Botanica Marina
Vol. XXIX, pp. 539–547, 1986

Morphological and Physiological Adaptations of the Cyphellaceous Fungus *Halocyphina villosa* (Aphyllophorales) to its Marine Habitat

S. Rohrmann and H. P. Molitoris[1]

Botanisches Institut, Arbeitsgruppe Pilzphysiologie, Universität, 84 Regensburg, Federal Republic of Germany

(Accepted 14 August 1986)

Abstract

Only 4 basidiomycetes are found among the 500 marine fungi known today. The marine basidiomycete *Halocyphina villosa* grows submerged on roots and wood of mangroves in brackish water of tropical/subtropical mangrove swamps. So far only material from the natural habitat of *Halocyphina villosa* has been investigated, mainly for morphological and systematic purposes. In vitro dependence of growth, basidiocarp- and enzyme-production on different factors, such as temperature (11–35 °C), salinity (0–200% natural seawater), pH (5–9) and composition of the medium are investigated in this paper.

Optimum colony growth was found at 27 °C, 25–75% natural seawater and pH 6–7. The composition of the medium was less important. Cyphellaceous basidiocarps were produced in vitro only between 22–27 °C, 25–100% natural seawater and pH 6–7. The addition of pieces of wood (various mangroves, also *Betula*) and/or of peptone as nitrogen source was required. Three types of basidiocarps were observed: funnel-shaped single basidiocarps, circular from above (type A); funnel-shaped single basidiocarps, laterally compressed (type B); composite basidiocarps (type C). The morphology of the type A basidiocarps is identical with those from the natural habitat. Only type A basidiocarps produced spores and were reported from the natural habitat also. Qualitative investigation of the production of 14 important and characteristic, predominantly extracellular enzymes revealed that *H. villosa* forms laccase, peroxidase, cellulase and pectinase (wood degradation), amylase and laminarinase (use of plant carbohydrates), lipase (reserve fats) and nitrate reductase (use of seawater nitrate, characteristic of marine fungi). The results are largely to be anticipated for fungi growing on woody substrates. The above in vitro findings lead to the conclusion that *Halocyphina villosa* in growth, fructification and enzyme composition is well adapted to its natural habitat.

Introduction

While terrestrial fungi have been known for a long time and are therefore thoroughly investigated, the first marine fungi were not discovered and described until the middle of the 19th century (see: Kohlmeyer and Kohlmeyer 1979). Marine mycology, the science dealing with marine fungi, began in the middle of this century with papers such as the one by Barghoorn and Linder (1944) on wood-destroying marine fungi.

By now about 500 marine fungi have been described, that is 'fungi, that can grow and sporulate in a marine habitat' (Kohlmeyer 1974). Only 4 marine basidiomycetes are known, two of which, *Halocyphina villosa* Kohlm. et Kohlm. and *Digitatispora marina* Doguet, belong to the Aphyllophorales.

H. villosa was first described by Kohlmeyer and Kohlmeyer in 1965 and classified as a fungus imperfectus. In a reinvestigation of Kohlmeyer's original material from the natural habitat, Ginns and Malloch (1977) were able to show clamp-connections, basidia with sterigmata and basidiospores. *H. villosa* there-

[1] Paper presented at the 4th International Marine Mycology Symposium, Portsmouth, 1985.

fore belongs to the basidiomycetes. This fungus produces basidiocarps up to 0.5 mm in diameter, among the largest basidiocarps found in marine fungi. By virtue of its cyphellaceous basidiocarps, it is placed in the order Aphyllophorales.

H. villosa has been collected in the Atlantic and Pacific Oceans, America, Africa and Sri Lanka, where it is mostly found growing on wood in brackish water of tropical and subtropical mangrove swamps. It therefore seems to be restricted to a quite specific habitat (Kohlmeyer and Kohlmeyer 1964–1969, 1977, 1979, Ginns and Malloch 1977, Koch 1982).

Up till now investigations on *H. villosa* have dealt mainly with morphology and systematics using material from the natural habitat (Kohlmeyer and Kohlmeyer 1965, Ginns and Malloch 1977). In addition, Kupka *et al.* (1981) isolated a culture of *H. villosa* and showed its ability to produce the antibiotic siccayne. In vitro investigations on colony growth, fructification and physiological parameters such as enzyme activities have not been conducted so far. Data on these parameters in particular, however, can provide information on the living conditions of *H. villosa* and its adaptation to a special habitat and are therefore the object of this paper.

Material and Methods

Organism

A strain of *Halocyphina villosa* was kindly supplied by T. Anke and J. Kupka (Abt. Biotechnologie, Universität, Kaiserslautern, GFR). The material was collected by F. Oberwinkler from a Columbian mangrove swamp. The culture was isolated and identified by Kupka *et al.* (1981). Stock cultures were grown on 'Moser b'-medium (Moser 1959) or cornmeal-agar at 22 °C (Esser 1974), stored at 6 °C and transferred at intervals of 3 months.

