Supporting Information

Melanin pathway determination in *Sclerotium cepivorum* Berk using spectrophotometric assays, inhibition compound and protein validation

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Supplementary Material File S1： Obtaining the Ethyl 2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate

Reagents and solvents used were obtained from commercial sources and were used without prior purification. Melting points were determined on an Electrothemal digital 90100 melting point apparatus and were uncorrected. The progress of reactions was monitored by thin layer chromatography (TLC) using silica gel 60-F_{254} coated aluminum sheets, in hexane/ethyl acetate (7:3) and visualized by a 254 nm UV lamp. Pure compounds were characterized, ^{1}H NMR and ^{13}C NMR were recorded for solutions in $CDCl_3$ with Me_4Si as internal standard on Bruker UltraShield (500 MHz) instrument. Chemical shifts are given in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet), or bs (broad singlet). CEM discovery SP microwave reactor was used for reactions.

To obtain this compound, the previously reported methodology was followed, which was modified to increase yield[1,2]. In a pressure tube for microwave reaction was placed salicylsaldehyde (1mmol), ethyl 4,4,4-trifluoroacetoacetate (1.2mmol) and morpholine (10% mmol). The reaction mixture was microwave-heated at 100 °C for 4 h. The end of the reaction was confirmed by TLC using Hex/Ethyl acetate (8:2). Once the reaction was completed, the crude was purified by recrystalization to obtain the product in 97% of yield.

Ethyl 2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate.

White solid; yield 92%; melting point 103.5-104.1 ℃ (Lit. 102-103 ℃[1], and 103.4-104.2 ℃[2]).

References:

[1]Chizhov, D.L., Sosnovskikh, V.Y., Pryadeina, M.V., Burgart, Y.V., Saloutin, V.I., and Charushin, V.N. (2008). The first synthesis of 4-unsubstituted 3-(trifluoroacetyl) coumarins by the Knoevenagel condensation of salicylaldehydes with ethyl trifluoroacetoacetate followed by chromene-coumarin recyclization. Synlett 19, 281-285.

[2]Xu, C., Yang, G., Wang, C., Fan, S., Xie, L., and Gao, Y. (2013). An efficient solvent-free synthesis of 2-hydroxy-2-(trifluoromethyl)-2H-chromenes using silica-immobilized L-proline. Molecules 18, 11964-11977.

