Journal Article

Whole genome alignment based development of molecular marker for detecting Leptosphaeria maculans and Leptosphaeria biglobosa, the causal agent of blackleg disease in Brassicas

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Running Title: Genome-alignment based molecular marker for blackleg fungus

Supplementary information

Table S1. Effector prediction of the top blast hits of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* specific LCBs using 'EffectorP' program.

Primers were designed from the selected LCBs. Details of those selected LCBs and primer specifications are shown in Table 3.

SL	Identifier	Prediction	Probability
***Leptosphaeria maculans* specific LCBs**			
1	Lm_LCB290_XP_003839331(LM2)	Effector	0.617
2	Lm-LCB11_XP_003842632	Non-effector	0.98
3	Lm-LCB12_XP_003844845	Non-effector	0.916
4	Lm-LCB13_XP_003839388	Non-effector	0.917
5	Lm-LCB13_XP_003839388	Non-effector	0.85
6	Lm-LCB149_OCK82192	Effector	0.712
7	Lm-LCB149_PSN75417	Non-effector	0.659
8	Lm-LCB149_XP_003845041	Non-effector	0.605
9	Lm-LCB15_OQV05678	Non-effector	0.661
10	Lm-LCB15_ORY18338	Non-effector	0.825
11	Lm-LCB15_RMD42378	Non-effector	0.742
12	Lm-LCB189_OQU97076	Unlikely effector	0.537

13	Lm-LCB189_XP_013274476	Non-effector	0.514
14	Lm-LCB247(2)_XP_003834629	Effector	0.641
15	Lm-LCB247(2)_XP_003834629	Effector	0.778
16	Lm-LCB247(2)_XP_003834629	Non-effector	0.731
17	Lm-LCB247_WP_049997181	Non-effector	0.554
18	Lm-LCB247_XP_003834627	Non-effector	0.9
19	Lm-LCB253_OHW98174	Non-effector	0.99
20	Lm-LCB253_THU82129	Non-effector	0.991
21	Lm-LCB264_EKG16596	Effector	0.825
22	Lm-LCB264_EKG16596	Non-effector	0.974
23	Lm-LCB264_KID95632	Effector	0.771
24	Lm-LCB264_KIW71946	Non-effector	0.723
25	Lm-LCB264_OAL44863	Non-effector	0.883
26	Lm-LCB264_RMZ83261	Effector	0.846
27	Lm-LCB274_XP_003837068	Non-effector	0.99
28	Lm-LCB274_XP_007716197	Non-effector	0.99
29	Lm-LCB290_RYF49765	Effector	0.713
30	Lm-LCB3_XP_003836666	Non-effector	0.761
31	Lm-LCB3_XP_003836666(1)	Unlikely effector	0.51
32	Lm-LCB46_KIH89307	Non-effector	0.838
33	Lm-LCB6_OAL47200	Effector	0.8
34	Lm-LCB6_RMZ85926	Non-effector	0.501
35	Lm-LCB6_XP_003841162	Non-effector	0.88
36	Lm-LCB6_XP_007686779	Effector	0.841
37	Lm-LCB9_EFQ86601	Effector	0.907
38	Lm-LCB9_OWY46227	Effector	0.598
39	Lm-LCB9_RAR00744	Non-effector	0.819
40	Lm-LCB9_XP_018388033	Non-effector	0.97
***Leptosphaeria biglobosa* specific LCBs**			
1	Lb_LCB247_KNG45253	Effector	0.877
2	Lb_LCB247_XP_001800097	Non-effector	0.733
3	Lb_LCB25_5T2N_A	Non-effector	0.992
4	Lb_LCB25_ACV41149	Non-effector	0.99
5	Lb_LCB25_QBM09653	Non-effector	0.902
6	Lb_LCB275_KUL86939	Non-effector	1
7	Lb_LCB275_OMP85366	Non-effector	1
8	Lb_LCB38_KZM19787	Non-effector	0.998
9	Lb_LCB38_OAL56669	Non-effector	0.993
10	Lb_LCB38_RMY82212	Effector	0.902
11	Lb_LCB38_XP_003838314	Non-effector	1
12	Lb_LCB43_CCT61205	Effector	0.878
13	Lb_LCB43_OCK73725	Effector	0.907
14	Lb_LCB43_OCK73998	Effector	0.775
15	Lb_LCB43_PWO12528	Effector	0.958
16	Lb_LCB43_RMD39683	Effector	0.664
17	Lb_LCB43_XP_008021303	Non-effector	0.764
18	Lb_LCB43_XP_008026396	Effector	0.547
19	Lb_LCB47_CZR57474	Effector	0.83
20	Lb_LCB47_EHK99800	Effector	0.949
21	Lb_LCB47_KFY49888	Non-effector	0.985
22	Lb_LCB47_OIW26414	Effector	0.982
23	Lb_LCB47_RDL36686	Effector	0.922
24	Lb_LCB47_RDL41425	Effector	0.899
25	Lb_LCB47_RYP32913	Non-effector	0.973
26	Lb_LCB47_RYP39691	Non-effector	0.695
27	Lb_LCB47_RYP56555	Non-effector	0.978
28	Lb_LCB47_SMR63353	Effector	0.646
29	Lb_LCB47_XP_022500824	Effector	0.801

