Growth and dimorphism of the mycoparasite *Tremella encephala* as affected by different nitrogen and carbon sources and the host presence

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**Abstract** – *Tremella encephala* is a haustorial biotrophic fungal parasite which grows both in the yeast and mycelial form during its life cycle. Biology of such non-commercial parasitic and dimorphic fungi is poorly known. We examined the effects of different nitrogen and carbon sources on growth and morphogenetic switch in *T. encephala*, as well as its host-specificity, host-parasite interaction and the effect of media on parasitism by culturing it alone, together with the known host *Stereum sanguinolentum* and with the possible host *Stereum hirsutum* on two different media. *Tremella encephala* utilized many organic and inorganic nitrogen sources and showed high variation in cellular nitrogen concentrations. Only glucose, glycerol and D-mannitol were exploited as the sole sources of carbon. Remarkable variation in dimorphism was observed between and within the strains. Parasitic interaction was not manifested in this laboratory study. *Tremella encephala* is not a strictly obligate parasite. Under laboratory conditions it grew prevalingly in the yeast form which may be more common in nature than currently known.

**Basidiomycota / biotrophy / fungal dimorphism / mycoparasitism / Tremellales**

**INTRODUCTION**

Biotrophic mycoparasites are fungi that parasitize other fungi. These fungi obtain nutrients from living cells of host fungi by means of special cells or branches (Barnett & Binder, 1973), such as haustoria (Barnett & Binder, 1973; Jeffries & Young, 1994). In the order Tremellales haustoria consist of a basal cell and one or a few haustorial filaments (e.g. Bandoni, 1961; Olive, 1946; Zugmaier & Oberwinkler, 1995) which penetrate outer layers of the host cell wall (Zugmaier et al., 1994; Zugmaier & Oberwinkler, 1995). Because biotrophs must transport nutrients through a complex interface, they absorb mainly amino acids and simple sugars (Hall & Williams, 2000; Jeffries & Young, 1994). They live in close contact with their hosts, and the relationship is physiologically stable: the biotroph is

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typically host-specific and causes only minor damage to the host over a long period of time (Jeffries, 1995). Taking into account that only few organisms do not have parasites (Begon et al., 1990) or any association with fungi (Jeffries & Young, 1994), mycoparasitism cannot be rare. In nature microbial competition and nutrient limitation favour characteristics advantageous in antagonistic interactions, and mycoparasitism is a powerful strategy in this respect (Jeffries & Young, 1994).

Fungal dimorphism, a common phenomenon over a wide range of taxonomic groups, includes – amongst others – the ability to grow as single cell yeasts or as multicellular mycelia in response to environmental conditions. Temperature, O₂ content, pH, nutrients, properties of the substrate, host vicinity or some other factor either alone or in combination may affect the dimorphic switch (e.g. Bandoni, 1961; Csank & Haynes, 2000; Fenwick, 1995; Gimeno et al., 1992; Pérez-Campo & Domínguez, 2001; Ruiz-Herrera et al., 1995; Ruiz-Herrera & Sentandreu, 2002; Sánchez-Martínez & Pérez-Martín, 2001; Zaragoza & Gancedo, 2000). Yeast colonies can grow as far as environmental conditions are favourable (Oberwinkler, 1987) and simple sugars are available (do Carmo-Sousa, 1969; Lachance & Starmer, 1999), but morphogenetic switch to mycelial growth form may be beneficial e.g. in colonisation of fluctuating environments and parasitism (Oberwinkler, 1987). Many dimorphic fungi parasitize animals, plants or fungi. For instance, the smut fungus Ustilago maydis (DC.) Corda is saprobic in the yeast form, but infects the host plant as mycelium (Gow, 1995; Ruiz-Herrera et al., 1995). In contrast, many human pathogens are infectious as yeasts (Gow, 1995). The yeast form is advantageous also in the insect-mediated colonization of trees and shrubs (Francke-Grosmann, 1939, 1967; Leufven, 1991; Rosa et al., 1994; Six, 2003; Whitney, 1982).

Species in the genus Tremella (Basidiomycota) are dimorphic and host-specific mycoparasites (Aptroot et al., 1997; Bandoni, 1987; Torkelsen, 1972, 1978, 1997) with Tremella encephala Pers.: Fr. being the first proven parasite. Bandoni (1961) demonstrated that the white firm core of the rose coloured fruit bodies is composed of hyphae of the host Sireum sanguinolentum (Alb. & Schwein.) Fr. to which haustoria of T. encephala are attached. Because species of Tremella have been successfully cultured in the absence of the host (e.g. Bandoni, 1961, 1963; Brough, 1974; Fenwick, 1995; Hanson & Wells, 1991), they cannot be strictly obligate parasites.

