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Supplementary Materials

Optimization of the Production and Characterization of An Antifungal Protein by *Bacillus velezensis* Strain NT35 and Its Antifungal Activity Against *Ilyonectria robusta* causing Ginseng Rusty Root Rot

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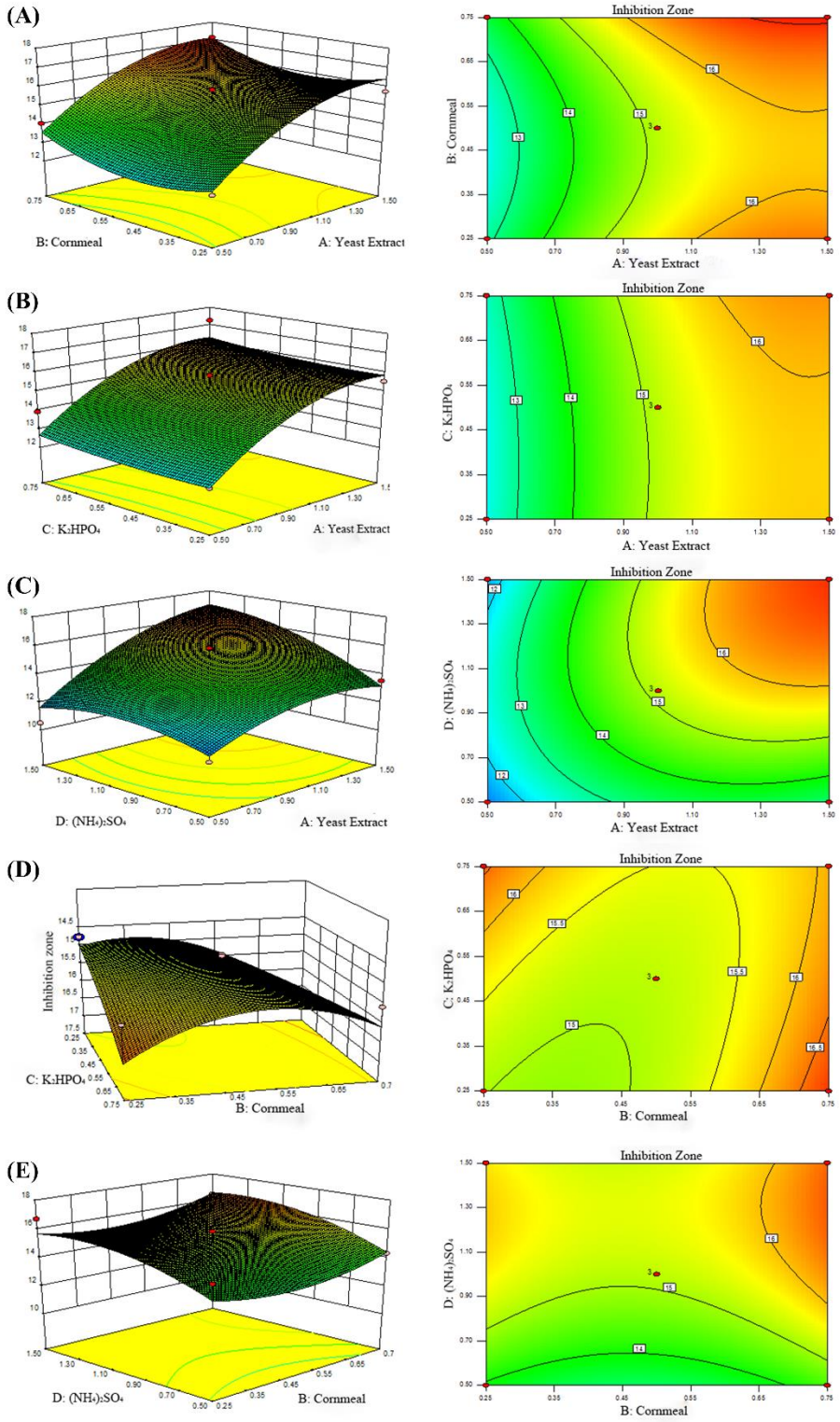

Figure S1. Response surface and contour of each test factor to inhibition zone. (**A**) The combined effects of yeast extract and cornmeal; (**B**) The combined effects of yeast extract and K_2HPO_4; (**C**) The combined effects of yeast extract and $(NH_4)_2SO_4$; (**D**) The combined effects of cornmeal and K_2HPO_4; (**E**) The combined effects of cornmeal and $(NH_4)_2SO_4$.