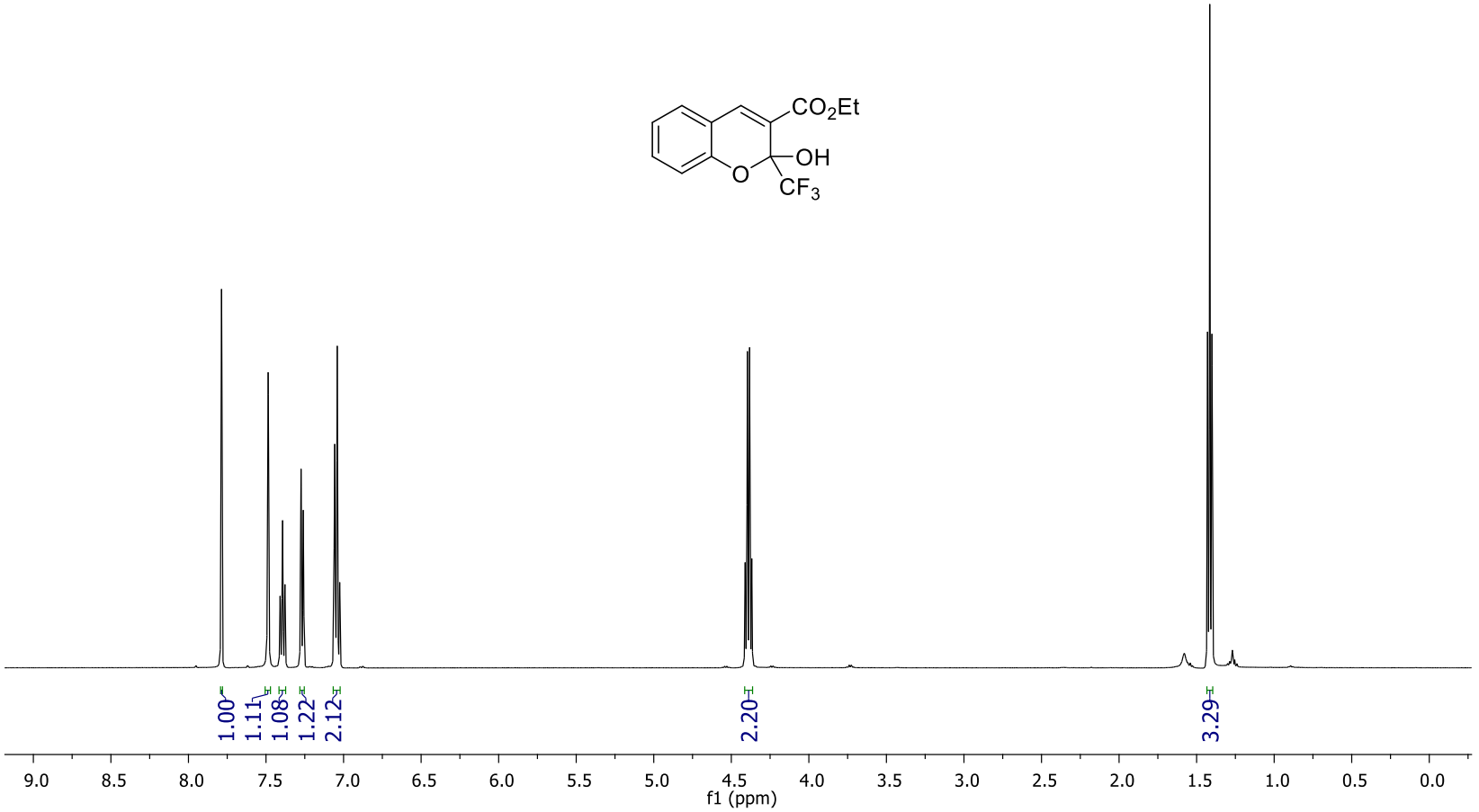

Figure S1. *Ethyl 2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate* ^{1}H NMR spectrum (500 MHz, $CDCl_3$) δ: 1.42 (t, *J* = 7.2 Hz, 3H, CH_3), 4.39 (q, *J* = 7.2 Hz, 2H, CH_2), 7.02-7.06 (m, 2H), 7.26 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.39 (m, 1H), 7.45 (s, 1H, OH), 7.79 (s, 1H).

