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Patterns of utilization of different carbon sources by Chytridiomycota

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Abstract A phylogenetically diverse set of seventeen isolates of Chytridiomycota were selected for a study of the utilization of common carbohydrates as sole carbon sources in synthetic media. *Rhizophlyctis rosea* AUS 13 is capable of the digestion of crystalline cellulose in the form of lens paper, filter paper and powdered filter paper and grows well with noncrystaline carboxymethyl cellulose or cellobiose, but cannot use starch or maltose as sole carbon sources in liquid and on solid media. None of the other sixteen isolates tested can digest crystalline cellulose, but all grow well on starch and maltose and several can also use cellobiose and/or sucrose as a sole carbon source. Four of the other sixteen isolates could also digest carboxymethyl cellulose slowly. Glucose is an excellent sole source of carbon in synthetic media for all seventeen isolates in the present study. In general, these data suggest variability in the ability of zoosporic true fungi to use carbohydrates other than glucose as sole sources of carbon. Four patterns of carbohydrate utilization emerged from this study of seventeen isolates. *R. rosea* degrades cellulose over a relatively wide pH range which suggests that the cellulase enzymes are stable over a wide pH range.

Keywords Carbon sources · Cellulose · Starch · Sucrose · Decomposition · pH · Chytrids

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Introduction

Zoosporic true fungi (including both Chytridiomycota and Blastocladiomycota) have frequently been

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observed growing as saprobes on many substrates in freshwater and soil ecosystems (Sparrow, 1960; Shearer et al., 2007). Descriptive studies designed to explore the roles of zoosporic true fungi in decomposition of organic matter from vascular plants have often suggested that many species of zoosporic true fungi are capable of growth on substrates containing cellulose along with other common carbohydrates. For example, Haskins (1939) concluded that the majority of zoosporic true fungi can grow on pollen grains, fruits, wood, roots and leaves of vascular plants. Sparrow (1960) provided a long list of plant parts which provide carbon sources for the growth of many species of zoosporic true fungi and which presumably contain complex mixtures of carbohydrates, including cellulose, hemicelluloses, pectin, starch and sucrose. Baits containing cellulose such as dried grass leaves, onion skin, filter paper, lens paper and cellophane are commonly used to capture zoosporic true fungi (Couch, 1939; Haskins, 1939; Willoughby, 1962; Dogma, 1969; Barr, 1987, 2001). However, the roles of zoosporic true fungi in the process of decomposition of carbohydrates in these substrates remain to be carefully investigated.

In preliminary studies with cellulosic baits, Haskins (1939) observed the growth of pure cultures of eight species of zoosporic true fungi on lens paper and cellophane: *Rhizophlyctis petersinii*, *Nowakowskiella elegans*, *Nowakowskiella* sp., *Septochytrium variabile*, *Catenochytridium carolinianum*, *Nowakowskiella ramosa* and *Cladochytrium replicatum*. Two years later, Whiffen (1941) carefully demonstrated cellulose decomposition using quantitative methods in three isolates of *Rhizophlyctis rosea* and in one isolate in each of six other genera: *Entophlyctis* sp., *Endochytrium operculatum*, *Nephrochytrium aurantium*, *Cylindrochytrium johnstonii*, *Nowakowskiella elegans* and *Septochytrium variabile*. When Whiffen (1941) reviewed the early research on cellulose decomposition, she proposed two possible roles for zoosporic true fungi in mixed cultures: these fungi are capable of either (1) directly utilizing cellulose as a carbon source or (2) indirectly through the action of other microbes. Whiffen (1941) hoped that the roles of these fungi in decomposition could be clarified by further research in the laboratory with pure cultures using techniques first developed by Couch (1939).

The roles of *R. rosea* in cellulose decomposition were studied further by Haskins & Weston (1950) using quantitative methods. Although many additional studies on the nutrition of zoosporic true fungi have been conducted in the laboratory since then, very little new information on cellulose decomposition has been provided. In fact, isolates from many species including some closely related to the ones tested by Haskins (1939) and Whiffen (1941) have been subsequently found to be unable to digest cellulose (Ajello, 1948; Goldstein, 1960; Willoughby, 1962; Barr, 1969, 1970; Hasija & Miller, 1971: Barr, 1984; Hassan & Catapane, 2000). Furthermore, these fungi were tested for ability to use cellulose as a carbon source using many different experimental protocols some of which did not include quantitative methods. Nonetheless, currently few species of zoosporic true fungi are thought to be able to digest crystalline cellulose.