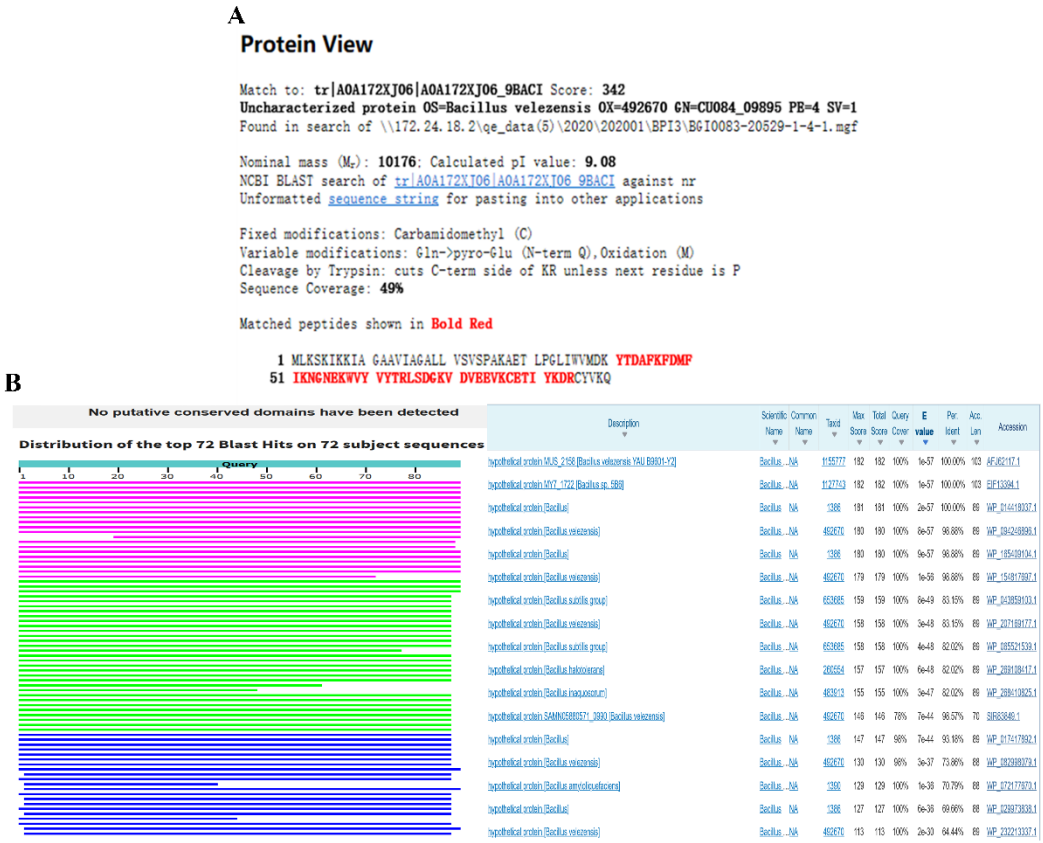

Figure S2. liquid chromatography tandem mass spectrometry (LC-MS/MS) results. (**A**) Identification results of antifungal protein 1-4-2F; (**B**) Amino acid sequence homology analysis of antifungal proteins.