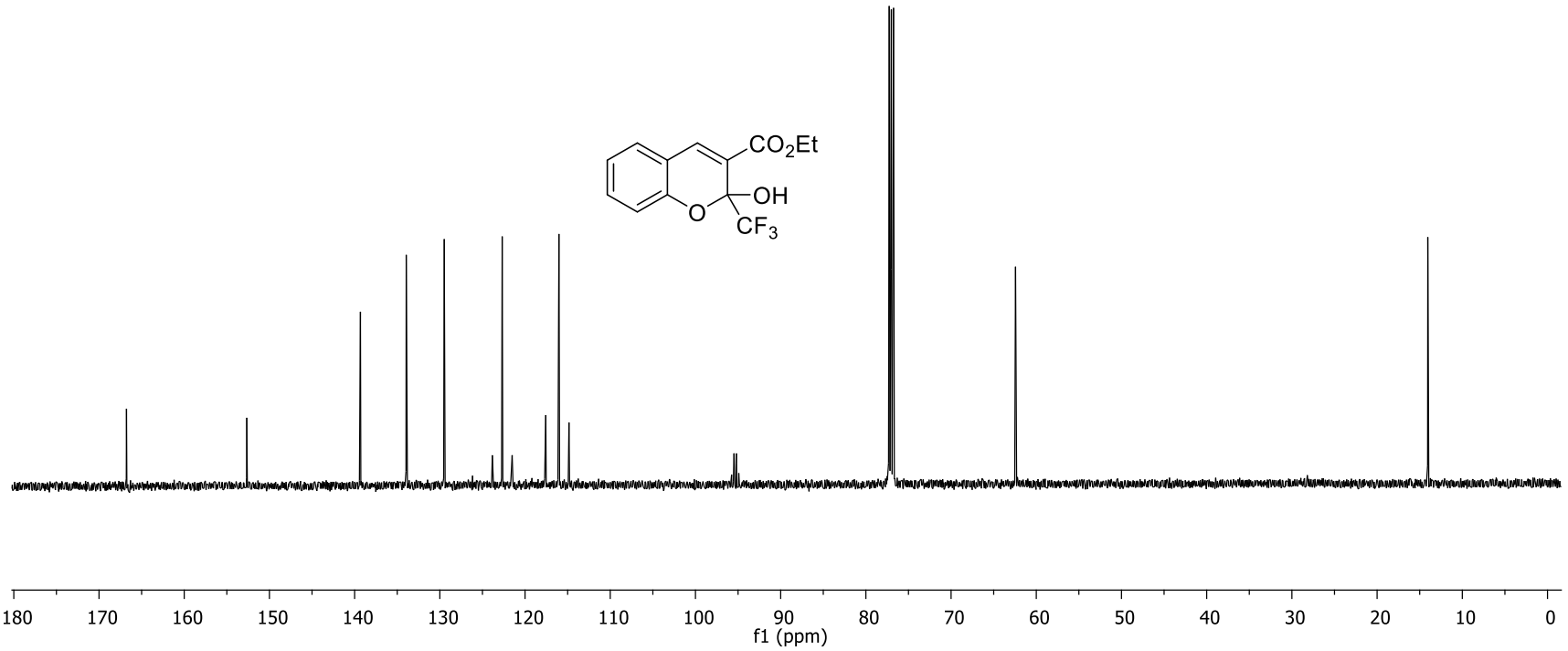

Figure S2. *Ethyl 2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate* ^{13}C-NMR spectrum ($CDCl_3$, 125 MHz) δ: 14.1 (CH_3), 62.4 (CH_2), 95.3 (q, *J*C,F = 34.8 Hz, *C*CF_3), 114.8, 116.0, 117.6, 121.5 (q, *J*C,F = 290 Hz, CF_3), 122.7, 129.5, 133.9, 139.3, 152.7, 166.8 (C=O).

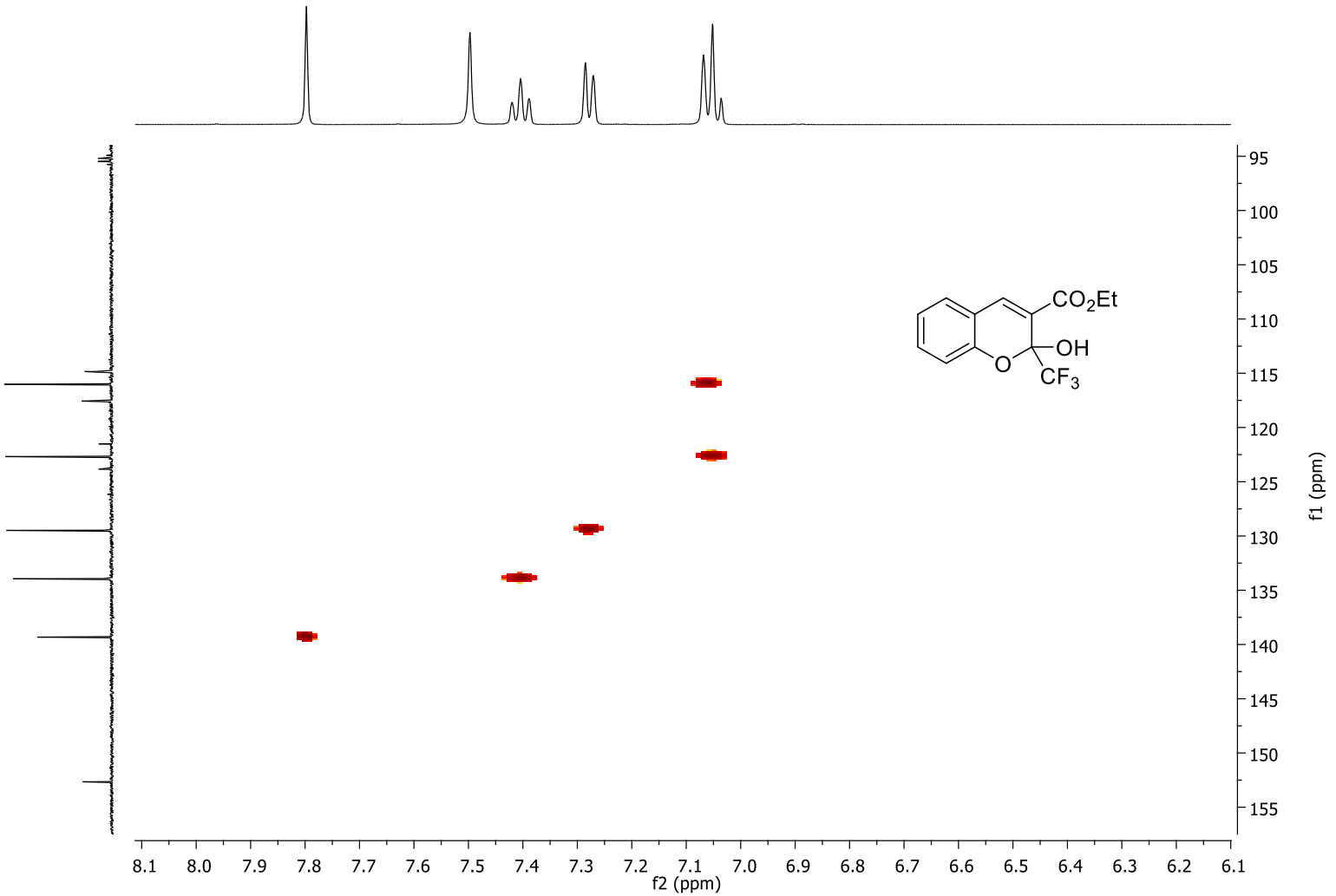

Figure S3. *Ethyl 2-hydroxy-2-(trifluoromethyl)-2H-chromene-3-carboxylate* HMQS spectrum of 2D-NMR ($CDCl_3$)