The few zoosporic true fungi which are thought to be able to decompose cellulose are currently placed in two orders: Cladochytriales and Rhizophlyctidales (Phylum Chytridiomycota, Kingdom Fungi) (Letcher et al., 2008; Mozley-Standridge et al., 2009). The Chytridiomycota include a very large number of phylogenetically diverse species (Sparrow, 1960; Shearer et al., 2007; Letcher et al., 2008). Most species of zoosporic true fungi in freshwater and soil ecosystems are saprobes (Sparrow, 1960), and almost all are obligate aerobes (Gleason et al., 2007). These fungi have access to complex carbohydrates such as cellulose, but utilization of this common source of energy remains to be firmly established in a range of taxa.

In contrast, the decomposition of plant tissues especially fibre in the digestive systems of herbivorous mammals by obligately anaerobic rumen fungi (Phylum Neocallimastigales, Kingdom Fungi) is well understood (Joblin, 1989; Akin & Borneman, 1990; Trinci et al., 1994; Rezaeian et al., 2004). All known species in the six genera of rumen fungi actively decompose cellulose. The pathways of carbohydrate metabolism have been most extensively studied in *Neocallimastix frontalis* (Orpin & Letcher, 1979; Trinci et al., 1994; Kown et al., 2009). Our knowledge of the ecology of rumen fungi may provide clues to our understanding of the roles of saprophytic zoosporic fungi growing in aquatic and soil ecosystems.

The aims of this study were to (1) determine which isolates in a genetically diverse sample of Chytridiomycota are capable of the use of cellulose as a sole source of carbon; (2) test major storage constituents

of plants such as starch and sucrose for potential use by these fungi as carbon sources; (3) study of the range of pH over which *R. rosea* actively decomposes cellulose; (4) putatively confirm the identity of the genera studied using rDNA sequences; and (5) achieve some insights into the role and mechanisms of cellulose decomposition by zoosporic true fungi in natural ecosystems.

Materials and methods

Selection and maintenance of isolates

Pure cultures of seventeen isolates of Chytridiomycota were selected for this study (Table 1). Data on the sources of these isolates is provided by Letcher et al. (2004), Commandeur et al. (2005), Gleason et al. (2007, 2010) and Digby et al. (2010). Partial sequences of the 18S, 28S and ITS1-5.8S-ITS2 ribosomal genes were previously recorded in Genbank for some of the isolates in this study (Letcher et al., 2004, 2006, 2008; Wakefield et al., 2010; Table 1). Prior to depositing cultures in the NITE culture collection in Japan, partial sequences of ribosomal genes were obtained for all of the isolates. These fungi are representatives of five orders in the Chytridiomycota. All of these fungi were maintained on solid PYG agar containing peptone 1.25 g, yeast extract 1.25 g, glucose 3 g and agar 20 g l^{-1}. Barr (1987) recommended the use of solid YpSs agar for the maintenance of all zoosporic fungi. However, we found that *R. rosea* (AUS 13) does not grow well on YpSs agar in contrast to the other isolates in our collection. Thus, PYG medium was used instead of YpSs (soluble starch rather than glucose is the primary source of carbohydrate in YpSs agar).

