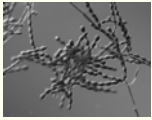


Conserving orchid and other fungi at the UAMH



NAOCC meeting June 25-26, 2012

Lynne Sigler, Curator & Professor
University of Alberta Microfungus Collection
and Herbarium (UAMH)
Devonian Botanic Garden, Edmonton, AB, Canada
www.uamh.devonian.ualberta.ca

UAMH Holdings

- 11,600 total accessions
- Includes significant collections of ecto- and ericoid mycorrhizal fungi
- 210 isolates of orchid mycorrhizal fungi
- 70 of these shown to initiate symbiotic seed germination
- 60 other orchid root endophytes of uncertain biological role



Images courtesy R.S. Currah

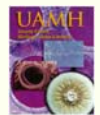
UAMH History

- Established in 1960 but earliest accession dates from 1934
- Long association with diagnostic and reference services in human and veterinary medicine
- Work on mycorrhizal and root associated fungi began in 1986 together with relocation to Devonian Botanic Garden and in partnership with R.S. Currah



Our roles

- Collect and catalogue fungal diversity.
- Identify, authenticate and characterize isolates.
- Archive strain data in a SQL database and disseminate through online + PDF catalogues.
- Annotate with sequences, images or other data from in-house and external research
- Preserve isolates by freeze-drying, freezing in liquid nitrogen or by other methods.



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Our roles

- Prepare dried colonies to back-up living cultures.
- Supply cultures and advise on strain selection.
- Educate through academic courses, workshops and individual training.
- Ensure quality management for a microbial collection.



Epulorhiza
specimens

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Preservation methods

- Freeze-drying (lyophilization) for sporulating fungi.
- Cryopreservation (LN vapor phase) main method for non sporulating & mycorrhizal.
- Water and oil storage also used for non sporulating
- Viability of frozen isolates may be affected by variables such as condition of source culture, rate of cooling, cryoprotectant used and presence of staling compounds.



Strain data

Data on preservation methods, location and viability results stored in database (not public).

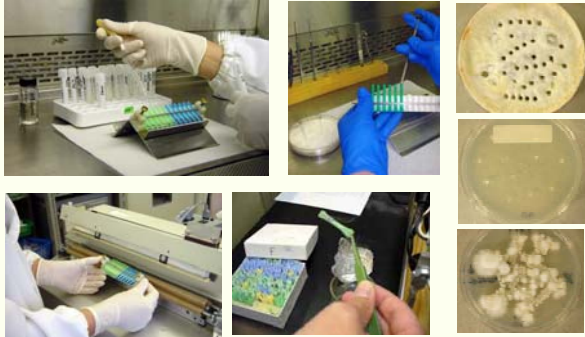
Strain ID	Date	Preservation
Epulorhiza sp. 1988	18 Sep 2012	LN
Epulorhiza sp. 1989	18 Sep 2012	LN
Epulorhiza sp. 1990	18 Sep 2012	LN
Epulorhiza sp. 1991	18 Sep 2012	LN
Epulorhiza sp. 1992	18 Sep 2012	LN
Epulorhiza sp. 1993	18 Sep 2012	LN
Epulorhiza sp. 1994	18 Sep 2012	LN
Epulorhiza sp. 1995	18 Sep 2012	LN
Epulorhiza sp. 1996	18 Sep 2012	LN
Epulorhiza sp. 1997	18 Sep 2012	LN
Epulorhiza sp. 1998	18 Sep 2012	LN
Epulorhiza sp. 1999	18 Sep 2012	LN
Epulorhiza sp. 2000	18 Sep 2012	LN
Epulorhiza sp. 2001	18 Sep 2012	LN
Epulorhiza sp. 2002	18 Sep 2012	LN
Epulorhiza sp. 2003	18 Sep 2012	LN
Epulorhiza sp. 2004	18 Sep 2012	LN
Epulorhiza sp. 2005	18 Sep 2012	LN
Epulorhiza sp. 2006	18 Sep 2012	LN
Epulorhiza sp. 2007	18 Sep 2012	LN
Epulorhiza sp. 2008	18 Sep 2012	LN
Epulorhiza sp. 2009	18 Sep 2012	LN
Epulorhiza sp. 2010	18 Sep 2012	LN
Epulorhiza sp. 2011	18 Sep 2012	LN
Epulorhiza sp. 2012	18 Sep 2012	LN
Epulorhiza sp. 2013	18 Sep 2012	LN
Epulorhiza sp. 2014	18 Sep 2012	LN
Epulorhiza sp. 2015	18 Sep 2012	LN
Epulorhiza sp. 2016	18 Sep 2012	LN
Epulorhiza sp. 2017	18 Sep 2012	LN
Epulorhiza sp. 2018	18 Sep 2012	LN
Epulorhiza sp. 2019	18 Sep 2012	LN
Epulorhiza sp. 2020	18 Sep 2012	LN
Epulorhiza sp. 2021	18 Sep 2012	LN
Epulorhiza sp. 2022	18 Sep 2012	LN
Epulorhiza sp. 2023	18 Sep 2012	LN
Epulorhiza sp. 2024	18 Sep 2012	LN
Epulorhiza sp. 2025	18 Sep 2012	LN
Epulorhiza sp. 2026	18 Sep 2012	LN
Epulorhiza sp. 2027	18 Sep 2012	LN
Epulorhiza sp. 2028	18 Sep 2012	LN
Epulorhiza sp. 2029	18 Sep 2012	LN
Epulorhiza sp. 2030	18 Sep 2012	LN