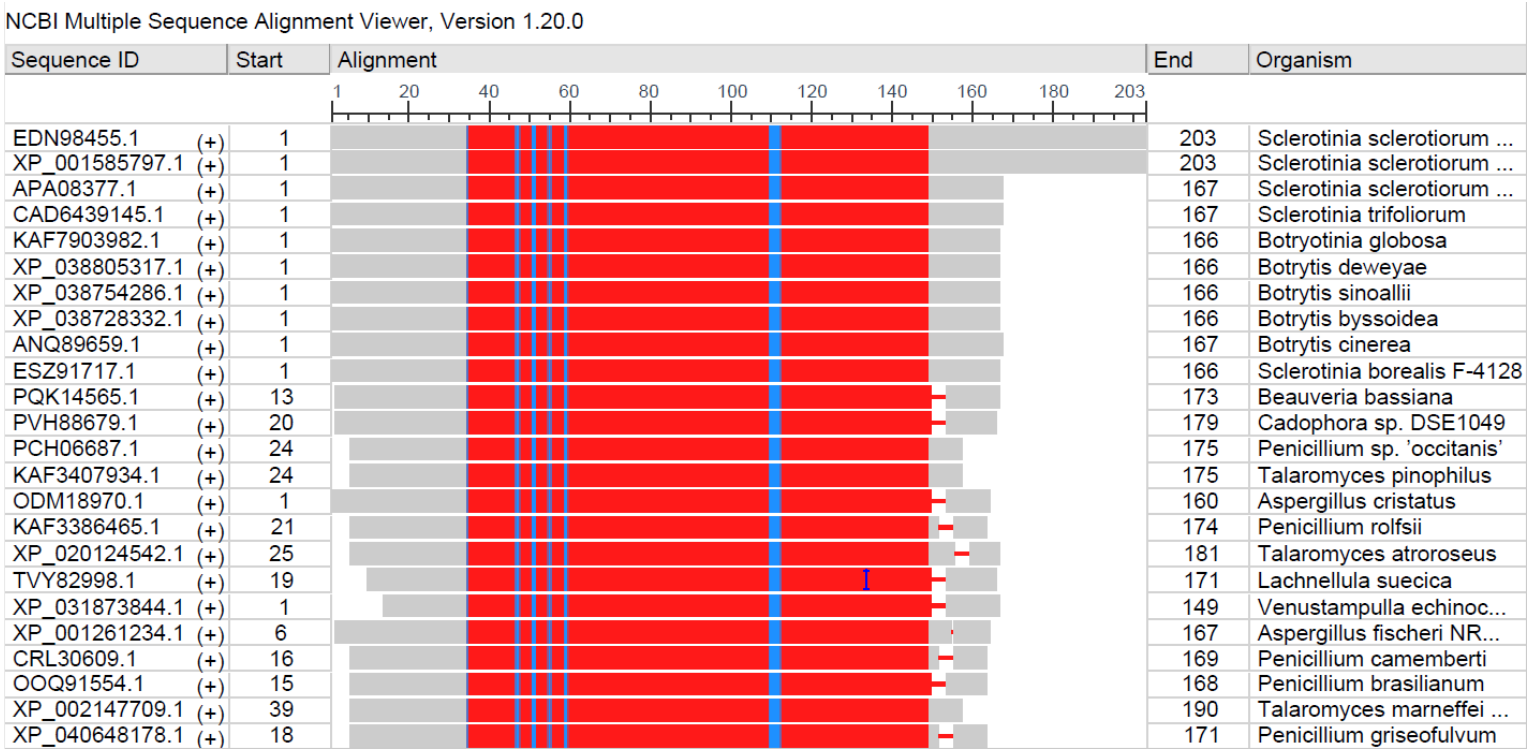

Figure S4. *Sclerotinia sclerotiorum* alignment. Comparison between SDH of *Sclerotinia sclerotiorum* (Sequence ID: EDN98455.1) and another SDH of different size. NCBI alignment.