Confirmation of identity

DNA isolation and PCR amplification

For DNA isolation, cultures were incubated on PYG agar plates for 1–2 weeks at 20°C. Total DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturers' instructions. The small subunit ribosomal DNA (18S rDNA) gene and D1 and D2 domains of the large subunit ribosomal DNA (28S rDNA D1/D2) gene sequences were amplified by polymerase chain reaction (PCR). The PCR were performed in 50 µl reaction volumes containing 23.5 µl of distilled water, 1 µl of KOD-Plus (Toyobo, Oosaka, Japan), 5 µl of 10× PCR buffer for KOD-Plus, 5 µl of dNTP, 4 µl of 25 mM $MgSO_4$, 0.75 µl of each primer (10 pmol/µl), and 10 µl of the extracted DNA as a template. Primer sets used were 18-F (5′-ATCTGGTTGATCCTGCCAGT-3′) and 18-R (5′-GATCCTTCCGCAGGTTCACC-3′) (Ueda-Nishimura & Mikat, 1999) for 18S rDNA, and NL1 (5′-GCATA TCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGT CCGTGTTTCAAGACGG-3′) (O'Donnell, 1993) for 28S rDNA D1/D2. Amplification was conducted in a GeneAmp PCR System 9700 (Applied Biosystems, Foster, CA, USA) under the following conditions: 18F and 18R—an initial denaturation at 94°C for 2 min, 30 cycles at 94°C for 15 s, 60°C for 30 s, and 68°C for 1.5 min, and the final extension period at 68°C for 10 min; NL1 and NS4—an initial denaturation at 94°C for 2 min, 30 cycles at 94°C for 15 s, 60°C for 30 s, and 68°C for 1.5 min, and the final extension period at 68°C for 10 min. Then, the amplified DNA was purified using Agencourt AMPure (Agencourt Bioscinece, Beverly, MA, USA) according to the manufacturer's instructions.

Sequencing

Sequencing reactions were conducted using a BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) following the manufacturer's instructions. For sequencing 18S rDNA in both directions, the primers 18-F, NS2 (5′-GGCT GCTGGCACCAGTCATGC-3′), NS3 (5′-GCAAGT CTGGTGCCAGCAGCC-3′), NS4 (5′-CTTCCGTCA ATTCCTTTAAG-3′), NS5 (5′-AACTTAAAGGAA TTGACGGA AG-3′), NS6 (5′-GCATCACAGACCT GTTATTGCCTC-3′), NS7 (5′-GAGGCAATAACA GGTCTGTGATGC-3′) and 18-R were used (White et al., 1990). Similarly, for the D1/D2 domains of 28S rDNA in both directions, the primers NL1 and NL4 (O'Donnell, 1993) were used. Sequencing reactions were conducted using BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) following the manufacturer's instructions with Biometra T-Gradient Cycler (Biometra, Göttingen, Germany), and the products were purified with CleanSEQ (Agencourt Bioscience). Sequences were analyzed with the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Table 1 Identity of the isolates of zoosporic true fungi analysed

Fungus name and isolate	NBRC number	Order	Accession numbers	Isolation substrate	Soil type
Terramyces sp. AUS 3	105431	R	AY439045 DQ485633	Pine pollen	Wet sclerophyll forest
Boothiomyces sp. AUS 6	105432	R	AY439046 DQ485634	Onion skin	Wet sclerophyll forest
Boothiomyces sp. AUS 7	105433	R	AY439047 DQ485635	Pine pollen	Open heath
Boothiomyces sp. AUS 8	105434	R	AY439048 DQ435636	Pine pollen	Dry sclerophyllforest
Boothiomyces sp. AUS 9	105435	R	AY439049 DQ485637	Snake skin	Mud from pond
Cladochytrium hyalinum AUS 11	105429	L	NA	Onion skin	Mountain forest
Rhizophlyctis rosea AUS 13	105426	T	EU379156 EU379199	Filter paper	University garden
Chytriomyces hyalinus AUS 14	105430	C	NA	Insect exoskeleton	Wet sclerophyll forest
Powellomyces sp. AUS 16	105427	S	NA	Pine pollen	Dry sclerophyll forest
Powellomyces sp. AUS 17	105428	S	FJ827635 FJ827671 FJ827709	Insect exoskeleton	Rain forest
Rhizophydium sp. Mar Ad 14	105437	R	DQ485570 DQ485647	Pine pollen	Fallow crop
Spizellomyces sp. Mar Ad 2-0	105424	S	FJ827669 FJ827691 FJ827727	Pine pollen	Fallow crop
Spizellomyces sp. Dec CC 4-10Z	105423	S	NA	Pine pollen	Crop
Spizellomyces sp. AVM1	106283	S	NA	Lens paper	Crop
Gaertneriomyces semiglobifer Mar C/C2	105425	S	FJ827645 FJ827701 FJ827738	Pine pollen	Crop