Biology of parasitic and dimorphic fungi is poorly known, and their significance in natural environments is probably underrated. Most of the studies dealing with nutrition, dimorphism or parasitism of such fungi were concerned with human and plant pathogens (e.g. Ernst, 2000; Ruiz-Herrera et al., 1995; Sánchez-Martínez & Pérez-Martín, 2001; Voegele & Mendgen, 2003) or species of economic interest (e.g. Gimeno et al., 1992; McIntyre et al., 2002; Ruiz-Herrera & Sentandreu, 2002; Zaragoza & Gancedo, 2000). There are only few detailed studies dealing with nutrition (Sampaio, 1999), dimorphism and/or compatibility (Bandoni, 1963; Brough, 1974; Fenwick, 1995; Hanson & Wells, 1991), and parasitism (Bandoni, 1961; Brough, 1974; Zugmaier et al., 1994; Zugmaier & Oberwinkler, 1995) of Tremella species.

As far as we know, this is the first study focusing on the nutrition and its effect on survival, growth, dimorphic switch, and parasitism in T. encephala. Specifically, we asked (1) whether different nitrogen (N) and carbon (C) sources and the host presence affect the growth and growth form of T. encephala, (2) whether T. encephala parasitizes only a single host species, (3) whether T. encephala has an effect on the growth of the host, and
(4) whether nutritional quality of the medium affects parasitism and host-specificity. These aspects were investigated under laboratory conditions in three experiments of which the nitrogen and carbon experiments concentrated on the effects of different N and C sources and the host-parasite experiment on the host-parasite interaction.

**MATERIALS AND METHODS**

We conducted three types of experiments: (1) culturing of *T. encephala* strains on media containing different N sources, (2) culturing of *T. encephala* strains on media containing different C sources, and (3) host-parasite experiment on two different media involving one well-established host *S. sanguinolentum* and the potential host *Stereum hirsutum* (Willd.) Pers.

**Species and strains**

*Tremella encephala* occurs in coniferous and mixed forests on conifers as a parasite of *S. sanguinolentum* which is a common, aphyloporoid decomposer of hard coniferous wood in the boreal zone (Eriksson et al., 1984; Strid, 1997). The related *S. hirsutum* decomposes deciduous (Eriksson et al., 1984; Strid, 1997) and occasionally coniferous wood (Eriksson et al., 1984) in the temperate zone (Jülich & Stalpers, 1980).

The strains of *T. encephala* (TE1, TE2, TE3), *S. sanguinolentum* (SS1, SS2, SS3), and *S. hirsutum* (SH1, SH2, SH3) that we used in our experiments originate from specimens collected in October 2004 and January 2005 in Oulu and Haukipudas, Northern Finland (65° 01’-65° 07’ N, 25° 25’-25° 27’ E). Each *T. encephala* strain was established from spore prints of two fruit bodies belonging to separate specimens. Instead, *Stereum* strains were established from a piece of a fruit body which was quickly rinsed in H$_2$O$_2$ or from mycelium in wood under the fruit body. All strains were freshly isolated prior to the experiments, and the ribosomal ITS region was sequenced at the Institute of Biotechnology (University of Helsinki, Finland). According to our blast comparison in EMBL Nucleotide Sequence Database – Fungi, the closest sequence homologs for all strains belong to the species that was expected. The specimens are deposited in the herbarium OULU and the strains in the Fungal Biotechnology Culture Collection (FBCC, Department of Applied Chemistry and Microbiology, University of Helsinki, Finland). The sequences were submitted to the NCBI GenBank. Collection numbers of the original specimens, FBCC codes of the strains, GenBank accession numbers, and the closest sequence homologs are shown in Table 1.