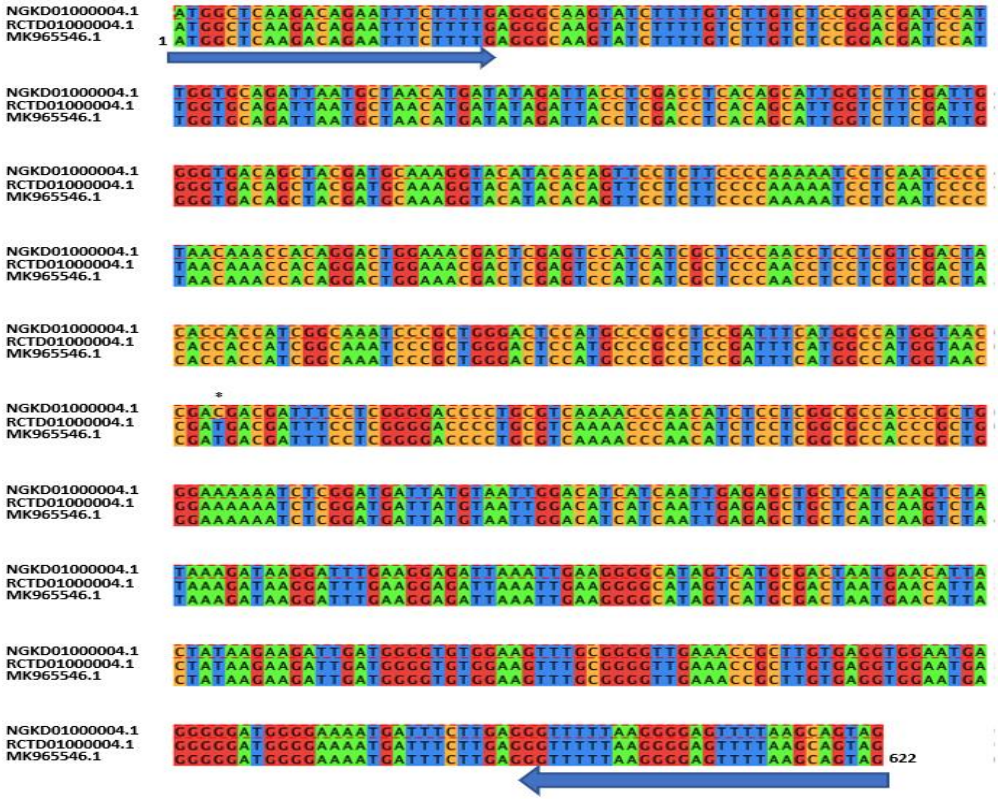

Figure S5. Multiple alignment of the Scytalone dehydratase gene from Sclerotium cepivorum in Gene Bank; NGKD01000004.1 genome sequenced by Kohn., RCTD01000004.1 genome sequenced by Valero-Jimenez et al.[3], and MK965546.1 complete SDH cds. * Show the only one difference, the blue arrows indicate the alignment site of the primers for PCR ScSDH amplification (Table S1).

[3] Claudio A. Valero-Jiménez, Maikel B.F. Steentjes, Jason C. Slot, Xiao qian Shi-Kunne, Olga E.Scholten, and Jan A.L. vanKan(2020). Dynamicsin Secondary Metabolite Gene Clustersin Otherwise Highly Syntenic and Stable Genomes in the Fungal Genus *Botrytis*. Genome Biol. Evol.12(12):2491–2507.doi:10.1093/gbe/evaa218