C Chytridiales, *L* Cladochytriales, *T* Rhizophlyctidales, *R* Rhizophydiales, *S* Spizellomycetales, *NA* data not available

Composition of growth media

Synthetic media

The basal CSM medium (chytrid synthetic media) contained macronutrients: $(NH_4)_2SO_4$ 200 mg, K_2HPO_4 600 mg, $Mg(NO_3)_2$ 200 mg, $CaCl_2{\cdot}2H_2O$ 50 mg, FeEDTA 500 μg and thiamine 133 μg l^{-1} and trace elements: $MnCl_2{\cdot}4H_2O$ 10 μM, $ZnCl_2$ 10 μM, H_3BO_4 33 μM, $CuSO_4{\cdot}5H_2O$ 1 μM, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.2 μM. In some media the mineral base was supplemented with a carbon source at 5 g l^{-1}: potato starch, powdered filter paper, carboxymethyl cellulose (sodium salt), cellobiose, maltose, or sucrose. Media were solidified by the addition of 20 g l^{-1} of agar, and 25 ml of solid media were placed into Petri dishes and 25 ml of liquid media into plastic centrifuge tubes.

Complex media

In experiments designed to test the effect of pH on the growth of *R. rosea* (AUS 13) the liquid media contained glucose or carboxymethyl cellulose 3.0 g l^{-1}, yeast extract 1.25 g l^{-1}, peptone 1.25 g l^{-1} and $MgSO_4{\cdot}7H_2O$ 0.12 g l^{-1}. Buffers were added to the media to set the pH after sterilization and prior to solidification. The chemical composition of the buffers is described in Gleason et al. (2010).

Inoculation and measurement of growth

The inocula for both synthetic and complex solid media consisted of zoospores, zoospore cysts and thalli obtained from cultures growing on PYG agar. In experiments designed to test the carbon sources with all isolates on solid synthetic media, the inocula were spread over the surface of the agar. In experiments designed to assess the response of AUS 13 to pH on solid synthetic basal media, 25 µl of a suspension of zoospores, zoospore cysts and thalli were placed on squares of Whatman lens paper (pure cellulose). The cultures were incubated at 20°C for 2 weeks. On solid media growth of all isolates was determined visually as an increase in the number of newly produced thalli and the size of the colonies.

The growth of 13 monocentric isolates was tested in liquid chytrid synthetic medium (CSM) basal media supplemented with maltose, cellobiose, or sucrose as carbon sources and in a control without carbon (Table 1). In addition, the growth of AUS 13 was tested on liquid synthetic media supplemented with either carboxymethyl cellulose or potato starch. The other isolates which grew well on cellobiose were also tested on carboxymethyl cellulose. Suspensions of zoospores, zoospore cysts and thalli were prepared by flooding Petri dishes containing 25 ml of PYG agar with 8 ml of sterile de-ionized (DI) water. After 1–2 h, 50-ml plastic centrifuge tubes containing 25 ml of media were inoculated with 200 µl of these suspensions. Five replicates of each of the cultures were grown on a Ratek orbital shaker (Model EOM5) (Digby et al., 2010; Gleason et al., 2010). After 2 weeks at 20°C, the cultures were centrifuged (830 g for 10 min at 4°C in a Sorvall Super T21 centrifuge). The pellet was washed with 25 ml of DI water, centrifuged again, and the pellet was placed into pre-weighed aluminium dishes. The thalli were oven-dried at 80°C until constant weight (24 h) and weighed (±0.1 mg). Cultures on solid PYG medium served as controls for viability of the inocula. In liquid media growth was measured as an increase in dry mass.