Media

Basic media: The quantities of the substances given below are calculated for 1000 ml water. pH was adjusted with HCl/NaOH before autoclaving (20 min, 121 °C, 1 bar). Cultures were grown in petri-dishes (diameter 9 mm), unless otherwise specified. *GPY-medium*: Glucose 1 g; peptone 0.5 g; yeast extract 0.1 g; agar 16 g; pH 6.0. *PYC-medium*: Peptone 0.5 g; yeast extract 0.1 g; Avicel 5 g; agar 16 g; pH 6.0. *GYS-medium*: Glucose 1 g; soluble starch 5 g; yeast extract 0.1 g; K_2SO_4 1 g; $MgSO_4 \times 7H_2O$ 0.5 g; agar 16 g; pH 7.4. *F 1003* (Sguros *et al.* 1962): Glucose 5 g; yeast extract 1 g; NH_4NO_3 2.4 g; $MgSO_4 \times 7H_2O$ 2.4 g; Tris(hydroxymethyl)aminomethane 1.2 g; pH 7.5. *Water agar*: Agar 16 g.

Salinity: The seawater used (Helgoland, position 'Nathurn', salinity 30.0 ± 2.0‰ was diluted with deionized water to concentrations of 75, 50 and 25% natural seawater, respectively. Water of a concentration of 200% natural seawater was prepared by evaporation of seawater down to half the original volume in a rotary vacuum evaporator.

pH: pH (5, 6, 7, 8, 9) was adjusted before autoclaving with HCl/NaOH. For the experiments on growth in GPY-medium the pH was also determined after autoclaving and then after 5 weeks of incubation using a surface electrode.

Addition of wood for fructification: Pieces of wood (length 2 cm, 3 or 4 per plate) or ground wood (0.5 g dry weight per plate) of the following species were added to the basic media: *Betula* spp. (birch applicator sticks); *Conocarpus erecta* L. (buttonwood); *Rhizophora mangle* L: (red mangrove); *Rh. racemosa* Meyer; *Tamarix pentandra* Pall. (five-stamen tamarisk).

Media and reagents for enzyme tests: Enzyme tests, composition of media, type of reaction and reaction time are described in detail by Molitoris and Schaumann (1986). The following enzyme activities are tested: Redox-metabolism: laccase, tyrosinase, peroxidase; nitrogen-metabolism: caseinase, gelatinase, nitrate reductase; fat-metabolism: lipase; carbohydrate-metabolism: amylase, cellulase, polygalacturonase, pectate transeliminase, chitinase, alginase, laminarinase.

Incubation

The petri-dishes and test-tubes were incubated at temperatures of 11 °C, 14.5 °C, 22 °C, 27 °C, 31 °C and 35 °C in incubators or incubation chambers with constant light and humidity during the whole incubation period.

Growth and fructification

Growth for each combination of factors was determined after 3, 7, 10, 14, 17, 21 and 28 days of incubation by measuring colony diameter on 3 replicate plates and calculating the mean value. Formation and morphology of basidiocarps were checked and documented at weekly intervals over a period of 16 weeks; the following methods were used:

Light microscopy: Axiomat NDC, Zeiss, Oberkochen.

Scanning electron microscopy: Fixation in 70% ethanol, 4% formaldehyde or buffered osmium tetroxide; dehydration in steps with increasing ethanol concentration; transfer into acetone; critical-point-drying with liquid CO_2 (CPA-II, Technics, Alexandria, Va., USA); sputtering with gold in a Hummer II Sputter (Technics, Alexandria, Va., USA); microscopy with Cambridge Stereoscan S 4-10 at a voltage of 10 KV.

Results

Colony growth

In order to characterize vegetative growth, colony growth was measured as an increase in diameter of the colony depending on the factors as shown in Table I.

Temperature: Of all factors investigated, temperature had the strongest influence on growth of *H. villosa*. Figure 1 shows the results. The following conclusions can be drawn:

Table I. Factors tested for effect on colonial growth of *H. villosa*

Temperature (°C)	11, 14.5, 22, 27, 31, 35
% seawater	0, 25, 50, 75, 100, 200
pH of medium[1])	5 (5.8), 6 (6.4), 7 (6.9), 8 (7.0), 9 (7.0)
Medium[2])	GPY (glucose, peptone, yeast extract)
	GYS (glucose, yeast extract, soluble starch)

[1]) pH of medium before autoclaving (after autoclaving)
[2]) see material and methods

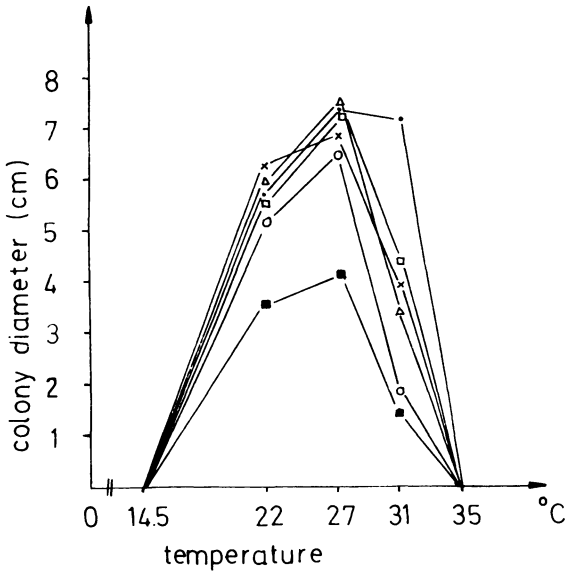


Fig. 1. Optimum temperature for colony growth of *Halocyphina villosa* at different salinities of medium GPY; pH 7.0; 21 days incubation. Each value represents the mean of 3 parallel plates with the seawater concentration given below.
seawater concentration: o—o 0% △—△ 25% ●—● 50% x—x 75% □—□ 100% ■—■ 200%