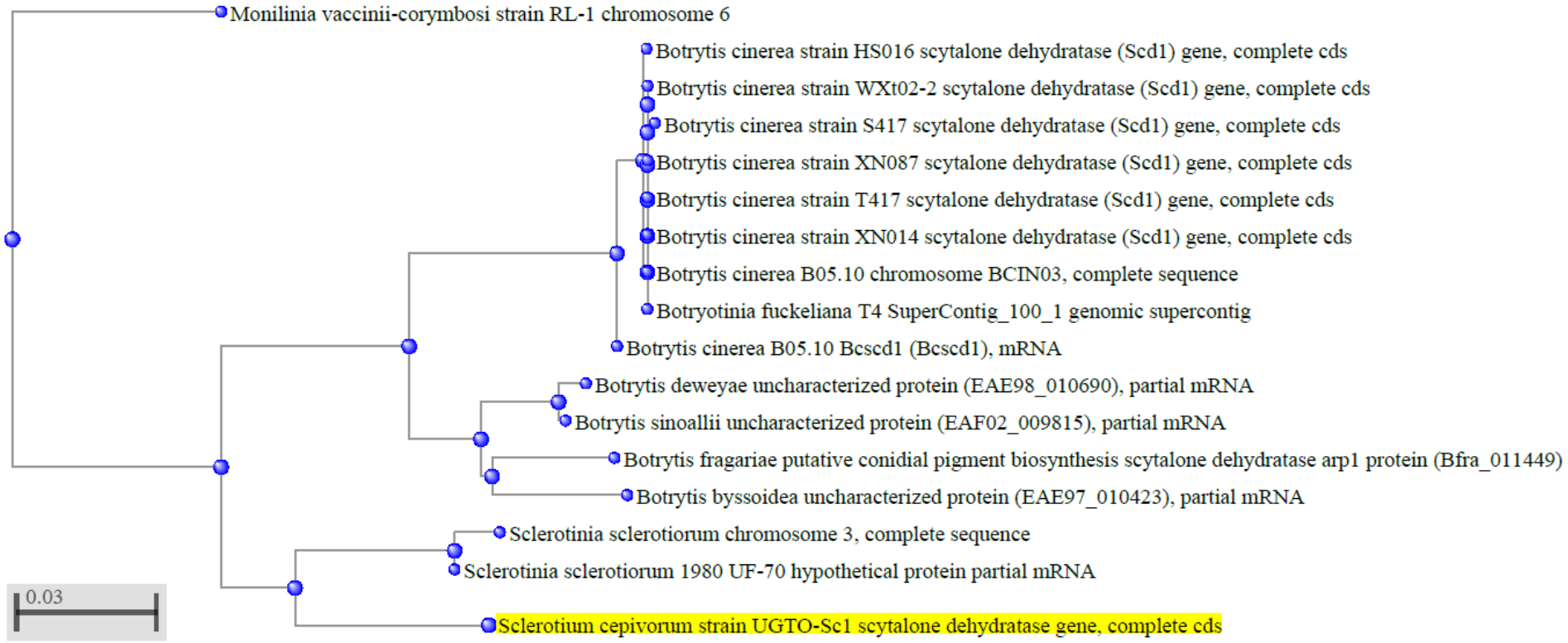

Figure S6. Distance tree SDH of *S. cepivorum* UGTO-Sc1 (GenBank: MK965546.1). *S. cepivorum* SDH sequence (yellow) seems to be more related to *Botrytis* species and *Sclerotinia sclerotiorum*, NCBI BLAST distance tree result using pairwise alignments. Fast Minimum Evolution was the algorithm used to produce the tree (Desper R and Gascuel O, Mol Biol Evol 21:587-98, 2004 PMID: 14694080). [4]

[4]Zheng Zhang, Scott Schwartz, Lukas Wagner, and Webb Miller (2000), "A greedy algorithm for aligning DNA sequences", J Comput Biol 2000; 7(1-2):203-14.