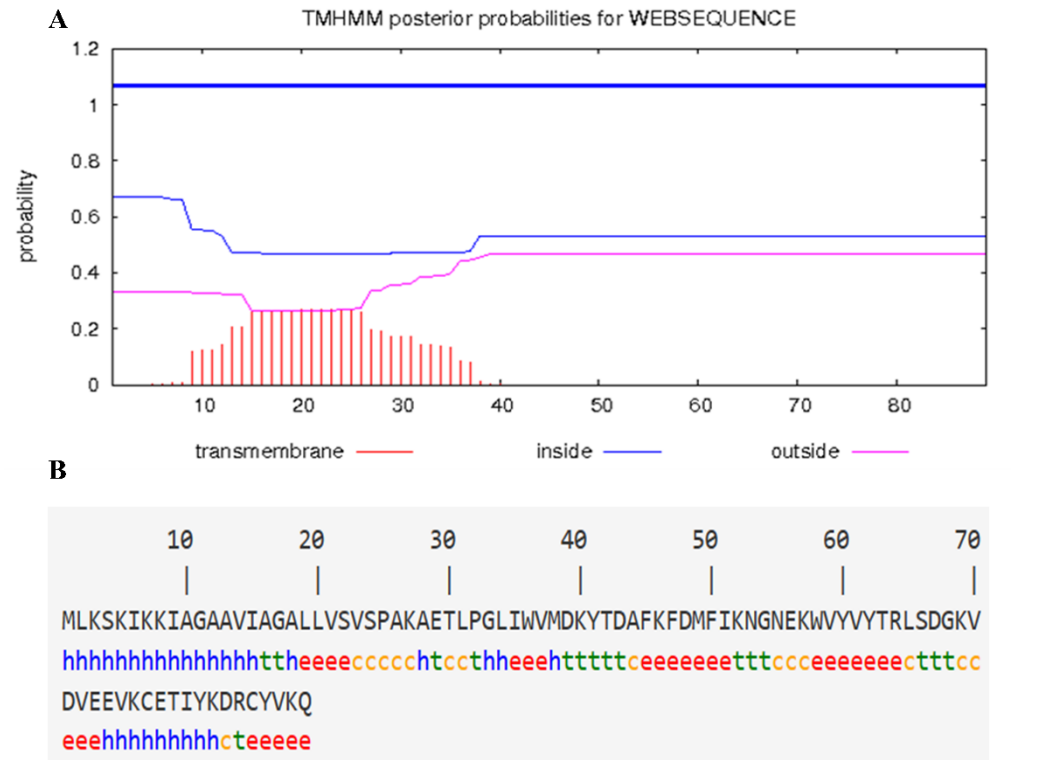

Figure S3. Bioinformatic analysis of antifungal protein 1-4-2F. (**A**) Transmembrane structure prediction; (**B**) Secondary structure prediction, where h is the α-helix, e is the extended polypeptide chain, t is expressed as β-turns, and c represents random coils.

Table S1. Plackett–Burman experimental design number and measured value. A, cornmeal, 10–20 g·L^{-1}; B, maltose, 10–20 g·L^{-1}; C, glucose, 10–20 g·L^{-1}; D, $(NH_4)_2SO_4$, 15–35 g·L^{-1}; E, beef extract, 15–35 g·L^{-1}; F, yeast extract powder, 15–35 g·L^{-1}; G, K_2HPO_4, 10–20 g·L^{-1}; H, KCl, 10–20 g·L^{-1}; and I, Na_2CO_3, 10–20 g·L^{-1}.

Test	A	B	C	D	E	F	G	H	I	Inhibition zone (mm)
1	35.00	35.00	35.00	10.00	10.00	10.00	20.00	10.00	20.00	13.59±0.11
2	35.00	15.00	35.00	20.00	20.00	10.00	10.00	10.00	20.00	13.33±0.07
3	35.00	35.00	15.00	10.00	10.00	20.00	10.00	20.00	20.00	13.78±0.04
4	15.00	15.00	15.00	20.00	10.00	20.00	20.00	10.00	20.00	14.23±0.07
5	15.00	35.00	15.00	20.00	20.00	10.00	20.00	20.00	20.00	12.62±0.18
6	35.00	15.00	35.00	20.00	10.00	20.00	20.00	20.00	10.00	15.56±0.04
7	15.00	15.00	35.00	20.00	20.00	20.00	10.00	20.00	20.00	12.27±0.03
8	35.00	35.00	15.00	20.00	20.00	20.00	10.00	10.00	10.00	14.56±0.04
9	15.00	35.00	35.00	20.00	10.00	10.00	10.00	20.00	10.00	8.65±0.05
10	35.00	15.00	15.00	10.00	20.00	10.00	20.00	20.00	10.00	13.56±0.04
11	15.00	35.00	35.00	10.00	20.00	20.00	20.00	10.00	10.00	12.23±0.07
12	15.00	15.00	15.00	10.00	10.00	10.00	10.00	10.00	10.00	7.82±0.08

Table S2. Measured value of steepest climbing test.

