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Note

# A senescence-delaying pre-culture medium for transcriptomics of *Podospora anserina*

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ABSTRACT

*Podospora anserina* is an efficient degrader of recalcitrant plant biomass but senesces quickly on most standard pre-culturing media. Among nine pre-culture media, sufficient growth without senescence was only observed on Luria-Bertani medium. The high quality RNA obtained from subsequent transfer cultures was suitable for transcriptomics.

## 1. Introduction

The coprophilous *Podospora anserina* is a model fungus used to study ageing (Osiewacz et al., 2013), prion mechanisms (Baxa et al., 2004) and sexual reproduction (Bennett and Turgeon, 2016; Grognet et al., 2014). *P. anserina* also has a distinctive potential for recalcitrant lignocellulose degradation due to its unusual habitat of herbivory dung and large repertoire of plant biomass degrading enzymes (Espagne et al., 2008; Richardson, 2002), which have potential biotechnological applications (Couturier et al., 2016). Transcriptomic analysis is widely used to understand different aspects of fungal physiology, e.g. the strategy to degrade plant biomass (Mäkelä et al., 2014), and the standardized approach adopted by the community to facilitate comparative analyses involves transfer of healthy pre-cultured mycelium to experimental conditions of interest. However, *P. anserina* is one of the few fungi that senesces quickly if cultivated on rich media (Griffiths, 1992) with senescence occurring from less than one week to 25 days (Lorin et al., 2006). Thus this species became a model to study ageing (Scheckhuber and Osiewacz, 2008).

Senescing mycelium could result in artefactual responses that override the natural regulatory response to the experimental conditions of interest. Senescence is caused by mitochondrial DNA instability (Scheckhuber and Osiewacz, 2008) and so far only three methods have been developed to delay senescence (Osiewacz et al., 2013), but these not particularly suitable for transcriptomic analysis. Senescence can be delayed by the use of fresh ascospores (Osiewacz et al., 2013) but these are complex, tedious and time-consuming to obtain. Calorie restriction (van Diepeningen et al., 2010) results in a type of thin and unhealthy mycelium which is generally not used in transfer experiment for physiology studies. Senescence-delaying mutants exist (Borghouts et al., 2001; Esser and Keller, 1976; Maas et al., 2004; Osiewacz and Nuber, 1996; Scheckhuber and Osiewacz, 2008), but the use of wild type strains is required to study the natural response of the fungus. We screened a range of pre-culture media for delayed senescence but with sufficiently healthy mycelia to be used for transcriptomic analysis. Here we present a novel and simple pre-culturing method to delay senescence and tested it for transfer cultures grown on plant biomass.

Methods, results and discussion.

*P. anserina* strain S mat+ (maintained on Corn Meal Agar (CMA) (Borghouts et al., 2001) (Table S1) plates stored at 4 °C after 2–3 d incubation at 27 °C) was tested on different media (Fig. 1) (Table S1 describes the media used), which were either among the most commonly used media for microbiological culturing or previously used for *P. anserina*. Duplicate plates inoculated with 5 mm mycelium plugs from fresh CMA plates were incubated at 27 °C for 5 d, and then serially sub-cultured seven times or until senescence appeared as black mycelium. Each medium was also tested with a polycarbonate (PC) membrane (diameter, 76 mm; pore size, 0.1 µm; Osmonics, GE Water Technologies, Trevose, PA), which can reduce the availability of nutrients and facilitates transfer of mycelial plugs without agar to liquid cultures. Only three of the media and PC membrane combinations delayed senescence: CMA + membrane, M2 + 0.02% D-fructose ( ± membrane) and Luria-Bertani agar (LA) ( ± membrane). Mycelium on CMA + membrane only delayed senescence for two sub-cultures whereas in the other two media ( ± membrane) senescence was delayed for all the seven sub-cultures tested. However, M2 + 0.02% D-fructose resulted in thin mycelium while in LA there was healthy growth and the Luria-Bertani medium was selected for further analysis.

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To our knowledge, there are no reports of other media that have shown such healthy growth without senescence as we obtained on LA.

To set-up liquid pre-cultures for transcriptomics analysis without senescence, Luria-Bertani Broth (LB) was tested and compared with the commonly used liquid minimal medium M2 (Espagne et al., 2008) and RNA from cultures was extracted as described previously (Klaubauf et al., 2016). Mycelium from a LA plate + membrane (incubated for 5 d at 27 °C) was harvested, diced to ~1 mm pieces, and used to inoculate 50 mL of LB or liquid M2 in 300 mL Erlenmeyer flasks (in triplicate) were incubated in a rotatory shaker (Infors HT Multitron Standard) at 27 °C, 120 rpm for 3 d (Fig. 2). LB cultures did not show any senescence, in contrast to liquid M2, and the mycelium appeared healthy (Fig. 2). LB broth was selected to use for transfer experiments. Static liquid LB cultures were also tested, but resulted in pellet-like mycelium (data not shown) instead of the dispersed mycelium from the shaking LB cultures. 2.5 ± 0.1 g of wet mycelium from LB pre-cultures was transferred to separate 250 mL flasks with either corn stover or soybean hulls at 1% concentration in 50 mL of M2, incubated at 120 RPM at 27 °C for 4 h, 24 h and 48 h. A total of 18 RNA samples were extracted and analyzed for quality, showing high integrity (determined using Agilent 2100 BioAnalyser) (Fig. 3) and suitability for transcriptomics (Benocci et al., 2018, unpublished results). Also of interest, genomic DNA could be easily extracted (Petrisko et al., 2008) from the LB pre-cultures which was of high quality suitable for genome sequencing (Fig. S1). For purity determinations of the nucleic acids, a Nanodrop ND-1000 UV–Vis Spectrophotometer (Thermo Scientific) was used to measure absorbance ratios at 260 nm/280 nm and 260 nm/230 nm. Carbon limitation may be an important feature of LB to avoid senescence in *P. anserina* cultures as previously suggested for other media (Maas et al., 2004; van Diepeningen et al., 2010). Sezonov et al. (Sezonov et al., 2007) showed that LB has low concentration of sugars, forcing *Escherichia coli* to catabolize the amino acids, suggesting a similar mechanism in *P. anserina*.