30	Lb_LCB47_XP_028507648	Non-effector	0.973
31	Lb_LCB52_KIP12204	Non-effector	1
32	Lb_LCB52_KNG51546	Effector	0.948
33	Lb_LCB52_KZP32518	Non-effector	1
34	Lb_LCB52_OAK96406	Non-effector	0.94
35	Lb_LCB58_KKK26246	Non-effector	0.936
36	Lb_LCB58_THW40983	Non-effector	0.545
37	Lb_LCB58_XP_007700085	Non-effector	0.954
38	Lb_LCB81_AKL78827	Non-effector	1
39	Lb_LCB81_CDP31273	Non-effector	1
40	Lb_LCB81_CRK12529	Non-effector	1
41	Lb_LCB81_KFX98083	Non-effector	1
42	Lb_LCB81_XP_003009645	Non-effector	1
43	Lb_LCB81_XP_025509315	Non-effector	1

LCBs. local colonial blocks.

a) *Leptosphaeria maculans* specific LCBs

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b) *Leptosphaeria biglobosa* specific locally collinear blocks (LCB)

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Figure S1. Nucleotide sequences of *Leptosphaeria maculans* (a) and *Leptosphaeria biglobosa* (b) specific 'local colonial blocks (LCBs)'.

Sequences that encodes for effector proteins are indicated as bold blue text and the designed forward and reverse primers are indicated by yellow and green highlighted text, respectively. List of primers are shown in Table 4.

