available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mycol

Teaching techniques for mycology: 24. Patterns of basidiospore germination in *Auricularia* (Heterobasidiomycetes)

Roland W. S. WEBER[a,*], *John WEBSTER*[b]

[a]*Department of Biotechnology, University of Kaiserslautern, Erwin-Schroedinger-Str. 56, 67663 Kaiserslautern, Germany*
[b]*12 Countess Wear Road, Exeter EX2 6LG, U.K.*

Keywords:
Auricularia auricula-judae
Germination patterns
Heterobasidiomycetes
Microconidia
Repetitious germination

ABSTRACT

'Basidiocarps of Auricularia can be stored dry, discharging ballistospores upon rehydration. This article describes methods to demonstrate basidiospore germination by repetition or formation of a primary mycelium and/or microconidia'

1. Introduction: features of interest

Heterobasidiomycetes (also called Phragmobasidiomycetes) comprise one of four classes of the phylum Basidiomycota (Wells & Bandoni 2001), and many species included here make up a sound monophyletic grouping as confirmed by DNA sequence analyses (Weiss & Oberwinkler 2001). They can be distinguished by a combination of several general features from Homobasidiomycetes (including mushrooms, toadstools, gasteromycetes), rusts (Urediniomycetes) and smuts (Ustilaginomycetes). Basidia of Heterobasidiomycetes are strongly lobed, and many of them are divided by transverse or longitudinal septa. Secondly, fruit-bodies of many Heterobasidiomycetes are jelly-like and can survive repeated drying and wetting, producing a fresh crop of viable basidiospores upon each rehydration event. This is a convenient feature if classes have to be held out of fruiting season. Only few Homobasidiomycetes (e.g. *Schizophyllum commune*) have basidiocarps which can be stored and used in this way. Thirdly, basidiospore germination of Heterobasidiomycetes is unusual in that it can proceed in several different ways, including repetitious germination to produce a further ballistospore, formation of homokaryotic microconidia, and emission of a germ-tube to give rise to a homokaryotic primary mycelium. The class Heterobasidiomycetes has been divided into two sub-classes, Heterobasidiomycetidae in which the homokaryotic spores are conidia, and Tremellomycetidae in which they take the shape of budding yeast cells (see Webster & Weber 2006).

Here we focus on *Auricularia auricula-judae* (Heterobasidiomycetidae). Fully hydrated basidiocarps are ear-shaped and somewhat rubbery or cartilaginous in texture (Fig. 1), hence the common name Jew's Ear fungus. The fungus produces basidiospores from cylindrical basidia which become divided into four cells by three transverse septa. Each cell emits an elongated epibasidium which produces a single basidiospore at the hymenial surface of the fruit-body.

The pattern of germination of freshly discharged basidiospores (Fig. 2) depends on the environment in which they find themselves (Brefeld 1888; Ingold 1982a, 1984). Spores

* Corresponding author. Tel.: +49 631 3167212; fax: +49 631 3167215.
E-mail addresses: rwsweber@rhrk.uni-kl.de (R. W. S. Weber), j.webster@exeter.ac.uk (J. Webster).
0269-915X/$ – see front matter © 2006 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.mycol.2006.07.022

Fig. 1 – Basidiocarps of *Auricularia auricula-judae* on a dead branch of *Sambucus nigra*.

remaining on the hymenium often germinate by repetition to produce a further ballistospore (Fig. 3), a phenomenon interpreted by Ingold (1982a) as a second escape chance. Basidiospores incubated on a nutrient-deficient medium such as tap-water agar (TWA) will lay down three transverse septa, and each of the resulting cells produces one or a few extensions (denticles) giving rise to clusters of sickle-shaped (lunate) microconidia (Figs 4,5). The precise role of these microconidia in the life-cycle of *A. auricula-judae* is uncertain; they may act in fertilisation but are also capable of hyphal germination (Fig. 6). If a basidiospore lands on a nutrient-richer medium such as cornmeal agar (CMA) or 0.2 % malt extract agar, it may emit a germ-tube. This branches to form a mycelium which may go on to produce lunate microconidia (Fig. 7). As far as is known, *Auricularia* spp. are heterothallic (Wong 1993), and their life-cycle is completed when two homokaryotic mycelia of compatible mating-types meet and establish a dikaryotic (heterokaryotic) secondary mycelium capable of forming fruit-bodies.

As outlined here, *A. auricula-judae* provides a useful demonstration of life-cycle flexibility in response to different environmental conditions. This complements an earlier consideration of the complex life-cycle of the zygomycete *Basidiobolus ranarum* (Weber & Webster 1998).

2. Source of material

Basidiocarps of *A. auricula-judae* are produced throughout the year on dead or moribund branches of elder (*Sambucus nigra*) and other broad-leaved trees. There is another common European species, *A. mesenterica* which fruits predominantly on elm (*Ulmus* spp.). A Far Eastern cultivated species, *A. polytricha* ('Mu-Erh'), is an essential ingredient of many Chinese soups. These and other species may also be used for the purposes of basidiospore germination. Fruit-bodies of *A. auricula-judae* and *A. mesenterica* can be collected from the field and stored dry for several years. Those of *A. polytricha* can be bought dry from Chinese food shops. *Auricularia polytricha* is probably the very first mushroom to have been cultivated, early records from China dating back to about A.D. 600 (Chang & Miles 2004).

3. Preparation of material

If dried fruit-bodies of *A. auricula-judae* are available, a demonstration of the various modes of spore germination involves very little preparation. However, it is advisable to test the viability of a new collection of basidiocarps before embarking on a practical course.

Day -7. Rehydrate basidiocarps of *A. auricula-judae* by placing them on moist filter paper in a closed chamber at room temperature (about 21–24 °C).

Day -6. Prepare a couple of thinly-poured agar plates (one TWA, one CMA) per group of students. Cut out fragments (about 5 × 10 mm) from the rehydrated basidiocarps and suspend them from the underside of a Petri dish lid with a little vaseline or petroleum jelly such that the smooth (hymenial) surface faces downwards. Mount the fragments to one side of the Petri dish lid. Repeatedly turn the dish lids at 15–30 min intervals like a dial, each time by about 1/8 revolution, in order to produce several basidiospore deposits on each agar plate.

Day -3/-2. Rehydrate a second lot of basidiocarps; suspend from Petri dish lids over fresh TWA and CMA plates and turn the lids as descibed above. Additionally, leave a few fruit-bodies incubating in the moist chamber with the hymenial surface facing *upwards*, thereby allowing liberated basidiospores to fall back onto the hymenium.

Day -1/0. Prepare a third crop of basidiospore deposits as described above.

Day 0. Present material to the students. Spore deposits should be viewed with a bright-field microscope by cutting a square of agar (15 mm side length) and mounting it in water under a coverslip. Freshly discharged basidiospores are aseptate and contain one haploid nucleus (Fig. 2). Spores incubated on TWA for 2 d will show germination by means of lunate microconidia (Fig. 4). After 6 d, the entire cytoplasm of basidiospores will have been evacuated as microconidia (Fig. 5), some of which may be showing hyphal germination (Fig. 6). In contrast, on CMA hyphal germination of basidiospores should predominate, the resulting mycelium producing lunate conidia after 6 d (Fig. 7). Germination by repetition can be demonstrated with basidiocarps incubated for 3 d with the hymenium facing upwards; wash the hymenium with