**Culture media**

All strains were maintained on the malt yeast peptone (MYP) medium (Bandoni 1972) with the exception that soytone was replaced by peptone (Sigma). They were initiated in dark at room temperature and stored at 4°C. The experiment media contained (per litre) a varying N or C source (N content 0.2 g, C content 2 g), CaCl$_2$ 0.05 g, MgSO$_4$ 0.15 g, NaCl 0.025 g,
FeCl$_3$$\cdot$6H$_2$O 0.05 g, thiamine 0.1 mg, agar 15 g, KH$_2$PO$_4$, and either glucose (the nitrogen experiment) or (NH$_4$)$_2$HPO$_4$ (the carbon and host-parasite experiment). The amounts of KH$_2$PO$_4$ and glucose (the nitrogen experiment) or (NH$_4$)$_2$HPO$_4$ (the carbon and host-parasite experiment) were determined by the N or C source to keep the N, C, and P (0.25 g/L) contents constant. Exceptionally in the carbon experiment the N content of the mixture of amino acids was higher (0.63 g/L) to achieve the accurate C content. In the nitrogen experiment the N source was either (1) mixture of amino acids, (2) the dipeptide glycyglycine (Merck), (3) casein enzymatic hydrolysate (CEH) (Sigma), (4) the protein bovine serum albumin (SAFC), (5) NaNO$_3$, (6) (NH$_4$)$_2$HPO$_4$, (7) NH$_4$NO$_3$, or (8) lacking (control). In the carbon experiment the C source was either (1) glucose, (2) glycerol, (3) D-mannitol (Riedel-de Haën), (4) sodium acetate (Merck), (5) mixture of amino acids, (6) tannic acid (Merck), (7) cellulose (Macherey-Nagel), (8) lignin (Department of Chemistry, University of Oulu, Finland), or (9) lacking (control). The mixture of amino acids contained L-asparagine, L-cysteine (Sigma), L-aspartic acid, L-glutamic acid, L-phenylalanine (Merck), L-leucin, L-threonine (British Drug Houses), L-metionin, L-valin (Fluka), and glycine (Riedel-de Haën). Every amino acid constituted an equal share of the total N (the nitrogen experiment) or C (the carbon experiment). The two media used in the host-parasite experiment were identical with the glucose and tannic acid media of the carbon experiment.

Prior to the addition of agar, the pH was adjusted to 5.6. Amino acids, dipeptide, CEH, protein, and tannic acid were filtered under sterile conditions into the autoclaved media and decanted into 9 cm diam. Petri dishes.

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Table 1. Collection numbers of the original specimens of which the strains of *Tremella encephala* (TE), *Sterile sanguinolentum* (SS), and *Sterile hirsutum* (SH) were established, FBCC codes of the strains, NCBI GenBank accession numbers, and the closest sequence homologs according to blast comparison in EMBL Nucleotide Sequence Database – Fungi.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Collection number(s)*</th>
<th>FBCC* code</th>
<th>GenBank accession number</th>
<th>Closest sequence homolog (identity, species)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE1</td>
<td>134 &amp; 143</td>
<td>FBCC1143</td>
<td>EU673081</td>
<td>AF410474 (98%, <em>T. encephala</em>)</td>
</tr>
<tr>
<td>TE2</td>
<td>177A &amp; 181</td>
<td>FBCC1144</td>
<td>EU673082</td>
<td>AF410474 (98%, <em>T. encephala</em>)</td>
</tr>
<tr>
<td>TE3</td>
<td>178 &amp; 179</td>
<td>FBCC1145</td>
<td>EU673083</td>
<td>AF410474 (98%, <em>T. encephala</em>)</td>
</tr>
<tr>
<td>SS1</td>
<td>123</td>
<td>FBCC1146</td>
<td>EU673084</td>
<td>AY618670 (99%, <em>S. sanguinolentum</em>)</td>
</tr>
<tr>
<td>SS2</td>
<td>153</td>
<td>FBCC1147</td>
<td>EU673085</td>
<td>AY618670 (96%, <em>S. sanguinolentum</em>)</td>
</tr>
<tr>
<td>SS3</td>
<td>175</td>
<td>FBCC1148</td>
<td>EU673086</td>
<td>AY618670 (99%, <em>S. sanguinolentum</em>)</td>
</tr>
<tr>
<td>SH1</td>
<td>205</td>
<td>FBCC1149</td>
<td>EU673087</td>
<td>AY854063 (100%, <em>S. hirsutum</em>)</td>
</tr>
<tr>
<td>SH2</td>
<td>211</td>
<td>FBCC1150</td>
<td>EU673088</td>
<td>AY854063 (100%, <em>S. hirsutum</em>)</td>
</tr>
<tr>
<td>SH3</td>
<td>213</td>
<td>FBCC1151</td>
<td>EU673089</td>
<td>AY854063 (100%, <em>S. hirsutum</em>)</td>
</tr>
</tbody>
</table>

*Collector: Emilia Pippola, herbarium: OULU.