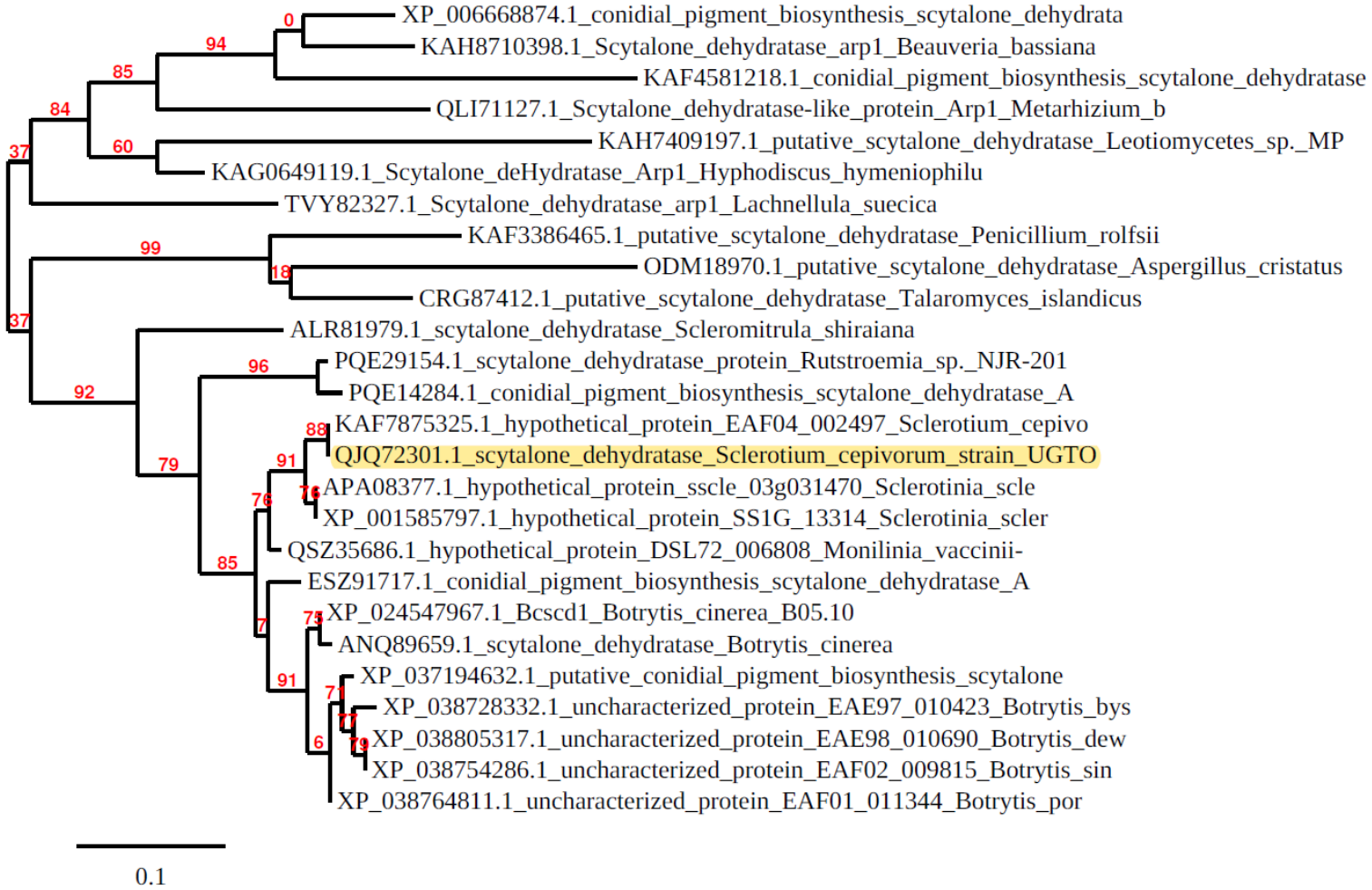

Figure S7. Phylogenetic tree of *S. cepivorum* UGTO-Sc1 SDH. It was constructed using the SDH aa sequence GenBank: QJQ72301.1. In yellow is represented the strain used in this article. Bootstrap values are presented in percentages.[5-11]

[5]Dereeper A., Audic S., Claverie J.M., Blanc G. *BLAST-EXPLORER helps you building datasets for phylogenetic analysis*. BMC Evol Biol. 2010 Jan 12;10:8. (PubMed)

[6] Dereeper A.*, Guignon V.*, Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M., Gascuel O. *Phylogeny.fr: robust phylogenetic analysis for the non-specialist.* Nucleic Acids Res. 2008 Jul 1;36(Web Server issue):W465-9. Epub 2008 Apr 19. (PubMed) *: joint first authors

[7] Edgar RC. *MUSCLE: multiple sequence alignment with high accuracy and high throughput.* Nucleic Acids Res. 2004, Mar 19;32(5):1792-7. (PubMed)

[8] Castresana J. *Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis.* Mol Biol Evol. 2000, Apr;17(4):540-52. (PubMed)

[9] Guindon S., Gascuel O. *A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood*. Syst Biol. 2003, Oct;52(5):696-704. (PubMed)

[10] Anisimova M., Gascuel O. *Approximate likelihood ratio test for branchs: A fast, accurate and powerful alternative.* Syst Biol. 2006, Aug;55(4):539-52. (PubMed)

[11] Chevenet F., Brun C., Banuls AL., Jacq B., Chisten R. *TreeDyn: towards dynamic graphics and annotations for analyses of trees.* BMC Bioinformatics. 2006, Oct 10;7:439. (PubMed)