Test	Yeast extract (g·L^{-1})	Cornmeal (g·L^{-1})	K_2HPO_4 (g·L^{-1})	$(NH_4)_2SO_4$ (g·L^{-1})	Inhibition zone (mm)
1	15.00	10.00	10.00	15.00	10.51±0.09
2	20.00	12.50	12.50	20.00	13.39±0.01
3	25.00	15.00	15.00	25.00	15.13±0.07
4	30.00	17.50	17.50	30.00	14.40±0.1
5	36.00	20.00	20.00	36.00	11.28±0.12

Table S3. Experiment design and results of response surface analysis. The inhibition zone values (mm) are expressed as mean ± standard deviation (SD).

Number	Yeast extract	Cornmeal	K_2HPO_4	$(NH_4)_2SO_4$	Inhibition zone (mm)
1	0	-1	0	-1	14.96±0.01
2	1	1	0	0	17.12±0.12
3	0	0	0	0	15.85±0.15
4	0	0	0	0	14.82±0.18
5	0	1	0	-1	14.34±0.16
6	0	0	1	1	16.43±0.22
7	-1	0	0	1	10.51±0.09
8	1	0	0	-1	13.56±0.04
9	0	1	1	0	15.59±0.01
10	-1	0	0	-1	11.02±0.02
11	0	1	0	1	16.46±0.04
12	1	-1	0	0	15.75±0.05
13	0	-1	0	1	16.71±0.09
14	0	-1	1	0	15.64±0.06
15	-1	0	1	0	13.93±0.07
16	1	0	0	1	16.59±0.24
17	0	0	-1	-1	13.92±0.08
18	0	0	0	0	14.78±0.22
19	0	1	-1	0	17.12±0.15
20	1	0	-1	0	15.54±0.10

21	0	0	1	-1	12.08±0.08
22	0	-1	-1	0	14.78±0.05
23	-1	1	0	0	14.05±0.05
24	-1	0	-1	0	12.34±0.05
25	0	0	-1	1	15.03±0.06
26	1	0	1	-1	17.25±0.25
27	0	1	1	0	14.26±0.06

Table S4. Response surface regression analysis (** $p < 0.01$).

Source of variance	Sum of squares	Degrees of freedom	Mean square	F value	P>F	Significant
Mold	77.51	1	5.54	6.60	0.0011	-
A- yeast extract	37.84	1	37.84	45.11	<0.0001	**
B- cornmeal	1.46	1	1.46	1.74	0.2113	-
C- K_2HPO_4	0.40	1	0.40	0.48	0.5034	-
D-$(NH_4)_2SO_4$	11.70	1	11.70	13.95	0.0028	**
AB	2.250×10^{-4}	1	2.250×10^{-4}	2.682×10^{-4}	0.9872	-
AC	3.600×10^{-3}	1	3.600×10^{-3}	4.292×10^{-3}	0.9488	-
AD	3.13	1	3.13	3.73	0.0772	-
BC	1.43	1	1.43	1.70	0.2164	-
BD	0.034	1	0.034	0.041	0.8433	-
CD	2.62	1	2.62	3.13	0.1023	-
A^2	5.44	1	5.44	6.49	0.0256	**
B^2	3.83	1	3.83	4.57	0.0539	**
C^2	0.13	1	0.13	0.16	0.6982	-
D^2	3.83	1	3.83	4.57	0.0539	**
Residual	10.07	12	0.84			-
Lack of Fit	9.33	10	0.93	2.54	0.3158	-
Pure Error	0.74	2	0.37			-
Cor Total	87.58	26				-

Table S5. Unknown protein amino acid sequence analysis.

amino acid	quantity	content (%)	amino acid	quantity	content (%)
Ala (A)	8	9.0	Lys (K)	13	14.6
Arg(R)	2	2.2	Met (M)	3	3.4
Asn (N)	2	2.2	Phe (F)	3	3.4
Asp (D)	6	6.7	Pro (P)	2	2.2
Cys (C)	2	2.2	Ser (S)	4	4.5
Gln (Q)	1	1.1	Thr (T)	4	4.5
Glu (E)	5	5.6	Trp (W)	2	2.2
Gly (G)	5	5.6	Tyr (Y)	5	5.6
His (H)	0	0	Val (V)	10	11.2
Ile (I)	6	6.7	Pyl (O)	0	0
Leu (L)	6	6.7	Sec (U)	0	0

Table S6. Unknown protein secondary structure prediction.

Name	Quantity	Content (%)
Alpha helix (h)	29	32.58
Extended strand (e)	29	32.58
Beta turn (t)	16	17.98
Random coil(c)	15	16.85