*Fungal Biotechnology Culture Collection.*

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Experimental design and experiments

In the nitrogen and carbon experiments there were four replicates for each *T. encephala* strain on every medium (treatment). In the host-parasite experiment each *T. encephala* strain was cultivated alone, together with every *S. sanguinolentum* and *S. hirsutum* strain, and *Stereum* strains were cultivated alone. Half of the cultures were on the glucose and half on the tannic acid medium. There were three replicates per each treatment.

While initiating *T. encephala* strains numerous spores from the two fruit bodies began to grow, and the colonies were mixed. Thus, every *T. encephala* strain was a heterogeneous yeast population. Inocula for the experiments were prepared by rinsing the mother culture of each *T. encephala* strain with 2 mL sterilized H₂O of which 50 μL (the nitrogen experiment) or 100 μL (the carbon and host-parasite experiment) was used to inoculate the experiment plates. The inoculum was placed in the middle (the nitrogen and carbon experiment) or 1 cm from the edge (the host-parasite experiment) of each plate. A 0.636 cm²-sized disc cut from the maintenance *S. sanguinolentum* and *S. hirsutum* plates was placed 1 cm from the opposite edge (the host-parasite experiment). Cultures were kept in the dark at room temperature.

Cell morphology at the colony edge and growth of *T. encephala* strains as well as growth of *Stereum* strains were examined immediately after the inoculation and after 15 wks. In the host-parasite experiment examinations were performed also after 5 d, and contact between *Stereum* and *T. encephala* strains (two classes: *Stereum* avoids and does not avoid *T. encephala*) was observed after 21 wks. Cell morphology was examined under the light microscope (Nikon Optihot-2) using × 400 magnification. The frequency of different structures was estimated by counting the first 300 structures and dividing them into yeast cells and hyphal structures including germ tubes, hyphae, and asexual reproductive spores, conidia (Fig. 1 a-d). The method was adopted from similar studies (Ruiz-Herrera et al., 1995; Ruiz-Herrera & Sentandreu, 2002). In addition, the occurrence of clamp connections was observed.

Microscopic structures and plates were photographed using Nikon COOLPIX880, and area measurements were performed using ImageJ (Abramoff et al., 2004). Relative growth rate (RGR, cm²/cm²/wk), i.e. the rate of increase in total area per unit of area, was calculated:

\[
RGR = \frac{\ln A_2 - \ln A_1}{t_2 - t_1}
\]

where A₁ and A₂ are areas (cm²) at times t₁ and t₂ (wk).

The nitrogen experiment was performed in the autumn 2005 except for the control which was carried out in the turn of the year 2005/2006. The carbon experiment was performed in the turn of the year 2005/2006 and the host-parasite experiment in the spring 2006. The viability of the cultures, which did not grow during the experiments, was checked by transferring them to the MYP medium. One of the *T. encephala* strains, TE2, was noticed to be unviable in the nitrogen experiment. After the experiments half of the replicates were incubated at room temperature and the other half at 4°C for three months. Macroscopic changes were then observed, and all uncontaminated cultures of the nitrogen experiment (tot. 27, 3-4 per treatment) were dried overnight at 60°C prior to the analysis of N and C concentrations. The analysis was made with the elemental analyzer EA 1110 (CE Instruments), and C/N ratios were calculated.
Statistical analyses

Statistical analyses were carried out using R for Windows (version 2.3.1) (Ihaka & Gentleman 1996). Variation in RGR (factors: strain, N or C source, and in the host parasite experiment also accompanying species) and N and C concentrations (factor: N source) were analyzed by ANOVA, followed by pairwise comparisons (Dunnett’s test) in which treatments were compared with the control. Q-Q and residual plots were examined to control the assumptions for normality and homogeneity of variances, and if the assumptions were violated, generalized linear models (GLZ, the R function glm) were used. Because of different growth rates of the species, RGR in S. hirsutum was analyzed after 5 d and RGRs in T. encephala and S. sanguinolentum after 15 wks. GLZ with binomial distribution and logit link was used to analyse variation in frequencies.
(frequency of hyphal structures and conidia) and yes/no variables (contact between *T. encephala* and *Stereum* strains) and with Gamma distribution and inverse link variation in C/N ratios. Factors were added into the models sequentially (first to last): strain, N or C source (the nitrogen and carbon experiment) or medium, strain, treatment (the host-parasite experiment). Deviance (D) was approximated by chi-squared distribution. In the case of C/N ratios GLZ was followed by contrast analysis in which every treatment was compared with the control. In all analyses related to the host-parasite experiment second order interactions and hierarchy between species and strains were taken into the models.