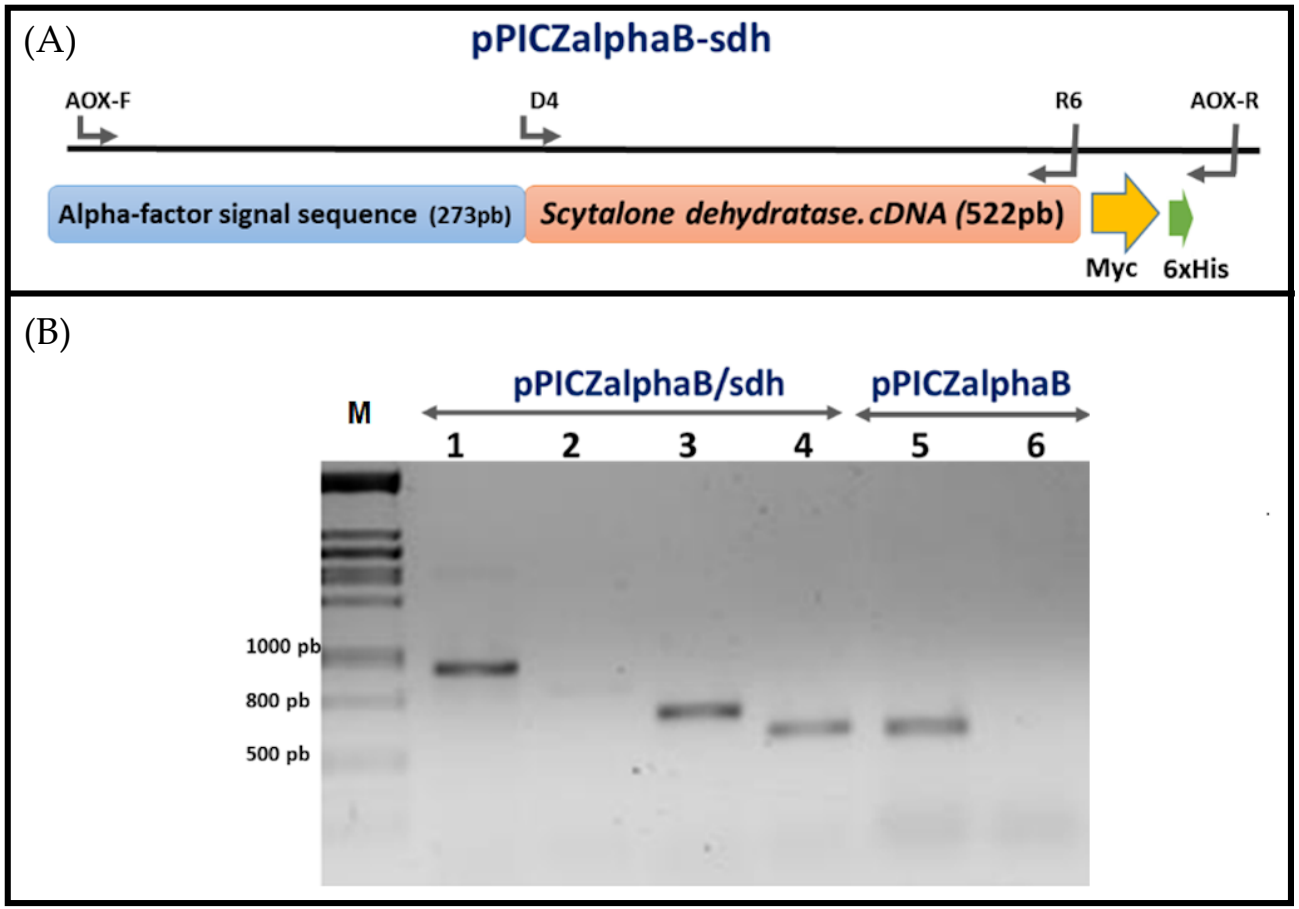

Figure S8. *Sclerotinia sclerotiorum* alignment. Comparison between SDH of *Sclerotinia sclerotiorum* (Sequence ID: EDN98455.1) and another SDH of different size. NCBI alignment. Figure S7. Analysis of P. pastoris transformants (pPICZαB/sdh or pPICZαB vector) A). Construction generated in pPICZαB and primers localization: AOX-F, AOX-R, D4, and R6. B) PCR products of pPICZαB/sdh or empty vector using different primers. Lines 1 and 5: AOX-F / AOX R. Lines 2 and 6: AOX-F / R6. Line 3: D4 / AOX-R. Line 4: D4 / R6. M=Molecular marker. 1% agarose gel electrophoresis.

Amplified product of line 2 is very weak, this is because of a low amplification efficiency in this reaction, however, the band of interest is observed [878 bp (AOX -F primer starts 86 bp upstream of the α-factor secretion signal)]. The rest of the lanes show the obtained pPICZαB-sdh *P. pastoris* transformants and they have a good amplification (for example complete construction on line 1 compared to empty vector on line 5).

List of primers used for isolation and expression for ScSDH gene					
Primer Name	**Sequence 5'---->3'**	**Tm (°C)**	**Restriction site**	**Amplicon size (bp)**	
PCR amplification of ScSDH					
SCBSDH-F05 (D4)	G**CTGCAG**CATCCACTATGGCTCAAGACAGAATTTCTTTTG	56	PstI	DNA	cDNA
SCBSDH-R05 (R6)	**GCGGCCGC**GTACTGCTTAAAACTCCCCTTAAAAACC	56	NotI	645	522
pPICZaB/ScSDH					
AOX-F	GACTGGTTCCAATTGACAAGC	55		DNA	cDNA
AOX-R	GCAAATGGCATTCTGACATCC	56		1238	1115
Sequences that are indicated in bold font correspond to Restriction enzyme site					

Table S1. Primers used for the expression of the SDH heterologous protein. Expression performed in *Pichia pastoris* by using a pPICZα plasmid (Invitrogen™).