Experimental procedure for visual observation of growth on lens paper

Sterile squares of Whatman No. 1 lens paper (pure cellulose) were chosen as a substrate because it is easy to see thalli growing among and under the fibres with the light microscope at 10× magnification, they allow rapid entry of oxygen by diffusion from the air and they retain moisture relatively well for at least 2 weeks. Four squares with the sides measuring approximately 4–8 mm were placed on solid basal CSM medium without an additional carbon source. One or two 25-µl drops of DI water with or without inocula were added in order to wet the lens paper. Observations were made daily after incubation at 20°C for 14 days. This procedure is similar to the one used by Haskins (1939).

Statistical analysis

One-way analysis of variance (ANOVA) was employed to explore differences in: (a) the growth of *R. rosea* (AUS 13) at different pH values in liquid media containing glucose or carboxymethyl cellulose, and (b) the growth of each isolate in liquid synthetic CSM basal media supplemented with different carbon sources (Zar, 1996). When differences were significant ($P < 0.001$), Tukey's test [performed with XLSTAT, version 2009; (Dunn, 1961)] was used to determine where the differences occurred.

Results

Confirmation of identity

After comparison with other known sequences for ribosomal genes in Genbank using the BLAST search program, the identity of all isolates was putatively confirmed at the generic level (Table 1).

Growth on solid media

In all isolates except AUS 13, the zoospores settled on and attached to all surfaces approximately at random, including the fibres of the lens paper and the surface of the solid basal synthetic agar medium. However, at the edges of the water drops a concentrated ring of zoospores developed into thalli with long rhizoids extending outward. All stages of the life cycle were present including zoospores actively swimming throughout the water drops after 14 days both in the presence and absence of lens paper (the control). Many zoospores of AUS 13 settled on the

cellulose fibres and developed into mature thalli which digested the fibres substantially altering their physical structure. There was no evidence that any of the other isolates digested the fibres or visibly altered their physical structure and therefore we assume that the only carbon sources available to these isolates were endogenous reserves.

On the solid basal synthetic agar media with lens paper at different pH values, the fibres became intensely red with large numbers of attached thalli of AUS 13 after 7 days at pH 4.7, 5.5, 6.7, 7.6 and 9.0. Growth was evident at pH 9.3 after 14 days, but no growth was observed at pH 2.8, 3.3 or 11.0.

All of the monocentric isolates except AUS 13 grew well on solid synthetic media containing starch or maltose as carbon sources. AUS 13 grew well on the media containing powdered filter paper and carboxymethyl cellulose. It was not possible to accurately assess the growth of some of the monocentric isolates on sucrose, cellobiose and carboxymethyl cellulose on solid media. Therefore, this method was abandoned. In contrast the growth of the polycentric isolate AUS 11 could be assessed on solid media by examining the diameter of the colonies. The diameters of the colonies increased on starch, maltose, cellobiose, and carboxymethyl cellulose but not on sucrose.

Growth in liquid media

All of the isolates except AUS 13 grew well on maltose. AUS 13 grew well only on cellobiose and carboxymethyl cellulose and did not grow at all on starch or maltose. Some of the other isolates grew well on cellobiose and/or sucrose, but in general in this sample of fungi the growth was variable on these substrates (Fig. 1). A small amount of growth was observed on carboxymethyl cellulose with AUS 3, AUS 14 and Mar Ad14 (Fig. 2), whereas AUS 6, AUS 9, Mar AD 2–0 and AVM1 did not grow on this substrate.

The pH range for growth of AUS 13

AUS 13 grew well on both glucose and carboxymethyl cellulose as carbon sources in liquid complex media. pH 4.7, 5.5, 6.7 and 7.6 allowed good growth on both substrates. However, significantly greater growth was observed at pH 6.7 and 7.6 than at pH 4.7 and 5.5 on both glucose and carboxymethyl cellulose ($P < 0.001$). Poor growth was observed at extreme pH values (Fig. 3).

Discussion

Rhizophlyctis rosea AUS 13 was isolated from filter paper baits. This species is capable of the digestion of crystalline cellulose in the form of lens paper, filter paper and powdered filter paper. In the present study we also found that AUS 13 grows well with noncrystaline carboxymethyl cellulose or cellobiose but not with starch or maltose as carbon sources in liquid and on solid media. Similar results were obtained in previous studies with other isolates of this fungus (Haskins, 1939; Whiffen, 1941; Stanier, 1942; Letcher et al., 2008), although in some cases, quantitative estimations were not made. This study is the first report of the growth of *R. rosea* on carboxymethyl cellulose suggesting that potentially many of the genotypes of this fungus have adapted to the utilization of both crystalline and noncrystalline cellulose in plant matter.