**RESULTS AND DISCUSSION**

**Range of nitrogen sources utilized**

Biotrophic mycoparasites and yeasts have restricted nutrition; they utilize mainly substances of relatively small molecular weight (e.g. Hall & Williams, 2000; Jeffries & Young, 1994; Lachance & Starmer, 1999). *Tremella encephala* was therefore expected to be selective in its nutrition as well. However, it grew on all N sources offered, surprisingly even on the control medium with no added N (Fig. 2).

Nitrogen and carbon concentrations and C/N ratios are shown in Table 2. The N concentrations were significantly affected by N source (*F*<sub>7,19</sub> = 8.175, *p* < 0.000). The mean N concentration on the control medium (0.12% dry mass) was significantly lower in comparison to the other media, except for protein, NaNO<sub>3</sub>, and NH<sub>4</sub>NO<sub>3</sub> (Table 3), and it corresponds to values reported by Levi and Cowling (1968) for mycelia grown under conditions of inadequate N supply (0.1-0.4% dry mass). The effect of N source on C concentrations (*F*<sub>7,19</sub> = 5.624, *p* = 0.001) and C/N ratios (*D*<sub>7</sub> = 29.852, *p* < 0.000) was also significant. Carbon concentrations on any of the media did not differ from the control (Table 3), but the mean C/N ratio for the control (ca. 345) was significantly higher compared to C/N ratios for cultures grown on the other media (in all pairwise comparisons *p* < 0.000). The C/N ratio for the control is high also in comparison with values reported for N deprived mycelia (160-278) (Dowding & Royle, 1971; Levi & Cowling, 1968).

Since the fungal N concentrations were low and C/N ratios high on the control medium, the evident growth probably does not indicate high performance, but at least the growth without N additions shows that *T. encephala* is tolerant to low N. The unicellular yeast form of *T. encephala* presumably cannot recycle N from senescing to growing parts unlike multicellular organisms (e.g. Aerts, 1990; Ellis *et al.*, 2005; Hyvärinen & Crittenden, 1998; Kytöviita & Crittenden, 2007). The growth without N additions is more likely explained by the ability to grow at low cellular N concentrations – a common ability among fungi (Crittenden *et al.*, 1994; Levi & Cowling, 1968; Merrill & Cowling, 1966) in particular those that occupy N deficient habitats (Cowling & Merrill, 1966; Crittenden *et al.*, 1994).

On other N sources than control, the mean N concentrations (0.68-2.71% dry mass) and C/N ratios (14.2-63.3) were quite similar to N concentrations (1.55-6.9% dry mass) and C/N ratios (8.6-41.3) reported for fungi grown at adequate N
Fig. 2. Relative growth rate (RGR, cm$^3$/cm$^2$/wk), frequency of hyphal structures, and frequency of conidia in two strains of *Tremella encephala* (TE1, TE3) grown without added nitrogen (control) and on different nitrogen sources. Data are mean values ± S.D., and the abbreviation CEH means casein enzymatic hydrolysate.
Table 2. Means of nitrogen (N) and carbon (C) concentrations and C/N ratios ± S.D. in *Tremella encephala* grown without added N (control) and on different N sources.

<table>
<thead>
<tr>
<th>N source</th>
<th>N concentration (% dry mass)</th>
<th>C concentration (% dry mass)</th>
<th>C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.120 ± 0.030</td>
<td>39.880 ± 0.353</td>
<td>345.019 ± 82.705</td>
</tr>
<tr>
<td>Amino acids</td>
<td>2.150 ± 0.705</td>
<td>39.530 ± 3.409</td>
<td>19.711 ± 6.054</td>
</tr>
<tr>
<td>Dipeptide</td>
<td>2.710 ± 0.220</td>
<td>38.443 ± 0.386</td>
<td>14.236 ± 1.085</td>
</tr>
<tr>
<td>CEH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.947 ± 0.918</td>
<td>39.007 ± 2.789</td>
<td>22.783 ± 8.923</td>
</tr>
<tr>
<td>Protein</td>
<td>0.678 ± 0.232</td>
<td>37.700 ± 1.338</td>
<td>63.328 ± 31.477</td>
</tr>
<tr>
<td>NaNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.813 ± 0.100</td>
<td>37.407 ± 1.630</td>
<td>46.525 ± 7.635</td>
</tr>
<tr>
<td>(NH&lt;sub&gt;4&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.650 ± 0.988</td>
<td>43.050 ± 0.524</td>
<td>31.299 ± 11.675</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.157 ± 0.023</td>
<td>42.353 ± 0.120</td>
<td>36.712 ± 0.788</td>
</tr>
</tbody>
</table>

<sup>a</sup>Casein enzymatic hydrolysate.

