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Control of human pathogenic microorganisms in plant production systems

Dairy Research Institute s.r.o. and Faculty of Agriculture, University of South Bohemia in České Budějovice, Czech Republic

Online training school on fungal taxonomy

Application deadline: 31 May 2021

Notification of acceptance: 04 June 2021

Training school period: 07 June – 31 August 2021 (lab work expected; live + recorded online lectures)

Dear friends,

The pandemic situation doesn't allow us to realize the "Training school on fungal taxonomy" in our labs. So, we are trying to manage the online course for those who are interesting in fungal taxonomy.

We can prepare videos, commented presentations, and lab protocols.

We hope you can work in your own lab and provide you below a list of material and chemicals*[1]. We can supervise your work on-line to approach the final results - determination of fungal strains by using DNA sequencing.

Each participant can choose one genus of fungi (includes four anonymized strains, marked as *Aspergillus* 1, 2, 3 and 4) and one genus of yeast (includes three strains, marked as *Candida* 1, 2 and 3) listed in Table 1. The fungal strains will be numbered. The final identification of each particular strain will be your task. To make sure there is no issue with potential lockdown,

access to lab facilities, quarantine, etc., you will have 12 weeks to perform this task, i.e. from 7th June until 31st August.

Our collection can provide fungal cultures for all participants to work on known samples. These strains are provided free of charge but their use is restricted to academic purpose only. There is just a manipulation fee (post fee). The fungal strains proposed for the study are listed in Table 1. The required primers are listed in Table 2. Please, check if you have access to required primers or have them synthesized*[2].

*[1]**Laboratory tools and equipment for molecular identification of fungi**

-Biosafety cabinet, protection equipment (gloves), autoclave for bioahazard waste

set of micropipets (10 ul, 100 or 200 ul)

- microtubes (200 ul, 1.5 ml)

- glass beads (require laboratory grinder or a vortex with microtube adaptor)

- pellet pestles, or tube homogenizers

- PCR cycler

- gel electrophoresis equipment

- gel imaging device or UV board

- access to sequencing facility or 3rd-party sequencing service

-computer with software (Finch (free), Geneous, BioEdit (free)) and Internet access

*[1]**List of chemicals, buffers, and enzymes**

- 0.5 M NaOH

- 10 mM Tris-Cl (pH 8.5) or TE buffer
- TAE or TBE electrophoretic buffer or chemicals necessary for preparation
- agarose
- DNA ladder
- *Taq* DNA polymerase master mix or *Taq* DNA polymerase kit (*Taq*, buffer, NTs)
- Exonuclease or PCR clean kit

*[2] In case the participant does not intend to deal with the molecular taxonomy of fungi after the course, we can provide the necessary amount of primers together with samples.

Table 1 - Strains proposed for the study during the training school

Genus	Species
Fungi	
Aspergillus	*A. cibarius; A. versicolor; A. tabacinus; A. montevidensis*
Penicillium	*P. crustosum; P. brevicompactum; P. commune; P. roqueforti*
Cladosporium	*C. ramotenellum; C. halotolerans; C. herbarum; C. cladosporoides*
Yeast	
Candida	*C. krusei; C. parapsilosis; C. intermedia*
Trichosporon	*T. domesticum; T. coremiiformis,*
Pichia	*P. fermentans; P. membranifaciens*

Table 2 - Primers required for the training school

Region	Primer	Sequence (5´→3´)	References
BenA	BT2a	GGTAACCAAATCGGTGCTGCTTTC	Glass & Donaldson, 1995
	BT2b	ACCCTCAGTGTAGTGACCCTTGGC	
Act	ACT-512F	CCGAGTACAAGGAGGCCTTC	Hong *et al*., 2006
	ACT-783R	TACCAGTCCTTCTGGCCCAT	
ITS	ITS1-f	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns, 1993; White *et al*., 1990
	ITS4	TCCTCCGCTTATTGATATGC	

Primers references

Gardes M., Bruns T. D. (1993) ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Mol Ecol. 2:113-8.

Glass N. L., Donaldson G. C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol. 61:1323-30.

Hong S. -B., Cho H. -S., Shin H. -D., Frisvad J. C. and Samson R. A. (2006) Novel *Neosartorya* species isolated from soil in Korea. Int. J. Syst. Evol. Microb. 56: 477–486.

White T. J., Bruns T. D., Lee S. B., Taylor J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. United States: Academic Press. pp. 315–322.

This training course on fungal taxonomy belongs to Dairy Research Institute Ltd., Czech Republic.

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Please, feel free to contact us and ask for details if you are interested in this course!!! The capacity of Online training school on fungal taxonomy is 10 persons.