1) In the experimental range of temperatures from 11 °C to 35 °C, the fungus was able to grow only between 22 °C and 35 °C; at 11 °C and 14.5 °C there was no growth.
2) Growth rate was best at 27 °C.
3) At 35 °C the colony grew only for a limited time.

Salinity: The salinity of seawater represents the most striking difference between the marine and the limnic-terrestrial environment. In order to show the salt-tolerance of vegetative growth of the brackish water fungus *H. villosa*, growth measurements were carried out at seawater concentrations of 0%, 25%, 50%, 75%, 100% and 200% at temperatures of 11 °C, 14.5 °C, 22 °C, 27 °C, 31 °C and 35 °C. From Figure 1 the following conclusions can be drawn:

1) In principle, growth of this fungus is possible at each of the salinities tested.
2) The optimum range of growth is between 25 and 100% seawater; at 0 and 200% seawater colony growth is slower.
3) No differences in colony growth between different salinities were observed at 35 °C, where growth was very slow.

pH of medium: The pH-values of 5, 6, 7, 8 and 9, as adjusted and measured before autoclaving, were again measured after autoclaving, after inoculation and after 5 weeks of incubation. The following results were obtained:

1) No pH-values above 7.0 were observed (see Table II).
2) In all cases (different initial pH, different salinity and temperature), the resulting pH-values after 5 weeks of growth were found to be around 8.
3) Within the limits tested, pH-values had only slight influence on colony growth.

Composition of medium: No difference in growth was found with the two semi-synthetic media, GPY and GYS.

Basidiocarps

Formation of basidiocarps: According to Kohlmeyer's definition (1974), one of the characteristics of marine fungi is the ability to fructify and propagate in the marine environment. As shown in Table II, in vitro tests on the influence on fructification of factors such as type of medium, additions to the medium, temperature, salinity and pH were conducted. It was tested

1) whether basidiocarps were formed,
2) under which conditions basidiocarp formation occurred,

3) after what time fructification was found and
4) whether basidiospores were produced.

As is evident from Table II in comparison with Table III and the observations on colony growth, conditions enabling fructification are more specific than for vegetative growth.

The following points are important:

1) It could be shown that basidiocarp formation occurs also in vitro. So far all basidiocarps investigated had been collected from natural habitats (Kohlmeyer and Kohlmeyer 1964–1969, 1977, Ginns and Malloch 1977).
2) Fructification is restricted to temperatures between 22 °C and 27 °C, whereas growth occurs between 22 °C and 31 °C (35 °C).
3) At 0 and 200% seawater no basidiocarps were formed; vegetative growth, however, occurred.
4) The pH-value of the medium in the range tested had no apparent influence on basidiocarp formation.
5) The composition of the media, however, seems to have decisive influence on basidiocarp formation: Basidiocarps were found only on media containing peptone and/or additions of wood.
6) Basidiocarps with basidiospores could be found only on plates with full strength seawater and the addition of wood (*Betula*). Basidiocarps producing basidiospores were formed faster than basidiocarps without basidiospores (5 weeks in comparison to 6 up to 8 weeks).

Table II. Effect of several factors on basidiocarp formation of *H. villosa*

Factors varied	Basidiocarp and basidiospore production under respective conditions						
Types of Media used[1])	F 1003 liquid	GYS	PYC (6)	GPY liquid (6)	GPY (8)	GPY + wood (5 S)	Agar + wood (8)
GPY + types of wood (pieces)	*Rhizophora racemosa*	*Tamarix pentandra*	*Betula* spp. (5 S)				
GPY + types of wood (ground)	*Rhizophora mangle* (7)	*Rhizophora racemosa* (7)	*Tamarix pentandra* (7)	*Conocarpus erecta* (7)			
Temperature (°C)	11	14.5	22 (5 S)	27 (5 S)	31	35	
% Seawater	0	25 (8)	50 (8)	75 (8)	100 (5 S)	200	
pH of medium[2])	5.8 (8)	6.4 (8)	7.0 (5 S)				