Table 3. Pairwise comparisons of nitrogen (N) and carbon (C) concentrations in *Tremella encephala* grown without added N (control) and on different N sources (treatments) between each treatment and control using Dunnett’s test.

<table>
<thead>
<tr>
<th>N source</th>
<th>N concentration (% dry mass) 95% CI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>t&lt;sub&gt;(7,48)&lt;/sub&gt;</th>
<th>p</th>
<th>C concentration (% dry mass) 95% CI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>t&lt;sub&gt;(7,48)&lt;/sub&gt;</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>0.726 – 3.334</td>
<td>–4.448</td>
<td>0.001</td>
<td>–4.173 – 3.473</td>
<td>–0.262</td>
<td>0.796</td>
</tr>
<tr>
<td>Dipeptide</td>
<td>1.371 – 3.809</td>
<td>–6.066</td>
<td>&lt;0.000</td>
<td>–5.013 – 2.138</td>
<td>–1.148</td>
<td>0.259</td>
</tr>
<tr>
<td>CEH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.523 – 3.130</td>
<td>–4.002</td>
<td>0.003</td>
<td>–4.696 – 2.949</td>
<td>–0.653</td>
<td>0.742</td>
</tr>
<tr>
<td>Protein</td>
<td>–0.662 – 1.777</td>
<td>–1.306</td>
<td>0.243</td>
<td>–5.756 – 1.396</td>
<td>–1.742</td>
<td>0.289</td>
</tr>
<tr>
<td>NaNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>–0.610 – 1.997</td>
<td>–1.519</td>
<td>0.243</td>
<td>–6.296 – 1.349</td>
<td>–1.848</td>
<td>0.289</td>
</tr>
<tr>
<td>(NH&lt;sub&gt;4&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.311 – 2.749</td>
<td>–3.584</td>
<td>0.007</td>
<td>–0.406 – 6.746</td>
<td>–2.533</td>
<td>0.095</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>–0.267 – 2.340</td>
<td>–2.271</td>
<td>0.086</td>
<td>–1.349 – 6.296</td>
<td>–1.848</td>
<td>0.289</td>
</tr>
</tbody>
</table>

<sup>a</sup>95% confidence interval (CI) shows the interval in which the difference between means occurs in 95% probability.

<sup>b</sup>Casein enzymatic hydrolysate.

supply (Ausmus et al., 1976; Newell & Statzell-Tallman, 1982; Rapior et al., 1988; Van Veen & Paul, 1979). Most of the N sources were probably utilized. Only the protein and nitrates, on which N concentrations did not differ significantly from the control, might have been unsuitable or poor N sources for *T. encephala*. An ability to break down proteins and other polymers is a rarity among yeasts (Deacon, 2006; Garraway & Evans, 1984), and some of them are unable to utilize nitrates (Barnett et al., 1990; Siverio, 2002), because they cannot synthesize the molybdopterin cofactor of nitrate reductase (Siverio, 2002).
Preference for simple carbon sources

*Tremella encephala* was selective with regards to carbon: it grew only on the glucose, glycerol, and D-mannitol media. The apparent growth on the other media (Fig. 3) is not real, but caused by a slight inaccuracy in the measurements which is within the margin of error. Effect of C source on RGR was significant ($F_{8,75}=154.644, p<0.000$), and according to pairwise comparisons, the mean RGR on each of the three media was significantly higher compared to the control (Table 4). According to a report of Centraalbureau voor Schimmelcultures, *T. encephala* is unable to exploit monosaccharides for assimilation. Thus, the utilization of glucose is no surprise. As far as the decomposition of glycerol is concerned, other biotrophs have been reported to prefer glycerol as their C source (Barnett, 1970; Cooke, 1977; Jordan & Barnett, 1978).

![Graph showing relative growth rate (RGR, cm²/cm²/wk) and frequency of hyphal structures in strains of *Tremella encephala* (TE) grown without added carbon (control) and on different carbon sources. Data are mean values ± S.D., and the abbreviation Na-acetate means sodium acetate.](image-url)
Table 4. Pairwise comparisons of relative growth rates (RGR, cm²/cm²/wk) in *Tremella encephala* grown without added carbon (control) and on different carbon (C) sources (treatments) between each treatment and control using Dunnett’s test.

