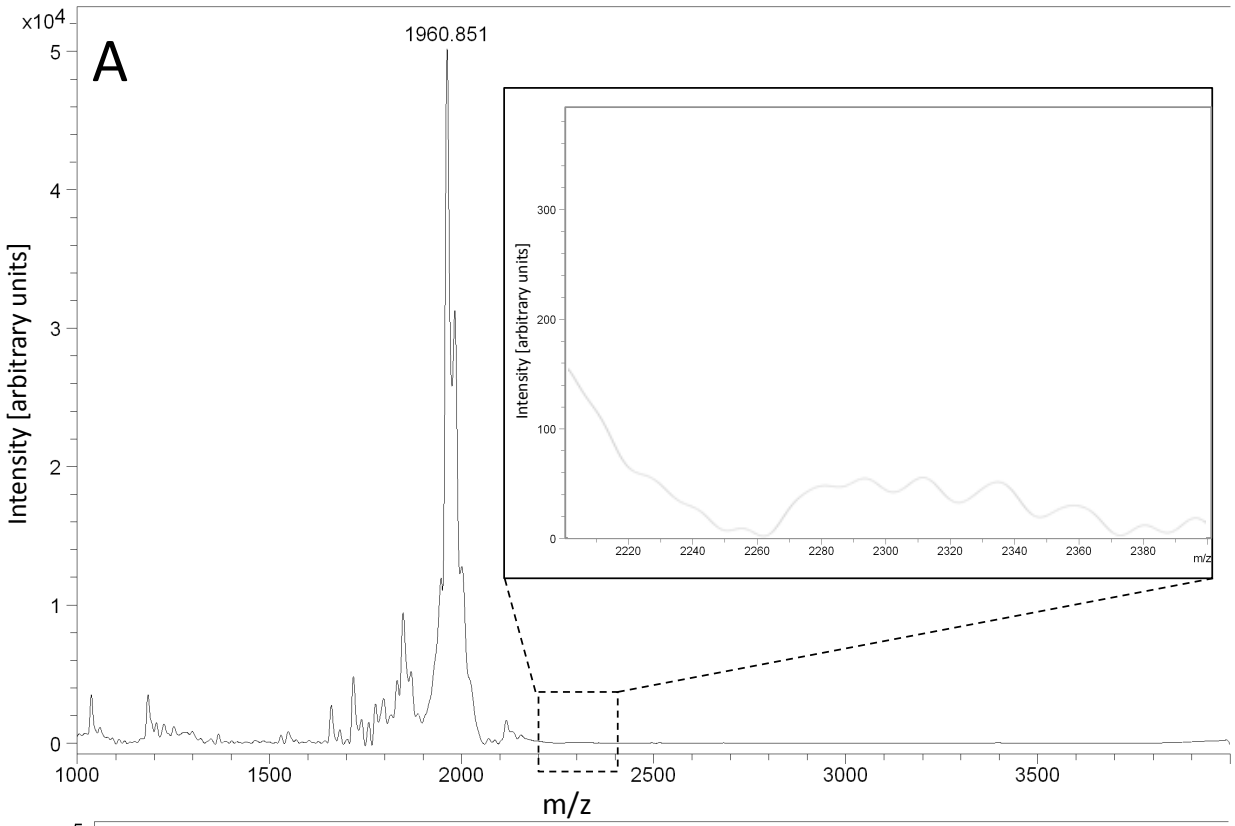
          axYS22-A1    1     GENLSYNFVEGDYVR   15
                             GE LSYN  EG+YVR
 protein YP_678322     100   GE-LSYN--EGEYVR   111
```

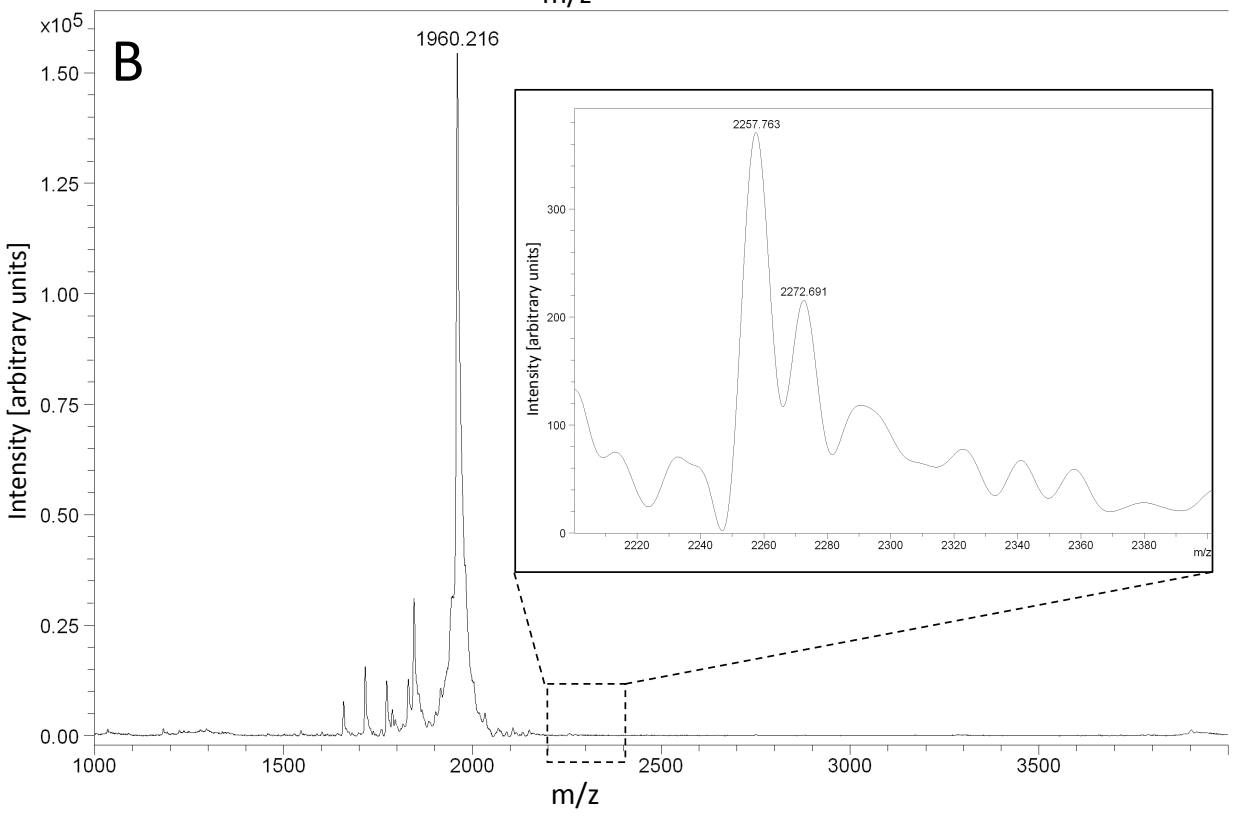
Supplemental Figure 1. Alignment of axY^{S}22-A1 and two naturally occurring proteins from *Lysinibacillus fusiformis* and *Cytophaga hutchinsonii.* The expectation-value scores for the two matches against eubacterial sequences are $5x10^{-4}$ and $7x10^{-4}$, respectively.



Supplemental Figure 2. flg22 dose response curve in the seedling protection assay. Ten-day old Arabidopsis wild-type Col-0 seedlings were grown in 96-well plates and either mock-treated or elicited with various concentrations of flg22 for 24 h and then inoculated with 10 µL of *P. syringae* pv. *maculicola* strain ES4326 (OD_{600} = 0.0002) carrying the LUX operon from *P. luminescens* as described in Danna et al., 2011. Inoculated seedlings were grown for an additional 29 h and then transferred (without surface sterilization) to a 96-well scintillation counter to determine the number of photons detected per min per well (CPM/well) as described in Danna et al. 2011. Control experiments showed that surface sterilization of seedlings prior to determining the luminescence does not significantly affect the results of the assay. Each data point represents the mean of 12 seedlings (12 wells). Error bars represent ± standard error of the mean. A t test showed that the p values corresponding to bacterial growth at various flg22 concentrations compared to mock-treated seedlings were: 1000 nM = 2.7×10^{-10}; 100 nM = 4.9×10^{-7}; 50 nM = 3.5×10^{-5}; 25 nM = 0.0002; 10 nM = 0.007; 1 nM = 0.10. Asterisks indicate statistically significant differences compared to mock ($p \leq 0.001$).