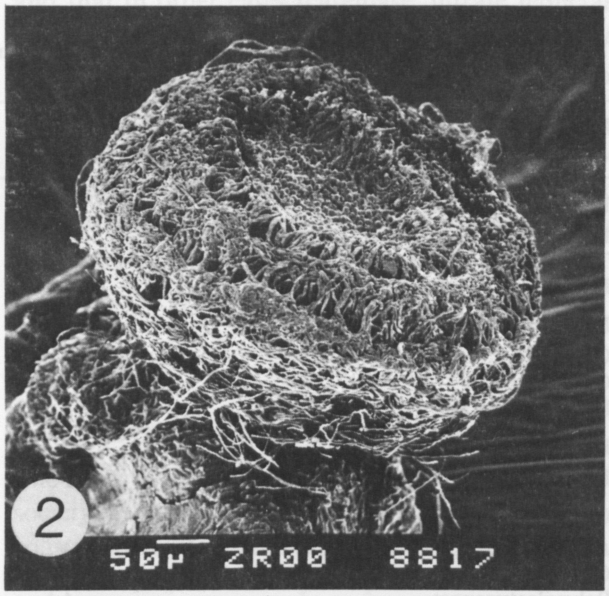
( ) Number in parentheses represents weeks lapsed until fructification
(S) Basidiocarps with basidiospores
[1]) For abbreviations and composition of media see material and methods
[2]) Measured after autoclaving

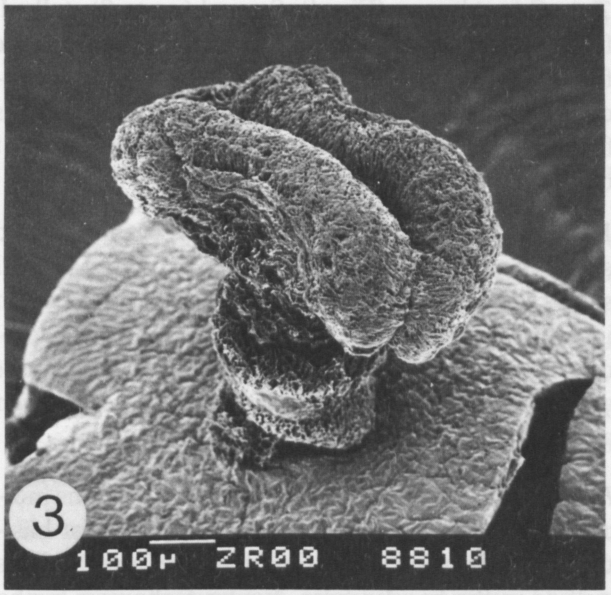
Table III. Formation and types of basidiocarps of *Halocyphina villosa*

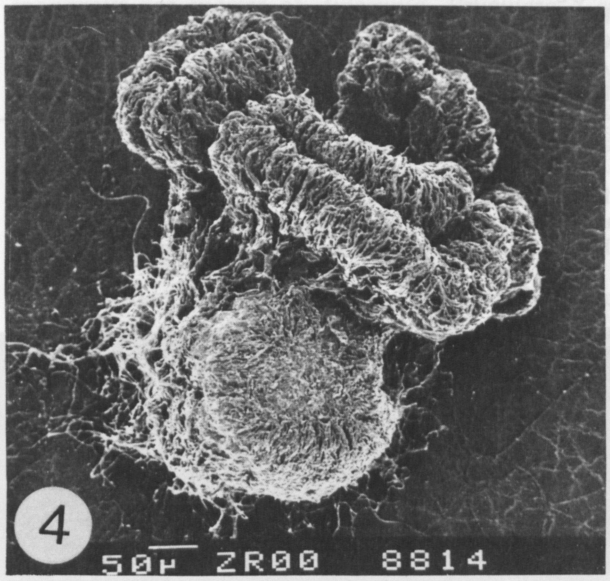
Natural habitat	In vitro		
	Type A	Type B	Type C
Conditions	mangrove swamps	all conditions allowing fructification	only on GPY with *Betula* wood	only on GPY with 50–100% seawater
Morphology of basidiocarp	single basidiocarp funnel-shaped circular from above interwoven hairs covering hymenium stalked basidiocarp diameter 0.5 mm		single basiodiocarp funnel-shaped laterally compressed interwoven hairs covering hymenium stalked basidiocarp diameter 0.5 mm	composite basidiocarp funnel-shaped as type A and/or B interwoven hairs covering hymenium stalked basidiocarp diameter 1.0 mm
Basidia	produced	produced	produced	produced
Spores	produced	produced	not produced	not produced

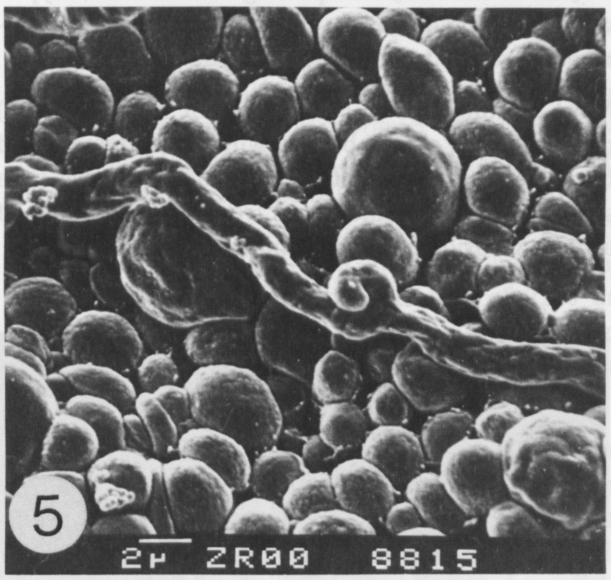
Basidiocarp morphology: As mentioned above, all basidiocarps of *H. villosa* described so far in the literature were collected from the natural habitat. They all belong to the same type: single structured, funnel-shaped and with a stalk. Wavy, hair-like hyphae grow towards the centre from the circular margin of the funnel, which is lined with hymenium. The interwoven hair-like hyphae cover the hymenium and protect it (Ginns and Malloch 1977). In vitro 3 types of basidiocarps, types A, B and C, were found.

