

A Universally Primed-PCR (UP-PCR) marker to discriminate *Clonostachys rosea* ACM941 from related strains

Zerihun A. Demissie¹, William G. Brown² and Michele C. Loewen^{1,3,4*}

¹Aquatic and Crop Resource Development, National Research Council Canada, Ottawa, ON, Canada; Zerihun.demissie@nrc.ca

²Adjuvants Plus Inc. 1755 Division Road N., Kingsville, ON Canada; bill@adjuvantsplus.com

³Department of Biomedical and Molecular Sciences, Queens University, Kingston, ON, Canada; michele.loewen@nrc.ca

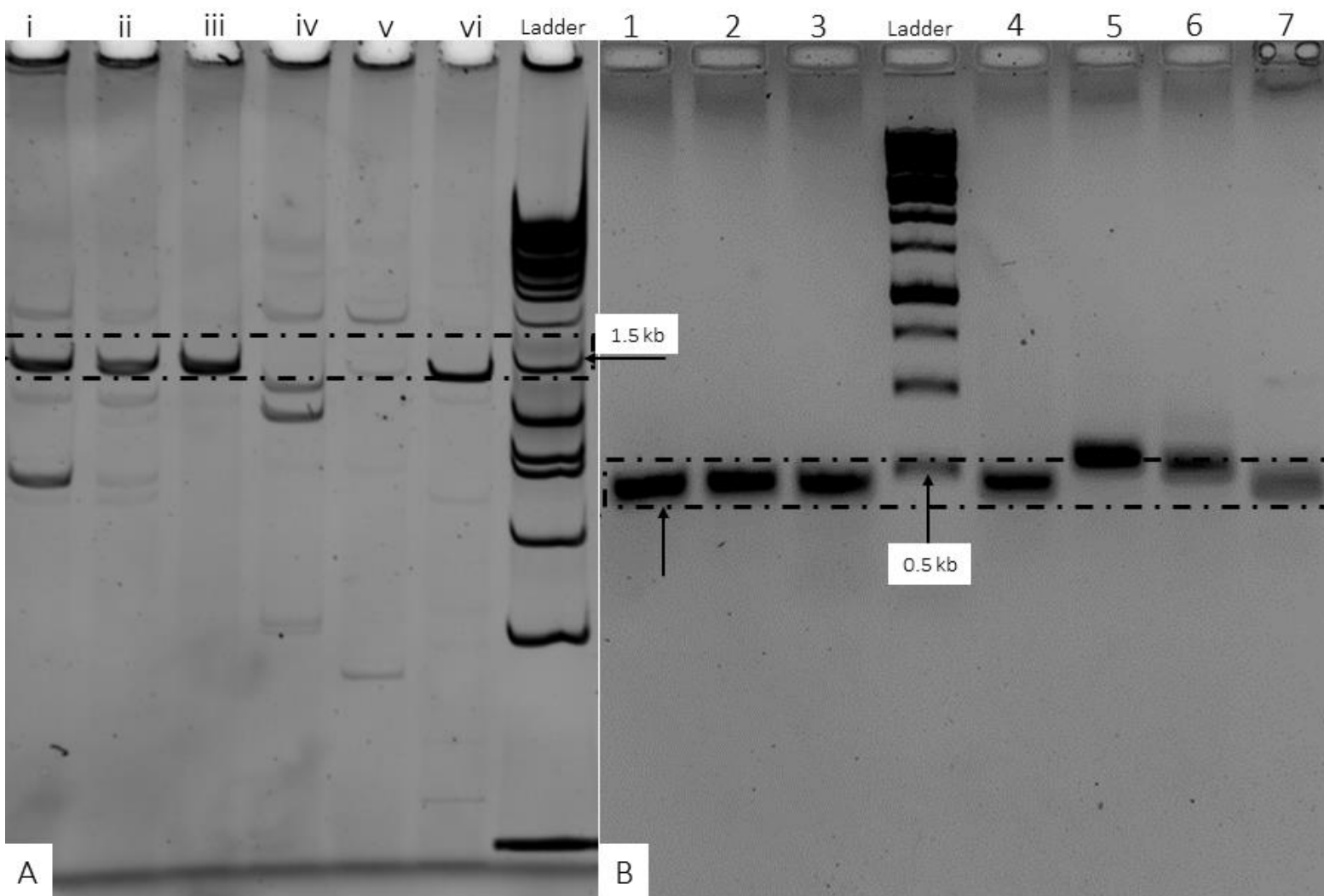
⁴Department of Chemistry and Biomolecular Sciences, University of Ottawa, 75 Laurier Ave E, Ottawa, ON K1N 6N5