<table>
<thead>
<tr>
<th>C source</th>
<th>95% CI (a)</th>
<th>( t_{(8,75)} )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.098 – 0.159</td>
<td>– 11.429</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.070 – 0.131</td>
<td>– 8.968</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>D-mannitol</td>
<td>0.115 – 0.176</td>
<td>– 13.000</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Na-acetate(^b)</td>
<td>– 0.014 – 0.047</td>
<td>41.000</td>
<td>0.438</td>
</tr>
<tr>
<td>Amino acids</td>
<td>– 0.030 – 0.031</td>
<td>– 0.060</td>
<td>0.953</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>– 0.041 – 0.025</td>
<td>– 0.681</td>
<td>0.840</td>
</tr>
<tr>
<td>Cellulose</td>
<td>– 0.028 – 0.033</td>
<td>– 0.257</td>
<td>0.953</td>
</tr>
<tr>
<td>Lignin</td>
<td>– 0.014 – 0.047</td>
<td>– 1.460</td>
<td>0.438</td>
</tr>
</tbody>
</table>

\(^a\)95% confidence interval (CI) shows the interval in which the difference between means occurs in 95% probability.

\(^b\)Sodium acetate.

In the course of evolution numerous transitions from a pathogenic to a saprobic nutritional mode, as well as the reverse, have occurred in the Basidiomycota (James *et al.*, 2006). If *T. encephala* had evolved from a saprobe, it could still possess some decomposition capability which is needed to exploit the offered C polymers: tannic acid, cellulose, and lignin. Fungi are not known to break down lignin without a supplementary C source (e.g. Deacon, 2006; Eriksson *et al.*, 1980; Garraway & Evans, 1984; Kirk *et al.*, 1976), but when given supplementary C in form of glucose and grown in optimal conditions, some yeasts can degrade lignin (Clayton & Srinivasan, 1981). *Tremella encephala* cannot assimilate certain lignin breakdown products (Sampaio, 1999), but according to the report of Centraalbureau voor Schimmelcultures, it can assimilate starch to some extent. Since no simple C supplements were supplied, *T. encephala*’s inability to degrade lignin is not proven, even though it is very likely. However, being unable to utilize a number of C polymers it cannot be an efficient decomposer.

**Variation in dimorphism**

In nature *T. encephala* is best known from its sexual stage (fruit bodies) with dikaryotic mycelia, basidia, and haustoria. Instead, under laboratory conditions *Tremella* species occur prevailingily in the yeast form (e.g. Bandoni, 1961; Bandoni & Boekhout, 1999; Brough, 1974; Fenwick, 1995). The yeast form was clearly dominant also in this study, and other structures were observed only to some extent. In the nitrogen experiment there was remarkable variation in dimorphism between the strains. Hyphal structures were noted mainly in the *T. encephala* strain TE3 (effect of strain significant \( D_1 = 28.465, p < 0.000 \)) on all media except for the control (effect of N source indicatively significant \( D_7 = 14.006, p = 0.051 \)) (Fig. 2). In the carbon experiment hyphal structures were
observed in all strains on the glycerol medium, and rarely on amino acid and tannic acid media (Fig. 3), but the frequencies were low in all cases (not significant). Hyphae should have formed also on the glucose medium identical with the \((\text{NH}_4)_2\text{HPO}_4\) medium on which hyphae occurred in the nitrogen experiment in the strain TE3. The different responses were probably caused by temporal factors or variation between subcultures of the same strain. Evidence of time dependent variation in fungal strains is available also for a few other species (Anderson et al., 2001; Cairney, 1999; Grelet et al., 2005).

Factors affecting morphogenetic switch observed mainly in the *T. encephala* strain TE3 remain inexplicable. Possibly neither N nor C sources were the actual regulators of dimorphism in *T. encephala*. Nutritional requirements of haustorial biotrophs are often complex (Jeffries & Young, 1994), and the ability to use various compounds is affected by many factors (Ruiz-Aguilar et al., 2004). A transfer to low temperature (5°C) induced dimorphic switch in *Tremella mesenterica* Retz.: Fr. (Fenwick, 1995). In this study incubation at 4°C after the experiments did not have any effect on *T. encephala*.