Type A basidiocarp (Fig. 2): Stalked, funnel-shaped single structured, circular when viewed from above. The funnel has an inside layer of hymenium. The hairs covering the hymenium appear to be arranged in concentric zones. Only basidiocarps of type A produce basidiospores, which are formed in the hymenium below the hairs and can also be found between those hairs in the centre of the basidiocarp (see Kohlmeyer and Kohlmeyer 1977, fig. 4). The in vitro basidiocarp type A has the same dimensions as basidi-









Figs 2–5
Fig. 2. Type A basidiocarp of *H. villosa* produced in vitro. GPY-medium with *Betula* wood; 100% seawater, pH 7.0, temperature 22 °C; SEM, fixation with osmium; bar = 50 µ. Fig. 3. Type B basidiocarp of *H. villosa* produced in vitro. GPY-medium with *Betula* wood; 100% seawater, pH 7.0, temperature 22 °C; SEM, fixation with formaldehyde; bar = 100 µ. Fig. 4. Type C basidiocarp of *H. villosa* produced in vitro. GPY-medium, 50% seawater, pH 9.0, temperature 27 °C; SEM, fixation with formaldehyde; bar = 50 µ. Fig. 5. Hypha with clamp-connection above the hymenium of a type A basidiocarp of *H. villosa*. GPY-medium with *Betula* wood; 100% seawater, pH 7.0, temperature 22 °C; SEM, fixation with formaldehyde; bar = 2 µ.

ocarps from the natural habitat. This applies also to the form and dimensions of basidia and basidiospores.

Type B basidiocarp (Fig. 3): Stalked, funnel-shaped single structure. The basidiocarp appears to be laterally compressed, almost no zonation is apparent in the hairs originating from the rim of the funnel and converging in the centre forming a distinct central slit. The basidiocarp viewed from above has a coffee-bean-like appearance. These basidiocarps exclusively were found on GPY-medium with wood (*Betula*) and did not form basidiospores.

Type C basidiocarp (Fig. 4): Several (up to 8) stalked, funnel-shaped basidiocarps of type A and B arise from a common basis. These basidiocarps are produced on GPY-medium with seawater concentrations from 50% to 100%. Basidiospore formation was not observed.

Basidiocarps of type B and C have not been described so far in material from the natural habitat. Table III shows the conditions allowing basidiocarp formation in nature and leading to in vitro production of basidiocarps type A, B and C. Morphology of basidiocarps and production of basidia and basidiospores is given as well.

Fine structure of basidiocarp: Clamp-connections indicating dicaryotic mycelium as a prerequisite for basidiocarp formation were found in the mycelium as well as in the basidiocarps. Figure 5 shows a hypha with a clamp-connection lying across the central hymenium of the basidiocarp.

Most basidiocarps (e. g. all those of type B and C) produced only simple basidia without sterigmata and basidiospores. Figure 6 shows some basidia with sterigmata and basidiospores from the hymenium of a type A basidiocarp (light microscopy).

Enzymes

Data on the enzymatic potential of *H. villosa* yield information on the ability of this fungus to degrade certain substrates specific to its natural habitat in a tropical/subtropical mangrove swamp. Production of a series of enzymes was tested using simple qualitative and semiquantitative tests on agar plates. Table IV shows the result of the enzyme tests carried out over a period of at least 4 weeks.

Among the redox-enzymes tested, *H. villosa* produced laccase and peroxidase. Tyrosinase was not found during the test period under the conditions employed.

Table IV. Enzyme production of *Halocyphina villosa* and influence of salinity of medium. Temperature 22 °C.

Enzyme	% Seawater 100%	0%
Redox-Metabolism		
Laccase	+	+
Tyrosinase	–	–
Peroxidase	+	+
Nitrogen-Metabolism		
Caseinase	+	+
Gelatinase	+	+
Nitrate reductase	+	+
Fat-Metabolism		
Lipase	+	–
Carbohydrate-Metabolism		
Amylase	+	+
Cellulase	+	+
Polygalacturonase	–	1
Pectate transeliminase	–	+
Chitinase	–	–
Alginase	u	–
Laminarinase	+	+

\+ Activity within 3 weeks
– no activity
1 activity after 3 weeks
u activity uncertain or not constant
For media and pH-values see material and methods