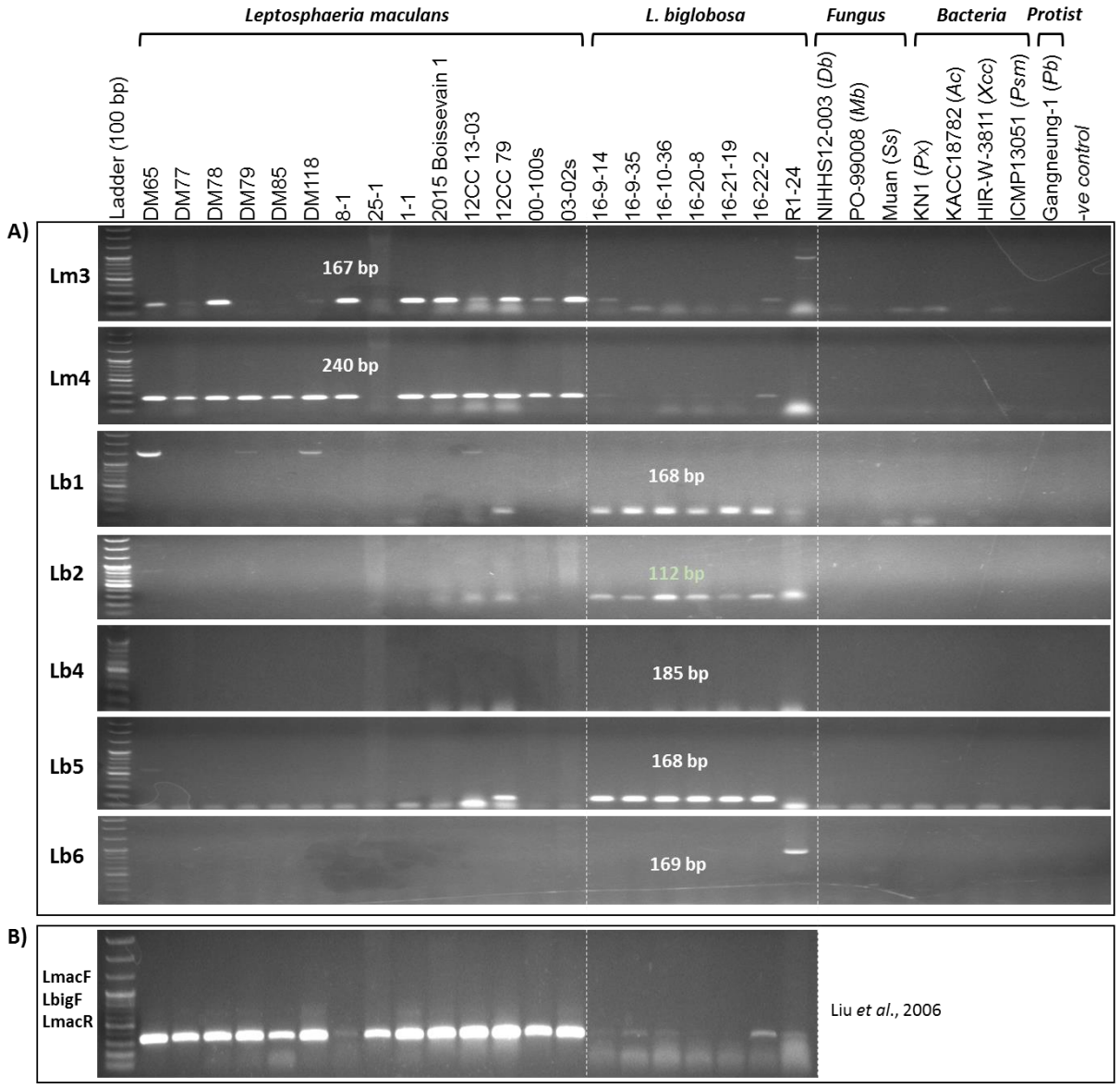

Figure S2. Amplification of pathogenic isolates by the designed *Leptosphaeria maculans* and *Leptosphaeria biglobosa* specific primers (A) and by previously published multiplex primers (B) in PCR assay.

None of these primers yielded complete specificity towards intended pathogenic isolates.
Genomic DNA (~100 ng/µL) of the fungal isolates was used as template. Primer sets Lm1,4 and Lb1,2,4,5,6 were intended to specifically amplify *Leptosphaeria maculans* and *Leptosphaeria biglobosa* isolates, respectively. *Db. Didymella bryoniae; Mb. Mycosphaerella brassicicola; Ss. Sclerotinia sclerotiorum; Px. Podosphaera xanthii; Ac. Acidovorax citrulli; Xcc. X. campestris pv. campestris; Psm. Pseudomonas syringae pv. maculicola; Pb. Plasmodiophora brassicae*.

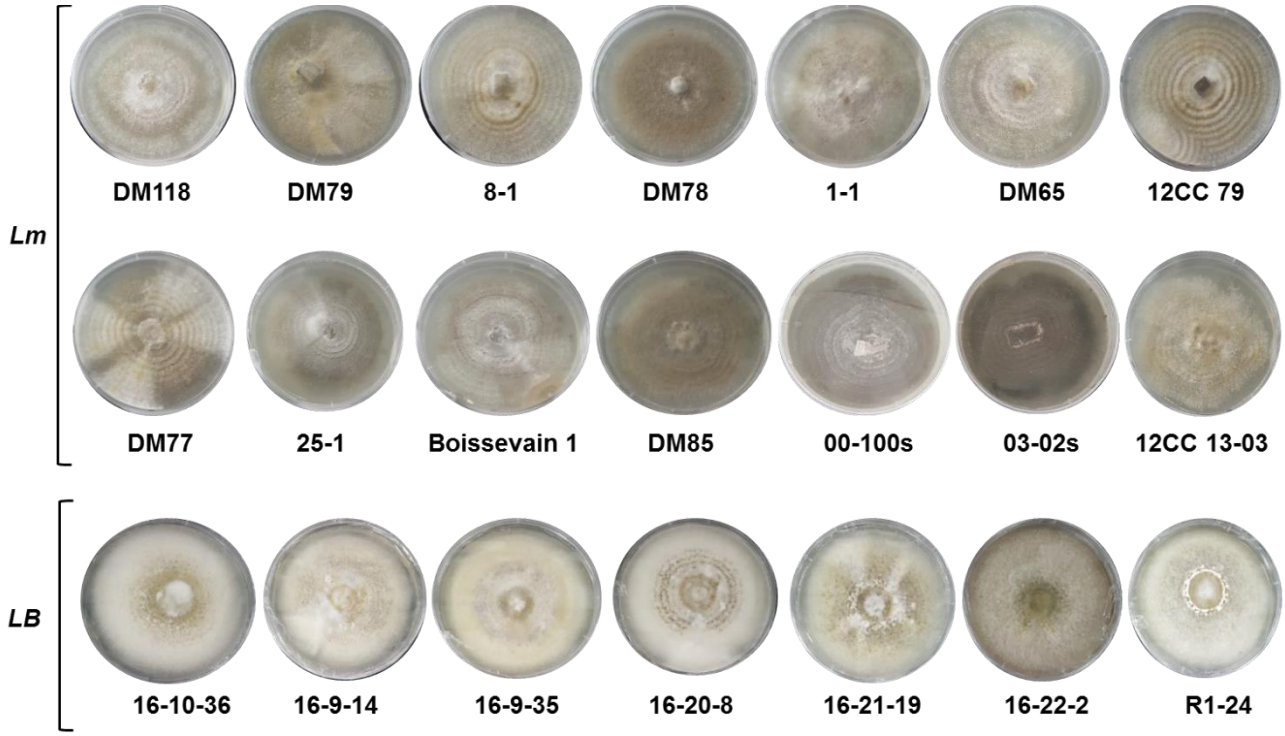

Figure S3: Culture plates of *Leptosphaeria maculans* (*Lm*) and *Leptosphaeria biglobosa* (*Lb*) isolates on V8 and PDA media, respectively.

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