In *T. mesenterica* germ tubes fuse to form dikaryotic mycelium if the isolates forming the tubes are from different fruit bodies (Bandoni, 1963). Because all *T. encephala* strains were established from multiple spores of two fruit bodies, each strain should have contained compatible mating types. For some reason conjugation failed, and only monokaryotic hyphae were observed. *Tremella encephala* has formed dikaryotic mycelium with haustoria in the presence of *S. sanguinolentum* (Bandoni, 1961; Zugmaier et al., 1994), but in the present study the host did not have any effect. In species of Tremellales mating does not necessarily occur readily, and if it fails, cells form haploid conidia and return to the yeast phase (Bandoni, 1987). Conidia were observed in the nitrogen experiment in the *T. encephala* strain TE3 on several media (Fig. 2). There seems to be some variation in the frequency of conidia on different media, but only the effect of strain was significant \((D_1 = 6.780, p = 0.009)\).

**Failure to parasitize**

There were no indications of parasitism, e.g. haustoria or active growth towards hosts, in the host-parasite experiment, and thus any definite interpretations of host-specificity of *T. encephala* or effect of the nutritional quality of the medium on parasitism and host-specificity cannot be made. The glucose medium was presumably too rich to encourage parasitism, and the tannic acid medium did not even support growth of *T. encephala*. In principle, media rich in N compared to C are known to enhance parasitism (Jeffries & Young, 1994; Kurtzman, 1968). On the other hand, many fungi grow equally well in the absence or in the presence of their potential hosts (Jeffries & Young, 1994).

In the host-parasite experiment *T. encephala* grew only on the glucose medium (Fig. 4), and its RGR was significantly affected by *Streptom* treatment \((F_{2,96} = 31.408, p < 0.000)\). According to pairwise comparisons, RGRs in *T. encephala* cultured with *S. sanguinolentum* \((t_{2,96} = -8.751, p < 0.000)\) and *S. hirsutum* \((t_{2,96} = -10.641, p < 0.000)\) were significantly lower than RGRs in *T. encephala* cultured alone. On average, RGRs in *S. sanguinolentum* were highest in absence of *T. encephala* \((F_{1,54} = 13.600, p < 0.001)\) (Fig. 4). RGRs in *S. hirsutum* were significantly affected only by medium \((F_{1,54} = 53.342, p = 0.000)\) and strain \((F_{2,54} = 16.035, p = 0.000)\) (Fig. 4).
Fig. 4. Relative growth rate (RGR, cm\(^2\)/cm\(^2\)/wk) in strains of *Tremella encephala* (TE), *Stereum sanguinolentum* (SS), and *Stereum hirsutum* (SH) in the different treatments (accompanying species absent or present) of the host-parasite experiment in which strains were grown on the glucose and tannic acid media. Data are mean values ± S.D. Note the discontinuous y-axis in the two lower graphs.
To sum up the host-parasite experiment, *T. encephala* grew fastest alone and suppressed the growth of *S. sanguinolentum*, but not the growth of *S. hirsutum*. Without evidence of parasitism low RGR in *T. encephala* and *S. sanguinolentum* in dual cultures refers to resource competition rather than to parasitism. Even though *T. encephala* did not parasitize its known host, *S. sanguinolentum*, our results do not imply that the host-parasite interaction does not occur in nature. Already Bandoni (1961) demonstrated that *T. encephala* is parasitic on *S. sanguinolentum*, and in nature the parasitic relationship can be observed in every fruit body of *T. encephala*.

**Notes on the life cycle**

According to the results, *T. encephala* is not highly dependent on the host. It survived on various media especially in the yeast form, but presumably it cannot reproduce sexually without *S. sanguinolentum*. It is important for any parasite such as *T. encephala* to maintain variation through new allele combinations created in recombination. By means of recombination *T. encephala* may keep track of the parasite-host coevolution and can better adapt to environmental changes. The asexually reproducing yeast forms in basidiomycetous dimorphic mycoparasites may occupy habitats different from the sexually reproducing filamentous form (Sampaio, 2004). Yeasts belonging to the genus *Tremella* have been isolated from the Tinto River (López-Archilla et al., 2004), decaying wood (González et al., 1989), and nests of an ant (Carreiro et al., 1997). Whether these identifications based on physiological and/or morphological criteria were correct or not remains to be shown with more rigorous testing. However, it seems that the yeast form of *T. encephala* and other *Tremella* species may well be more common under natural conditions than currently known.

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Growth and dimorphism of *Tremella encephala*


