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Original paper: Changes in carbohydrates and organic acids in the mycelium during vegetative growth of *Lyophyllum shimeji* and *Lyophyllum fumosum*

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Changes in the distribution of low molecular weight carbohydrates, polysaccharides, and organic acids in mycelia during the vegetative growth of *Lyophyllum shimeji* and *Lyophyllum fumosum* were studied. On a dry-weight basis the low molecular weight carbohydrate content during vegetative growth was in the range of 2.9–9.8% in *L. shimeji* and 1.9–10.0% in *L. fumosum*. Trehalose, glucose, fructose, mannitol, arabitol and glycerol were identified in the mycelia, of which trehalose was the major component. On the same basis, the polysaccharide content was in the range of 54.0–62.4% in *L. shimeji* and 48.4–62.9% in *L. fumosum*. The acetic acid-soluble (glycogen) fraction was predominant in the polysaccharide fractions in the vegetative mycelia of both species. Trehalose and glycogen accumulated in the vegetative mycelia were thought to serve as carbohydrate reserves. The organic acid content was in the range of 1.3–1.6% in *L. shimeji* and 1.8–2.2% in *L. fumosum*, and eleven organic acids were identified. Malic, citric, pyroglutamic and fumaric acids were predominant in *L. shimeji*, and lactic, malic, citric, pyroglutamic and fumaric acids were predominant in *L. fumosum*. Lactic acid was found to accumulate in the mycelium of *L. fumosum* during vegetative growth.

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Original paper: Change of glutamate dehydrogenase activities during fruit-body formation in *Lentinus edodes*

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The activities of NAD- and NADP-dependent glutamate dehydrogenases (GDH) in vegetative mycelia of *Lentinus edodes* increased in the earlier growth stages, i.e., 5 to 10 days after inoculation, as well as the time of fruit-body formation when strain No. 68 was cultivated in peptone-glucose-vanillin (PGV), peptone-glucose-glucuronic acid (PG-GlcU) and peptone-glucose-vanillin-glucuronic acid (PGVGlcU) liquid media. The GDH activities also increased in young fruit-bodies. No significant increase was observed in mycelial GDH activities and fruit-body yield when the strain was grown in PG liquid medium. The addition of 1.0 mM diethyldithiocarbamate (DETC) to the culture medium on the 7th day of incubation, when GDH activity was at a peak, inhibited the activity of NAD-GDH as well as fruit-body formation. However, no inhibition was observed when DETC was added on the 21st day of incubation. When vanillin (50 μg/ml) and glucuronic acid (2 mg/ml) were added on the 10th and 15th day of incubation in PG medium, the activity of NAD-GDH and mycelial dry weight increased with production of fruit-bodies, but the addition of DETC at the same time inhibited the activity of NAD-GDH, mycelial growth and fruit-body formation. No effect was observed when vanillin and glucuronic acid were added on the 20th day of incubation. Addition of ferulic acid to the glutamate medium (Glu) enhanced mycelial growth and GDH activities on Glu medium. These results indicated that the reproductive process of *L. edodes* starts by the 20th day of incubation and the stimulated GDH activities in the eary growth stages in the presence of phenolic compounds and uronic acids may be responsible for the induction of fruit-body formation.

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Original paper: Nutritional requirements for the vegetative growth of *Lyophyllum fumosum*

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The nutritional requirements for the vegetative growth of *Lyophyllum fumosum* (Pers.: Fr.) P. D. Orton were investigated by use of a synthetic liquid medium. A wide range of carbohydrates served as carbon source in the medium which supported growth of *L. fumosum*. Glucose, mannose, fructose, sucrose, dextrin, glycogen, pectin and soluble starch were especially good carbon sources for mycelial growth. Yeast extract, soyton, peptone, meat extract, casamino acid and the amino acids mixture of the basal medium were acceptable nitrogen sources for the growth, while ammonium and nitrate salts were poor nitrogen sources. The amino acids mixture in the culture medium could be replaced by L-valine and L-citrulline. The vegetative mycelium did not grow in the absence of KH_2PO_4 and $MgSO_4$, and the yield of mycelium was decreased by the omission of $ZnSO_4$,

$FeSO_4$, $CuSO_4$, $MnSO_4$, thiamine and nicotinamide. The vegetative mycelium yield was increased in the basal medium containing 0.1–1 mg/l of indole-3-acetic acid (IAA), kinetine, gibberellic acid, l-naphthyl acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D), but it was decreased in the basal medium containing 10 mg/l of IAA and NAA.