Many of the other zoosporic true fungi included in this study were isolated into pure culture from pine pollen grain and onion skin baits which contain cellulose as well as other carbohydrates. However, none of these isolates digested crystalline cellulose (in lens paper), though all grew well on starch and maltose. Several of these isolates can also use cellobiose and/or sucrose as a carbon source. AUS 11, AUS 3, AUS 14 and Mar Ad 14 also digested carboxymethyl cellulose slowly. This study represents the first report of the growth of these genera in the Chytridiomycota on carboxymethyl cellulose. Since the conditions for growth were identical for all isolates, qualitative comparisons between species were possible.

Willoughby (1962) found that *Cladochytrium replicatum* could grow on grass leaves but not on pure cellulose baits in lake ecosystems and was unable to digest cellulose in pure culture. Our isolate of *Cladochytrium hyalinum* (AUS 11) digested carboxymethyl cellulose in pure culture which is a noncrystalline and soluble form of cellulose. We isolated *Spizellomyces* sp. (AVM1) on filter paper from soil (unpublished data), but this isolate could not digest filter paper in pure culture. Couch (1939) also observed that a number of species could grow on

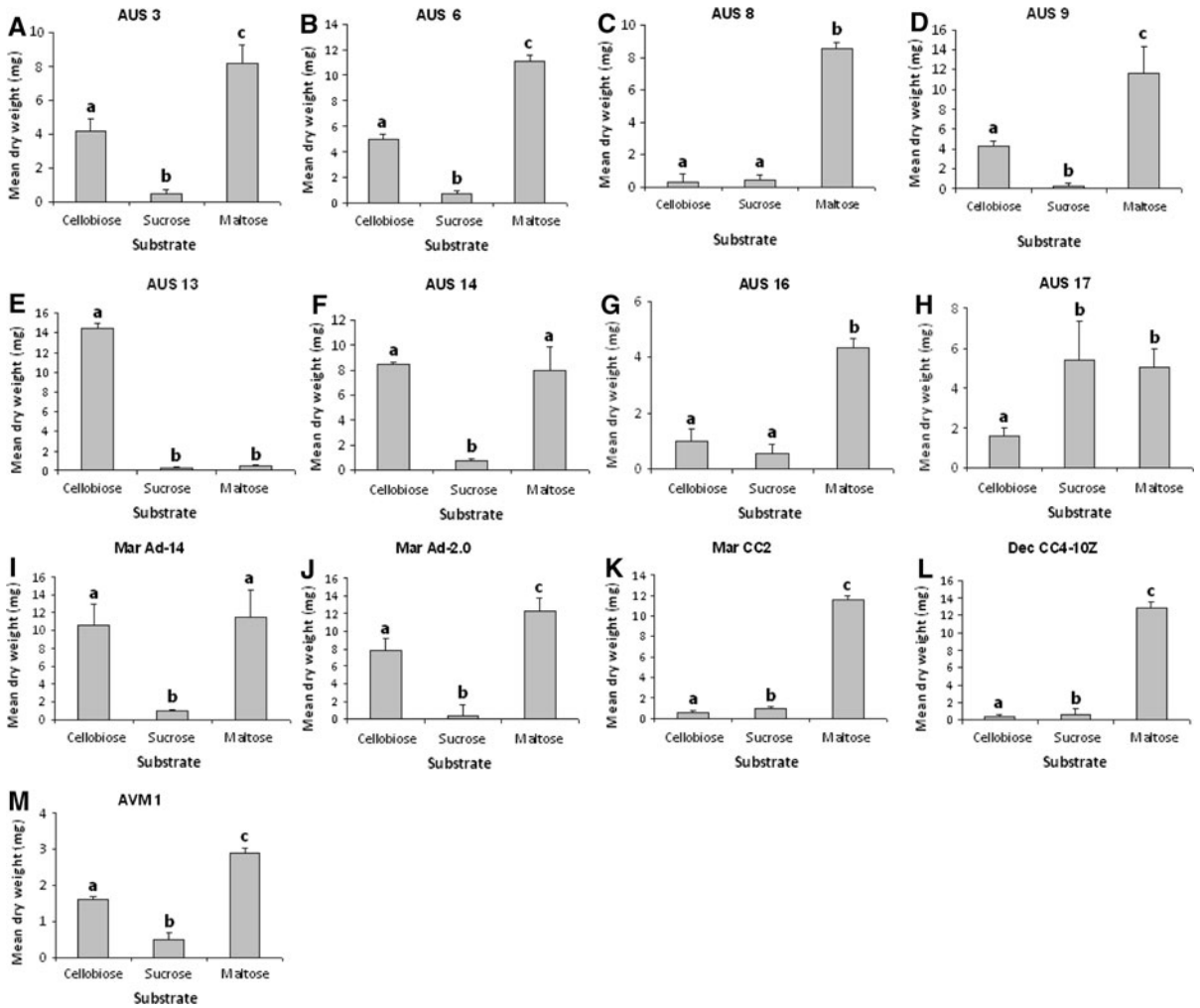

Fig. 1 Growth of 13 monocentric isolates on liquid synthetic media with maltose, cellobiose and sucrose. Growth is expressed as mean dry weight. The *same letters above the bars* showed that the values do not differ significantly ($P > 0.001$)

filter paper in mixed cultures, but they were unable to grow in pure culture on this substrate.