Cryopreservation method

- Cryoprotectant 10% v/v glycerol (or DMSO)
- Straw method of Stalpers et al 1987¹
- Cut 4 mm polypropylene straws (Stone or Sweetheart brand) into 4 cm lengths
- Seal straws at one end and sterilize
- Label 6 straws and place in holding rack
- Fill ½ full with sterile cryoprotectant
- Use 3 mm sterile push pin to transfer 5-7 plugs of mycelium + agar to straws; seal at other end

1. Stalpers JA et al, Mycologia 79:82-89, 1987

Cryopreservation protocol



Racks and push pins manufactured according to Stalpers et al 1987

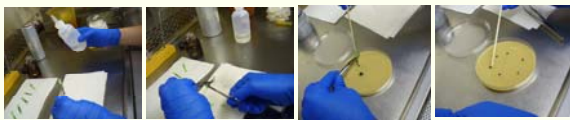
Water & oil storage

- Fill Nunc vials $\frac{3}{4}$ full with sterile water or light mineral oil.
- Use sterile disposable transfer tube to transfer plugs of mycelium and agar to vial.
- Seal caps; store WS in frig, OS at room temp.
- No purity or viability checks done at set-up.



LN reconstitution for purity & viability

- Hold straw over sterile towel or petri dish.
- Clean with 70% EtOH, snip both ends of straw.
- Use a sterile applicator to push plugs onto a labelled agar plate (PDA or other).
- Spread the plugs apart and away from the residual cryoprotectant.



Preservation and identification problems

- Orchid fungi can be difficult to preserve, particularly *Ceratorhiza* group
- Do not freeze well nor survive in water
- Current experimental work trying charcoal filter paper method of Stielow et al¹ (C. Gibas)
- Molecular work needed; current ITS study of 48 *Epulorhiza* isolates ongoing by student of R. Currah



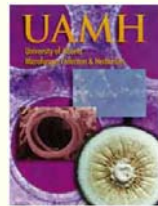
1. Stielow JB et al, Mycologia 104:324-330, 2012

Selected fungal & orchid taxa

<i>Armillaria</i> species	<i>Eulophia alta</i>
<i>Ceratobasidium obscurum</i>	<i>Orchis rotundifolia</i>
<i>Ceratorhiza goodyerae-repentis</i>	<i>Coeloglossum viride</i> , <i>Platanthera obtusata</i> , <i>P. praeclara</i> , <i>Spiranthes lacera</i>
<i>Ceratorhiza pernacatena</i>	<i>Platanthera praeclara</i>
<i>Ceratorhiza</i> species	<i>Dactylorhiza incarnata</i> , <i>Campylocentrum micranthum</i> , <i>Habenaria macroceratitis</i> , <i>Platanthera lacera</i> , <i>P. praeclara</i> , <i>P. leucophaea</i> , <i>Rodriguezia compacta</i> , <i>Spiranthes eatonii</i> , <i>S. floridana</i>

Orchid isolates complete data

- Available in PDF or online Catalogues
- Search substrate or characteristics for 'orchid'



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Selected fungal and orchid taxa (cont.)

<i>Epulorhiza albertaensis</i>	<i>Platanthera orbiculata</i>
<i>Epulorhiza anaticula</i>	<i>Calypso bulbosa</i> , <i>Platanthera dilatata</i> , <i>P. obtusata</i> , <i>P. hyperborea</i> , <i>Coeloglossum viride</i>
<i>Epulorhiza calendulina</i>	<i>Amerorchis rotundifolia</i>
<i>Epulorhiza inquilina</i>	<i>Platanthera integrilabia</i>
<i>Epulorhiza repens</i>	<i>Dactylorhiza majalism</i> <i>D. maculata</i> , <i>Orchis morio</i> , <i>Spiranthes caseii</i> , <i>S. lacera</i>
<i>Epulorhiza</i> species	<i>Calypso bulbosa</i> , <i>Goodyera oblongifolia</i> , <i>Encyclia tampensis</i> , <i>Epidendrum conop-seum</i> , <i>Habenaria macroceratitis</i> , <i>H. repens</i> , <i>Platanthera ciliaris</i> , <i>P. grandiflora</i> , <i>P. holochila</i> , <i>P. leucophaea</i> , <i>P. praeclara</i> , <i>Spiranthes brevilabris</i> , <i>S. odorata</i> , <i>S. tuberosum</i>

Thanks to our orchid fungi depositors

- 1986 - **R.S. Currah** & associates C. Zelmer, K. Richardson, A. Smreciu, S. Hambleton
- 2000 - **L. Zettler** & associates K. Piskin, W. Kutosky, D. Mauer, N. Huber, C Pollack, & others
- 2000 - S.L. Stewart
- 2000 - J. Sharma
- 2006 - B. Keel