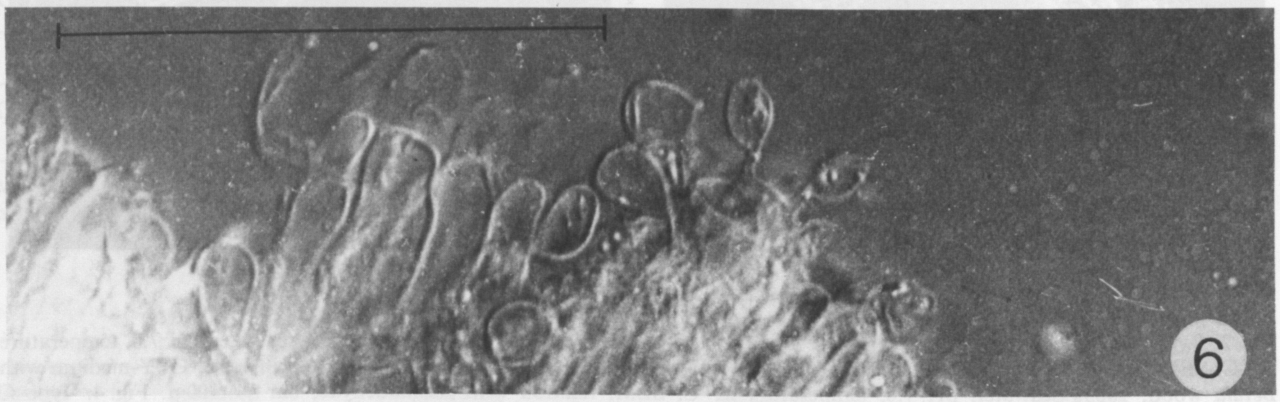


Fig. 6. Hymenium of a type A basidiocarp of *Halocyphina villosa* with basidia, sterigmata and basidiospores. GPY-medium with *Betula* wood; 100% seawater, pH 7.0, temperature 22 °C; light microscopy, phase contrast; bar = 50 µ.

Nitrate reductase and the proteases gelatinase and caseinase, enzymes of the nitrogen-metabolism, were produced on media of 0% and 100% seawater.

As representative for the fat-metabolism, lipase was found to be produced at 100% seawater.

Enzymes representing carbohydrate-metabolism in this fungus were amylase, cellulase, polygalacturonase (late enzyme formation), pectate-transeliminase and laminarinase. Although *H. villosa* must be considered as a marine fungus, it was observed that in media with 0% seawater enzyme formation in some cases was faster (laccase on guaiacol, laminarinase), and that in other cases (caseinase, nitrate reductase, amylase) enzyme activity was stronger than in seawater media.

The only enzymes that were produced neither in seawater nor in deionized water medium under our experimental conditions were tyrosinase, polygalacturonase (activity only after 3 weeks), chitinase and alginase (uncertain or very weak activity).

Discussion

Colony growth

The mangrove is a characteristic marine coastal plant society. It appears as brackish water (estuary) and coastal mangrove and is restricted to tropical/subtropical areas. Woody representatives of the mangrove are species of *Avicennia, Bruguiera, Ceriops, Conocarpus, Laguncularia, Rhizophora, Sonneratia, Tamarix*. The lowest monthly mean temperature in mangrove swamps is around 18 °C; the daily average temperature deviations are less than 10 °C (see West 1956, Tischler 1976).

Colony growth of *H. villosa* was found from 22 °C to 31 °C with an optimum at 27 °C. The results of our in vitro experiments with a relatively high temperature optimum and a relatively narrow temperature range coincide well with the conditions prevailing at the natural habitat of this fungus. Similar temperature ranges between 20 °C and 30 °C were found generally for marine wood-destroying fungi by Johnson and Sparrow (1961), for *Nia vibrissa* Moore et Meyers by Doguet (1968), and for an African species of *Lulworthia* (above 15 °C) by Schaumann (1974).

In mangrove swamps, drastically different salinities from almost freshwater up to full strength seawater (estuary) or even higher (evaporating ponds) are found. Euryhaline plants could therefore be expected. Indeed, vegetative growth of *H. villosa* was found over the full range of salinities with an optimum between 25% and 100% seawater. This coincides well with the conditions in the natural habitat, as found by in vitro experiments by Lee and Baker (1972) for marine fungi from mangrove swamps in Hawaii.

The pH-value of seawater ranges from 8.1 to 8.3 (Kohlmeyer and Kohlmeyer 1979). The water of mangrove swamps has a slightly acidic pH between 6 and 7 (Tischler 1976) because of its high content of humic acids and tannins. Depending on the amount of seawater, pH-values between 6 and 8 could be expected in mangrove swamps. This is also the pH-range of the media in our experiments. This could explain the fact that colony growth and fructification were rather independent of the pH of the medium.

Formation of basidiocarps

The presence of clamp-connections in the mycelium and other criteria led Ginns and Malloch (1977) to include *H. villosa* in the basidiomycetes. Formation of clamp-connections in our culture of *H. villosa* was indicative of dicaryotic mycelium, which is a prerequisite of basidiocarp formation. Under certain experimental conditions, basidiocarp formation was found indeed. As shown in Figure 5, clamp-connections were also found in the basidiocarps, confirming earlier reports (Ginns and Malloch 1977).