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Original paper: Increase of urediniospores in the telium of *Zaghouania phillyreae* (Uredinales)

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The number of urediniospores in the telium of *Zaghouania phillyreae* increased from early winter (January) to early spring (March). Finally the telium was completely filled with urediniospores and had apparently become a uredinium. A spore-like body showing some of the characteristics of both basidiospores and urediniospores was occasionally observed at the bottom of a teliospore.

In room experiments, the ratio of numbers of urediniospores to basidiospores was not directly related to air temperature. However, the rate of increase of urediniospores was slow at low temperature (5–10°C) when test leaves were collected while teliospores and basidiospores were formed abundantly in the field.

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Original paper: An attempt of classification by biological characteristics of some pathogenic water moulds from freshwater fishes

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The identification of water moulds of the family Saprolegniaceae has been based on their asexual and sexual characteristics. In this study, we attempted to classify the three genera *Saprolegnia, Achlya* and *Aphanomyces* in the Saprolegniaceae from some of their biological characteristics. The optimal temperatures for growth were 20–25°C, 30–35°C and 25–30°C for *Saprolegnia, Achlya* and *Aphanomyces*, respectively. The three genera also differed in their sensitivities to malachite green, NaCl, K_2HPO_4, sorbose and Polyphenon–100^R. The strains tested were decreasingly sensitive of NaCl and K_2HPO_4 in the order of *Aphanomyces, Achlya* and *Saprolegnia*, and to malachite green, Polyphenon–100^R and sorbose in the order of *Aphanomyces, Saprolegnia* and *Achlya*. From the sensitivity tests of *Aphanomyces* to Polyphenon–100^R and sorbose, the pathogenic strain *A. piscicida* could be distinguished from the saprophytic strains. The three genera in the Saprolegniaceae and some species in these genera could thus be classified on the basis of certain biological characteristics. Biological tests might thus be applicable for the classification of fungi belonging to certain genera of the Saprolegniaceae.

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Original paper: Nutritional requirements for the vegetative growth of *Lyophyllum shimeji*

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The nutritional requirements for the vegetative growth of *Lyophyllum shimeji* were investigated by use of a synthetic liquid medium. A wide range of carbohydrates served as carbon source in the medium which supported growth of *L. shimeji*. Glucose, xylose, mannose, fructose, sucrose, dextrin, glycogen, pectin and soluble starch were especially good carbon sources for mycelial growth. Meat extract, Soyton, yeast extract, polypeptone, casamino acids and the amino acids mixture of the basal medium were acceptable nitrogen sources for the growth, while ammonium and nitrate salts were poor nitrogen sources. The amino acids mixture in the culture medium could be replaced by L-alanine, L-isoleucine, L-valine, L-glutamine, L-citrulline or L-serine. The vegetative mycelium did not grow in the absence of KH_2PO_4 and $MgSO_4$, and the yield of mycelium was decreased by the omission of $ZnSO_4$, $FeSO_4$, thiamine and nicotinamide. The vegetative growth was accelerated in the basal medium containing 0.1–1 mg/l of indole-3-acetic acid (IAA), kinetine, gibberellic acid, 1-naphthyl acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid, but it was inhibited in the basal medium containing 10 mg/l of IAA and NAA.

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Original paper: Analysis of yeast cell constitution by thin layer chromatography using diatomaceous earth as a support.

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Methylated saccharides from yeast cells were analysed by thin layer chromatography (TLC) using diatomaceous earth as a support. Residues from which fatty acids had been extracted for analysis of yeast cell constitution were used as samples. Freeze-dried yeast cells (50 mg) were hydrolysed with 5% methanolic hydrochloride acid, and fatty acid methyl ester was extracted from the hydrolate with n- hexane. Chloride ions were removed from the residual solution with ion exchange resin. The solution was concentrated and used as a sample. Thin layer plates were prepared with mixture of diatomaceous earth (Wako Pure Chemical Indus.) and plaster (9 : 1). A mixture of 67% isopropanol (35 ml) and ethyl acetate (65 ml) was used as the solvent for the one-step development. Anthrone-sulfuric acid reagent was used for detection. The R*f* value ($\times 100$) of the light yellowish brown spot of xylose was 60. Mannose gave a dark brown major spot of R*f* value 47 and a

minor spot of R*f* value 25, and glucose gave a bluish purple spot of *Rf* value 40. For galactose, two clear, light yellow spots (R*f* value, 25 and 28) and two minor spots (R*f* value, 45 and 55) were observed. The presence of minor spots indicating anomers of mannose and galactose did not disturb the detection of major spot of methylated saccharides in mixed solution. Mannose, glucose and galactose were detected in strains of *Geotrichum, Kluyveromyces, Pichia* and *Schizosaccharomyces* by this thin layer chromatography. Glucose and galactose were detected in the strain of *Cryptococcus* and *Trichosporon*, together with minor spots of xylose. The methylated saccharide mixture could not be separated on a thin layer plate prepared with Kieselgur G of Merck (Germany). This one-step TLC may be suitable to the case of a few samples whose constituent saccharides need to be known immediately, as it needs no multiple development.