Cellobiose and maltose are known to be products of the extracellular digestion of cellulose and starch, respectively, and released into the environment by this process. Therefore, we would expect that fungi which degrade cellulose and starch to be able to use cellobiose and maltose, respectively, as carbon sources. However, cellobiose and maltose released by the digestion of cellulose and starch by other microbes in mixed cultures might also be absorbed and used by zoosporic true fungi. We would expect starch reserves in dead plant tissues to be excellent substrates for some saprophytic zoosporic true fungi. Furthermore, it is possible that some of these fungi may be able to use sucrose stored in plant tissues. In this study, we observed significant differences in the abilities of zoosporic fungi to use cellobiose and sucrose as carbon sources. This was previously observed in some species of *Spizellomyces* by Barr (1984), although dry weights were not provided.

The enzymatic digestion of cellobiose as well as noncrystalline and crystalline cellulose, all of which have β-1,4-glycoside bonds, is necessary for complete digestion of plant cell walls. Only *R. rosea* AUS 13 digests all of these substrates. Our data indicates a partial mechanism for digestion of cellulose in some of the other isolates tested in the present study, but the amount of cellulose digested may be too small to allow these zoosporic fungi to compete with other

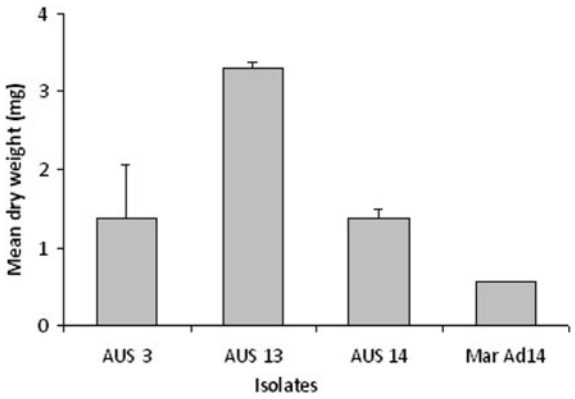

Fig. 2 Growth of AUS 13, AUS 14, AUS 3 and Mar Ad14 on carboxymethyl cellulose. Growth is expressed as mean dry weight