Danna, C.H., Millet, Y.A., Koller, T., Han, S.W., Bent, A.F., Ronald, P.C., and Ausubel, F.M. (2011). The Arabidopsis flagellin receptor FLS2 mediates the perception of the *Xanthomonas* Ax21 secreted peptides. Proc. Natl. Acad. Sci. USA **108:**9286-9291.


A
x10^4
1960.851
Intensity [arbitrary units]
m/z
0
1
2
3
4
5
1000
1500
2000
2500
3000
3500
Intensity [arbitrary units]
m/z
0
100
200
300
2220
2240
2260
2280
2300
2320
2340
2360
2380


B
x10^5
1960.216
2257.763
2272.691
Intensity [arbitrary units]
m/z
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0.25
0.50
0.75
1.00
1.25
1.50
1000
1500
2000
2500
3000
3500
Intensity [arbitrary units]
m/z
0
100
200
300
2220
2240
2260
2280
2300
2320
2340
2360
2380

Supplemental Figure 3. Mass spectrometry analysis of mixtures of flg22 and axY^{S}22-A1 peptides. Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Karas and Hillenkamp, 1988) analyses were performed on a Bruker Daltonics, Microflex instrument that was operated at an accelerated voltage of 20kV for detection of positive ions in linear mode. The matrix solution was α-cyano-4-hydroxycinnamic acid (α-CHCA) (Sigma-Aldrich, 10 mg/ml) in 50% acetonirile/0.1% trifluoroacetic acid. Solutions of varying concentrations of pure axY^{S}22-A1 (referred to as A1), flg22, or mixtures of the two peptides were prepared in Milli-Q water. 1 µL of matrix solution and 1 µL of sample solution were mixed directly on a polished steel target (Bruker Daltonics, Inc.) and air-dried at room temperature. The lowest concentration of flg22 that could be detected when spotted alone was 0.01 µM (data not shown). However, in the mixtures of A1 and flg22, the limit of detection of flg22 was 1.0 µM, presumably a consequence of ion suppression (Burkitt et al., 2003). Thus, flg22 could be detected in a mixture of 1 mM A1 + 1 µM flg22 but not in a mixture of 100 µM A1 + 0.1 µM flg22. Only the nonsulfated peak corresponding to A1 was detected in these experiments (peaks at 1960.851 and 1960.216 in panels A and B, respectively), likely because the sulfate group is lost from A1 during the ionization process (P. Ronald and S.W. Han, unpublished data). Due to the utilization of the linear mode of detection, masses observed were within 2-3 mass units of the calculated molecular weights (1959.91 for the non-sulfated derivative of A1 and 2272.7 for flg22). (A) Mass spectrum of 1 nanomole of A1 (1 µL spotted from a 1 mM solution). Insert shows a magnified portion of the spectrum in the region of the mass of flg22 (mass ranges 2200-2400) showing that flg22 could not be detected. (B) Mass spectrum of a mixture 1 nanomole of A1 + 1 picomole flg22 (1 µL spotted from a solution of 1 mM A1 + 1 µM flg22). Insert shows a magnified portion of the spectrum in the region of the mass of flg22 (mass ranges 2200-2400). The peak at 2272.691 corresponds to the expected mass of flg22 (2272.7). The peak at 2257.763 corresponds to pyroGLU-flg22 (expected mass 2255.2). The N-terminal glutamine residue of flg22 is cyclized to pyroglutamate during storage.

Burkitt, W.I., Giannakopulos, A.E., Sideridou, F., Bashir, S. and Derrick, P.J. (2003). Discrimination effects in MALDI-MS of mixtures of peptides—Analysis of the Proteome. Australian J. Chem. **56:**369-377.

Karas M. and Hillenkamp F. (1988). Laser desorption ionization of proteins with molecular masses exceeding 10,000 Daltons. Anal. Chem. **60:**2299-2301.