*Corresponding author: michele.loewen@nrc.ca

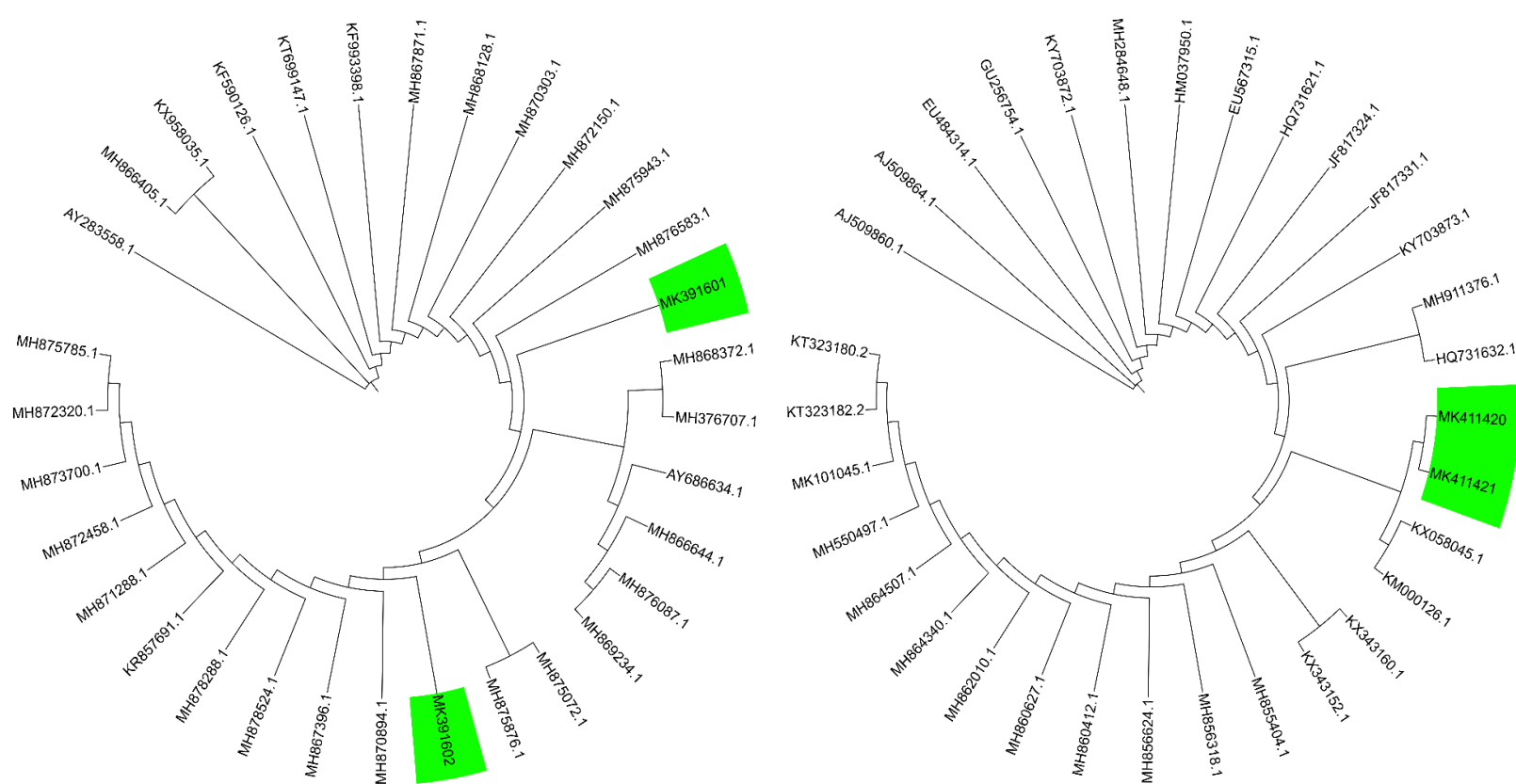
Supplementary Tables and Figures

		BLASTn analysis against				
		IK726 genome		CBS125111 genome		NCBI nucleotide database
ID	Size (bp)		Location		Location	
SCAR-1	466	+ve	scf_003: 1010111 - 1010558	+ve	Scaffold- 1:2996275- 2996690	-ve
SCAR-2	453	-ve	N/A	-ve	N/A	-ve
SCAR-3	404	+ve	scf_547: 1756- 2148	-ve	N/A	+ve
SCAR-4	375	+ve	scf_033: 132172- 132536	+ve	Scaffold- 14:1082620- 1082973	-ve