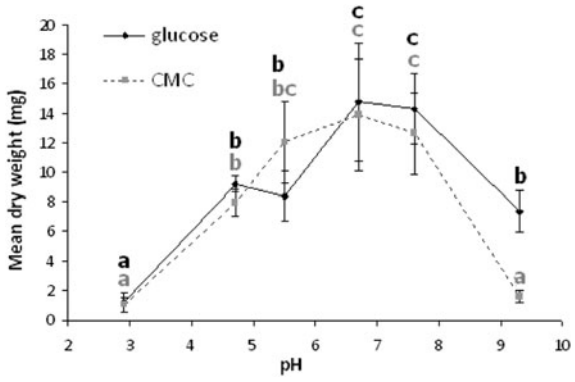

Fig. 3 Growth of *Rhizophlyctis rosea* (AUS 13) in liquid complex media at different pH values using glucose and carboxymethyl cellulose, as carbon sources. Growth is expressed as mean dry weight. The *same letters above the bars* showed that the values do not differ significantly ($P > 0.001$)

microbes which rapidly digest cellulose. However, it is possible that the release of small amounts of cellulase enzymes may soften plant cell walls in order to facilitate penetration of rhizoids into the cytoplasm of plant cells, so that they can reach stored carbohydrates more easily. Although *R. rosea* appears to synthesize and release cellulases (Haskins & Weston, 1950), nothing is known about the specificity of these enzymes.

Glucose is an excellent source of carbon in synthetic media for all isolates selected for the present study (Digby et al., 2010). Significant variability in the ability of zoosporic true fungi to use carbohydrates other than glucose as sole sources of carbon is indicated from this study. Four patterns emerged among the isolates selected for this study: (1) *R. rosea* digests crystalline cellulose, (2) the other fungi show a strong preference for starch, (3) a few of these fungi can use cellobiose and carboxymethyl cellulose (a partial mechanism for cellulose digestion) and (4) some of these isolates can use sucrose. In general, cellobiose and sucrose are poor substrates. Since zoosporic fungi have different patterns of carbohydrate metabolism, these species might play different roles in the digestion of plant materials in natural ecosystems. The relationship of the ability of zoosporic fungi to grow on plant materials in natural ecosystems and the ability to grow on carbohydrates in pure culture needs further clarification.

Rhizophlyctis rosea degrades cellulose over a relatively wide pH range. We observed that the pH ranges for the use of glucose, filter paper and carboxymethyl cellulose as carbon sources are similar. This suggests that the extracellular enzymes necessary for the digestion of cellulose are relatively stable with a wide range of pH values. Slow growth at extremes of pH (2.8 and 9.3) probably insures survival, but the rates of growth at these pH values may not be sufficient to compete with other extremophiles. The use of carboxymethyl cellulose allows quantitative growth studies on cellulose degradation because it is soluble as opposed to crystalline cellulose which is insoluble in water. Dry weights are difficult to obtain when insoluble carbon sources are present in the media.

The initial response to the lack of exogenous usable nutrients is the continuation of the life cycle at the expense of whatever endogenous or exogenous resources are available. This phenomenon was previously observed by Lilje & Lilje (2008) and Gleason & Lilje (2009). This may improve the chances that zoospores will encounter usable substrates, but this is not to be considered as net new growth.

Single genotypes of zoosporic fungi have never been found in the absence of other microbes in natural ecosystems. The information available at present on the decomposition of cellulosic materials by members of the Chytridiomycota suggests that very few species are capable of decomposing crystalline cellulose as a sole source of carbon in pure culture. However, research on pure cultures only indicates the potential nutritional capacities of individual species and does not take into account the

complexity of interactions between species and the environment. Cellulose decomposition by these fungi in the presence of other species of fungi, protists and bacteria in microbial communities may be significant. Couch (1939) suggested that the presence of bacteria was necessary for the growth of some species of zoosporic fungi on cellulose. Ultimately, a thorough understanding of the role of zoosporic true fungi in carbohydrate metabolism will require stepwise research using physiological, biochemical, molecular and morphological approaches. Metagenomics currently provides tools to facilitate the analysis of genomic heterogeneity in environmental contexts with uncultured microbes (Handelsman, 2004). Analysis of functional genes involved in carbohydrate metabolism has presently begun with one isolate of *Neocallimastix* (Kown et al., 2009). These data apply to the rumen ecosystem but similar studies need to be conducted in other ecosystems.

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