In conclusion LA/LB can delay senescence, prolonging the health of the mycelium. In addition it allowed us to extract good quality RNA (Fig. 3) from plant biomass cultures suitable for transcriptomics. LA/LB media should also be useful to perform transcriptomics of transfer experiments to other physiological conditions with *P. anserina* wild type

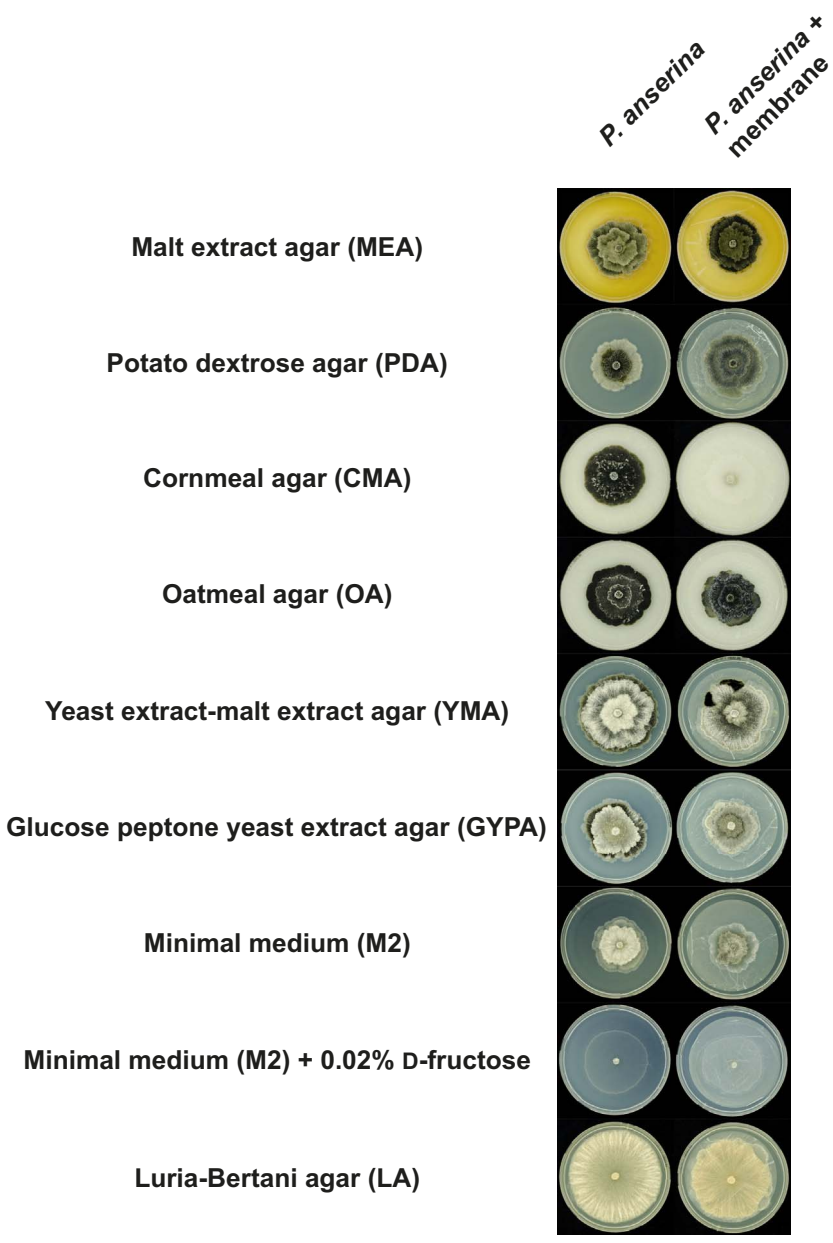


**Fig. 1.** Growth on solid media at the first sub-culture. Senescence, displayed as black mycelium, was observed in all media with the exception of CMA + membrane, M2 + 0.02% D-fructose ( ± membrane) and LA ( ± membrane). CMA + membrane at the first sub-culture did not show any senescence, but it appeared after serially sub-culturing twice more (not shown). M2 + 0.02% D-fructose resulted in thin mycelium, while mycelium in LA was healthy. Note that "Minimal medium (M2)" contained 0.55% of fructose.