Supplementary Table 1. BLASTn analysis of the four SCAR fragments cloned and sequenced. SCAR-1 is the higher molecular weight fragment from strain ACM941 (boxed in Figure 1), SCAR-2 is the lower molecular weight fragment from strain ACM941 (boxed in Figure 1), SCAR-3 is the higher molecular weight fragment from strain 88-710 (boxed in Figure 1) and SCAR-4 is the lower molecular weight fragment from strain 88-710 (boxed in Figure 1).



Supplementary Figure 1. Amplification of rRNA regions from *C. rosea* strains and other biocontrol fungi. (a) PCR products of ITS1 + LR7 primers resolved on 6% SDS-PAGE and (b) PCR products of ITS1-X + ITS1-Y primers resolved on 1.5% agarose gel. Label descriptions: **i** - *C. rosea* strain 88-710; **ii** - *C. rosea* strain ACM941; **iii** - *C. rosea* strain DAOMC175083; **iv** - *T. citrinoviride* strain Tricho.12; **v** - *T. harzianum* strain Tricho.18; **vi** - *G. Catenulatum* strain J1446; **1** - *C. rosea* strain 88-710; **2** - *C. rosea* strain ACM941; **3** - *C. rosea* strain DAOMC175083; **4** - *C. rosea* strain DAOMC241828; **5** - *T. citrinoviride* strain Tricho.12; **6** - *T. harzianum* strain Tricho.18 and **7** - *G. Catenulatum* strain J1446.



Supplementary Figure 2. Phylogenetic relationships among different *C. rosea* strains and other selected species based on (a) partial D1/D2 region and (b) Partial ITS-1 and ITS-2 and complete 5.8S regions.