The temperature range (22 – 27 °C) and salinity range (25 – 100% seawater) in which basidiocarp production was observed are relatively narrow. They are however within the range of vegetative growth near the growth optimum of 27 °C and about 50% seawater. For the two marine wood-inhabiting basidiomycetes *Digitatispora marina* Doguet and *Nia vibrissa* Moore et Meyers, Doguet (1964, 1968) could also demonstrate coincidence of optimum temperature for vegetative growth and fructification. Basidiocarp formation of *H. villosa* in our in vitro experiments requires a minimal seawater concentration of 25% and proves that *H. villosa* is an obligate marine fungus according to the definition of Kohlmeyer and Kohlmeyer (1979).

An increasing salinity tolerance with increasing temperature, showing a combined influence of temperature and salinity on colony growth, was first found by Ritchie (1957) for several species of the marine imperfect genus *Phoma* and was correspondingly called 'Phoma-pattern'. Two observations in *H. villosa* point in the same direction: The salinity optimum of colony growth at 31 °C (50 – 75% seawater) was higher than at 27 °C (25 – 50% seawater). The highest number of basidiocarps per plate was obtained at 22 °C with a salinity of 25% to 50% seawater; at the

higher temperature of 27 °C the highest number of basidiocarps occurred at the higher seawater concentration of 50% to 100%. However, these are isolated observations, and since this phenomenon was not found over the whole temperature range, it possibly should not be called 'Phoma-pattern' sensu stricto in contrast to the observations with *Digitatispora marina* Doguet (Doguet 1964, 1968).

At temperatures from 22 °C to 27 °C and salinities from 25% to 100% seawater, basidiocarps were formed only provided the medium contained peptone as a nitrogen source and/or wood and cellulose, respectively. Therefore, the conditions necessary for fructification also designate *H. villosa* as a wood-inhabiting fungus. It would be interesting in this context to find out the specific substance or substances responsible for basidiocarp formation.

Depending on the medium used, basidiocarp formation began at the earliest 4 weeks after inoculation with primordium formation, leading to basidiocarp production within another week. With certain media in vitro basidiocarp production came to a standstill at different stages (primordia, basidiocarps without basidiospores, basidiocarps with basidiospores).

Morphology of basidiocarps

Basidiocarps of type A, B and C were formed in culture. In nature only the occurrence of the circular, funnel-shaped, stalked basidiocarps of type A has been reported in the literature so far (Kohlmeyer and Kohlmeyer 1964–1969, 1965, 1977, Hughes 1975, Ginns and Malloch 1977, Koch 1982).

Since only in vitro basidiocarps of type A produce basidiospores, this seems to be the natural propagation structure of *H. villosa*. As types B and C were only found under experimental conditions, and since they do not produce basidiospores, they apparently are not of biological significance. Basidiospores in type A basidiocarps were only produced on GPY-media containing *Betula*-wood. It is not known which structural or chemical properties of *Betula*, a species which does not occur in mangrove swamps, are responsible for basidiospore-formation in type A basidiocarps. Gramss (1979) was able to show the influence of some media on basidiocarp formation of several wood-destroying basidiomycetes; however, no marine fungi were included in this study. Depending on the medium, basidiocarp formation proceeded to different stages of development. Doguet (1968) reported for the marine basidiomycete *Nia vibrissa* Moore et Meyers the influence of light on basidiocarp formation. Since in *H. villosa* no differences were found in basidiocarp formation between incubation in constant light, in constant darkness and in light/darkness cycles (Rohrmann and Molitoris, unpublished results), light apparently does not influence basidiocarp formation in this fungus.

Enzymes

The investigation of the ability to produce certain enzymes can provide important data for physiological characterization of an organism. The mainly extracellular enzymes tested here were chosen for ecological relevance, physiological importance and suitability for investigation by availability of simple qualitative and semiquantitative tests on agar plates.

As a wood-inhabiting fungus, *H. villosa* should be able to degrade wood. That was proved by the presence of laccase and peroxidase, enzymes involved in wood degradation and typical for white rot fungi degrading both lignin and cellulose. Until now, among the marine fungi white rot type of wood degradation as indicated by presence of the typical enzymes and by microscopical investigations could be shown only for the basidiomycete *Nia vibrissa* Moore et Meyers (Leightley and Eaton 1979). In most other cases wood degradation in marine fungi followed the soft rot type, i.e. wood degradation by dissolution of secondary walls of wood cells (see Leightley 1980). Investigating the carbohydrate metabolism, the presence of cellulase and pectinase, two more enzymes important for wood degradation, could be demonstrated. The presence of amylase and laminarinase (possibly also alginase, see results) indicates the ability of the fungus to use other reserve materials of plants. Similarly, Gessner (1980) could show the presence of certain enzyme activities by means of which marine fungi could use the plants of salt marshes as substrates.

The spectrum of usable substrates in the natural habitat was furthermore increased by the ability of *H. villosa* to degrade fats (lipase) and proteins (gelatinase, caseinase).

Seawater contains up to 100 mg m^{-3} nitrate. The production of nitrate reductase would allow the use of nitrate as an additional nitrogen source. *H. villosa* synthesizes this enzyme irrespective of the salinity of the medium. That is in agreement with results of Molitoris and Schaumann (1986), showing that all obligate marine fungi tested so far in their survey are able to produce nitrate reductase.