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Short Communication: On the *Inocybe* collected by Kumagusu Minakata (1)

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Two taxa of *Inocybe* collected by Kumagusu Minakata were identified as *I. glabrodisca* and *I.* aff. *dunensis*, respectively, and are new to the Japanese mycoflora. Their microscopic features are reported with illustrations.

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Short Communication: A trial cultivation of *Lyophyllum shimeji* on solid media

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Fruit-body formation of *Lyophyllum shimeji* was achieved on a solid medium. The solid medium was prepared in bags by adding 750 ml of liquid medium (recipe: soluble starch, 100 g; D-glucose, 25 g; pectin, 1 g; yeast extract, 3 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; thiamine-HCl, 1 mg; $CaCO_3$, 5 g; charcoal powder, 5 g; water, 860 ml) to 120 g of peat moss. Cultures were incubated in darkness for 90 days at 23°C, then under fluorescent lamp illumination (50 lux.) for 30 days at 16°C. Following transfer to light at 16°C, primordia appeared on the media after 13–15 days and grew into fruit-bodies after 14–17 days. All of 27 strains of *L. shimeji* formed fruit-bodies, and fruit-body yields were 14.6–62.7 g/bag.

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Note: Two species of yeasts isolated from wood vinegar

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Two species of yeast were isolated from wood vinegar and identified. One species, isolated from wood vinegar extracted from Japanese oaks, was *Dipodascus capitatus*. The other species, isolated from wood vinegar extracted from North American maples, was *Rhodotorula minuta*.

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Review: How do *Phycomyces* sporangiophores respond to gravity? —Present knowledge and perspectives—

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Gravity is one of the factors guiding growth in fungi. We summarize what is known about gravitropism in *Phycomyces* sporangiophore—one of the most developed model systems considering "stimulus perception—stimulus transduction—response" system in fungi.

Compared with response to light, gravitropism in *Phycomyces* appears to be less effective in respect to the latency period and the speed of response. Maybe this is the reason why studies on gravitropism have been less intensive, resulting in obscure and sometimes contradictory information on receptors and involved mechanisms.

There are several parameters influencing the gravitropic response: developmental stage and diameter of the sporangiophore, absolute growth rate, ratio of differential growth. The interplay between gravity and light is expressed in two ways: influence of light on gravisensitivity and tropic equilibrium when both stimuli are present simultaneously.

Attempts to dissect the mechanism of gravitropic response and to model its kinetics involve experiments in a microgravity environment as well as application of centrifugal force. The gravireceptor is suspected to be localized in the growth zone but has not yet been identified. Further progress in the gravitropic studies could be achieved using mutants with impaired or enhanced perception of gravitational stimuli.

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Review: The 18S and 26S rRNA partial base sequencings of yeasts and yeast-like fungi from the phylogenetic and taxonomic points of view

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In the studies on the 18S and 26S rRNA partial base sequencings (one region in 18S rRNA and two regions in 26S rRNA) over the past six years, the following conclusions were obtained. 1) In the partial base sequencings of 18S rRNA (position 1451–1618, 168 bases), the separate genus or genera can be set up, when the calculated number of base differences was five or more. 2) Generally, the base substitutions occurred more frequently in the 26S rRNA (positions 1611–1835, 225 bases and

positions 493–622, 130 bases) than those in the 18S rRNA (position 1451–1618, 168 bases). Between *Nematospora coryli* (Q-5 or Q-6) and *Holleya sinecauda* [Q-9(Q-8)], however, the base differences were large and numbered seven in the partial base sequencings of 18S rRNA (positions 1451–1618, 168 bases) but only two in those of 26S rRNA (positions 1600–1835, 236 bases). 3) The genus *Williopsis* Zender, delimited by Kurtzman, was divided into three groups or more (*Zygowilliopsis* and *Komagataea*). 4) The nitrate-assimilating *Pichia* species, which were once classified in the genus *Hansenula* Sydow et Sydow, were divided into seven groups, and *Ogataea, Kuraishia* and *Nakazawaea* were proposed. 5) Of the methanol-assimilating yeasts, *Pichia pastoris* was phylogenetically distant and *Komagataella* was proposed. 6) Several problems in the partial base sequencings were presented and discussed phylogenetically and taxonomically.

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