Accession number descriptions of A (arranged based on their similarity to *C. rosea* strain ACM941 D1/D2 region from high to low): MK391601 *C. rosea* strain_ACM941 partial D1/D2 region; MK391602 - *C. rosea* strain_88-710 partial D1/D2 region; MH878524.1 - *C. rosea* strain CBS 907.72F large subunit ribosomal RNA gene; MH876583.1 - *C. rosea* strain CBS 128894 large subunit ribosomal RNA gene; MH875943.1 - *C. rosea* strain CBS 127294 large subunit ribosomal RNA gene; MH875785.1 - *C. rosea* strain CBS 126933 large subunit ribosomal RNA gene; MH873700.1 - *C. rosea* strain CBS 710.86 large subunit ribosomal RNA gene; MH872458.1 - *C. solani* f. *nigrovirens* strain CBS 469.73 large subunit ribosomal RNA gene; MH872320.1 - *C. rosea* strain CBS 907.72B large subunit ribosomal RNA gene; MH872150.1 - *C. rosea* f. *catenulata* strain CBS 125.72 large subunit ribosomal RNA gene; MH871288.1 - *G. aureum* strain CBS 998.69 large subunit ribosomal RNA gene; MH870303.1 - *C. rosea* f. *catenulata* strain CBS 443.65 large subunit ribosomal RNA gene; MH868128.1 - *C. rosea* strain CBS 277.50 large subunit ribosomal RNA gene; MH867871.1 - *C. rosea* strain CBS 226.48 large subunit ribosomal RNA gene; KR857691.1 - *C. rosea* strain CBS 710.86 28S ribosomal RNA gene; KF993398.1 - *C. rosea* isolate PAV-M 1.025 28S ribosomal RNA gene; KT699147.1 - *C. rosea* culture-collection MUT<ITA>:4900 28S ribosomal RNA gene; KF590126.1 - *Gliocladium* sp. G3-1 28S ribosomal RNA gene; AY283558.1 - *B. ochroleuca* strain CCFC226708 large subunit ribosomal RNA gene; MH878288.1 - *C. rosea* strain CBS 906.72A large subunit ribosomal RNA gene; MH876087.1 - *C. rosea* strain CBS 127642 large subunit ribosomal RNA gene; MH875876.1 - *C. rosea* strain CBS 127143 large subunit ribosomal RNA gene; MH870894.1 - *C. rosea* strain CBS 438.68 large subunit ribosomal RNA gene; MH869234.1 - *C. rosea* strain CBS 194.57 large subunit ribosomal RNA gene; MH867396.1 - *C. rhizophaga* strain CBS 202.37 large subunit ribosomal RNA gene; MH866644.1 - *N. camerunensis* strain CBS 225.31 large subunit ribosomal RNA gene; MH866405.1 - *C. rosea* f. *catenulata* strain CBS 154.27 large subunit ribosomal RNA gene; MH875072.1 - *C. rhizophaga* strain CBS 125416 large subunit ribosomal RNA gene; AY686634.1 - *B. ochroleuca* 28S ribosomal RNA gene; MH376707.1 - *C. krabiensis* voucher MFLUCC 16-0254 28S large subunit ribosomal RNA gene; MH868372.1 - *C. rosea* strain CBS 271.51 large subunit ribosomal RNA gene; and KX958035.1 - *C. rosea* isolate 26R-8-F03 internal transcribed spacer 1. Accession number descriptions of B (arranged based on their similarity to *C. rosea* strain ACM941 ITS-5.8S-ITS region from high to low): MK41142 - *C. rosea* strain_ACM941 ITS-1 and ITS-2 partial and 5.8S complete rRNA sequences; MK411421 - *C. rosea* strain_88-710 ITS-1 and ITS-2 partial and 5.8S complete rRNA sequences; MH284648.1 - *Trichoderma* sp. isolate yi1252_1 small subunit ribosomal RNA gene; KY703872.1 - *C. rosea* strain pC1 18S ribosomal RNA gene; GU256754.1 - *B. ochroleuca* strain ATCC 48395 18S ribosomal RNA gene, partial sequence; EU484314.1 - *B. ochroleuca* isolate SB_Bac6 18S ribosomal RNA gene, partial sequence; AJ509864.1 - *Nectria gliocladioides* 5.8S rRNA gene; KY703873.1 - *C. rosea* strain pP3 18S ribosomal RNA gene; KX343160.1 - *Clonostachys* sp. isolate GETM11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1; KX343152.1 - *C. rosea* isolate 22PDA-4C2 18S ribosomal RNA gene; KX058045.1 - *C. rosea* strain qlz6 18S ribosomal RNA gene; KM000126.1 - *Fungal* sp. BVK-SMA-2 18S ribosomal RNA gene; JF817331.1 - *C. rosea* strain CanS-56 18S ribosomal RNA gene; JF817324.1 - *C. rosea* strain CanS-48 18S ribosomal RNA gene; HQ731632.1 - *Bionectria* sp. Papochf 04 18S ribosomal RNA gene; HQ731621.1 - *Bionectria* sp. Macof 02 18S ribosomal RNA gene; HM037950.1 - *B. ochroleuca* strain wxm110 18S ribosomal RNA gene; EU567315.1 - *B. ochroleuca* strain EXGF-1 18S ribosomal RNA gene; AJ509860.1 - *N. gliocladioides* 5.8S rRNA gene; KT323182.2 - *C. rosea* isolate HP113 18S ribosomal RNA gene; KT323180.2 - *C. rosea* isolate HP111 18S ribosomal RNA gene; MK101045.1 - *C. rosea* isolate UCR3250 small subunit ribosomal RNA gene; MH550497.1 - *Clonostachys* sp. strain daef27 small subunit ribosomal RNA gene; MH911376.1 - *C. rosea* strain MF22417 small subunit ribosomal RNA gene; MH864507.1 - *C. rosea* strain CBS 127294 small subunit ribosomal RNA gene; MH864340.1 - *C. rosea* strain CBS 126933 small subunit ribosomal RNA gene; MH862010.1 - *C. rosea* strain CBS 710.86 small subunit ribosomal RNA gene; MH860627.1 - *C. rosea* strain CBS 907.72B small subunit ribosomal RNA gene; MH860412.1 - *C. rosea* f. *catenulata* strain CBS 125.72 small subunit ribosomal RNA gene; MH856624.1 - *C. rosea* strain CBS 277.50 small subunit ribosomal RNA gene; MH856318.1 - *C. rosea* strain CBS 226.48 small subunit ribosomal RNA