In summary it can be stated that the marine basidiomycete *Halocyphina villosa* is well adapted to the

climate, water and substrate conditions in its natural habitat, the tropical/subtropical mangrove swamp. This follows from the results of in vitro experiments on optimal conditions for vegetative growth, basidiocarp formation and production of necessary enzymes for substrate degradation.

Acknowledgements

This paper is part of a thesis by S. R. performed in the laboratory and under the guidance of Prof. Dr H. P. Molitoris. The authors thank Prof. Dr T. Anke, University of Kaiserslautern and Dr J. Kupka, Basel, for a culture of *Halocyphina villosa*, and Mr R. Summers, M. A., for correcting the English text.

References

Barghoorn, E. S. and D. H. Linder. 1944. Marine fungi: Their taxonomy and biology. *Farlowia 1*: 395–467.

Doguet, G. 1964. Influence de la temperature et de la salinité sur la croissance et la fertilité du *Digitatispora marina* Doguet. *Bull. Soc. Fr. Physiol. Veg. 10*: 285–292.

Doguet, G. 1968. *Nia vibrissa* Moore & Meyers Gastéromycete marin. 1. Conditions générales de formation des carpophores en culture. *Bull. Soc. Mycol. Fr. 84*: 343–351.

Esser, K. 1974. *Podospora anserina. In*: (C. R. King, ed.) *Handbook of Genetics*, Vol. 1. Plenum Press, New York. pp. 531–551.

Gessner, R. V. 1980. Degradative enzyme production by saltmarsh fungi. *Bot. Mar. 23*: 133–139.

Ginns, J. and D. Malloch. 1977. *Halocyphina*, a marine basidiomycete (Aphyllophorales). *Mycologia 69*: 53–58.

Gramss, G. 1979. Die Fruchtbildung höherer Pilze. II. Holzzerstörende Basidiomyceten. *Z. Mycol. 45*: 195–208.

Hughes, G. C. 1975. Studies of fungi in oceans and estuaries since 1961. I. Lignicolous, caulicolous and foliicolous species. *Mar. Biol. A. Rev. 13*: 69–180.

Johnson, T. W. and F. K. Sparrow. 1961. *Fungi in Oceans and Estuaries*. Cramer, Weinheim.

Koch, J. 1982. Some lignicolous marine fungi from Sri Lanka. *Nord. J. Bot. 2*: 163–169.

Kohlmeyer, J. 1974. On the definition and taxonomy of higher marine fungi. *Veröff. Inst. Meeresforsch. Bremerh. Suppl. 5*: 263–286.

Kohlmeyer, J. and E. Kohlmeyer. 1964–1969. *Icones Fungorum Maris*. Cramer, Weinheim und Lehre.

Kohlmeyer, J. and E. Kohlmeyer 1965. New marine fungi from mangroves and trees along eroding shorelines. *Nova Hedwigia 9*: 89–104.

Kohlmeyer, J. and E. Kohlmeyer. 1977. Bermuda marine fungi. *Trans. Br. Mycol. Soc. 68*: 207–219.

Kohlmeyer, J. and E. Kohlmeyer. 1979. *Marine Mycology. The Higher Fungi*. Academic Press, New York, San Francisco, London.

Kupka, J., T. Anke, W. Steglich and L. Zechlin. 1981. Antibiotics from basidiomycetes XI. The biological activity of siccayne, isolated from the marine fungus *Halocyphina villosa* J. & E. Kohlmeyer. *J. Antibiotics 34*: 298–304.

Lee, B. K. H. and G. E. Baker 1972. Fungi associated with the roots of red mangrove, *Rhizophora mangle*. *Mycologia 65*: 894–906.

Leightley, L. E. 1980. Wood decay activities of marine fungi. *Bot. Mar. 23*: 387–395.

Leightley, L. E. and R. A. Eaton. 1979. *Nia vibrissa* – a marine white rot fungus. *Trans. Br. Mycol. Soc. 73*: 35–40.

Molitoris, H. P. and K. Schaumann 1986. Physiology of marine fungi. A screening program for enzyme activities. *In*: (S. T. Moss, ed.) *The Biology of Marine Fungi*. Cambridge Univ. Press, Cambridge pp. 35–48.

Moser, M. 1959. Beiträge zur Kenntnis der Wuchsstoff-Beziehungen im Bereich ectotropher Mycorrhizen I. *Arch. Microbiol. 34*: 151–169.

Ritchie, D. 1957. Salinity optima for marine fungi affected by temperature. *Am. J. Bot. 44*: 870–874.

Schaumann, K. 1974. Experimentelle Untersuchungen zum Einfluß des Salzgehaltes und der Temperatur auf das Mycelwachstum höherer Pilze aus dem Meer- und Brackwasser. *Veröff. Inst. Meeresforsch. Bremerh. Suppl. 5*: 443–474.

Sguros, P. L., S. P. Meyers and J. Simms 1962. Role of marine fungi in the biochemistry of the oceans. I. Establishment of quantitative technique for cultivation, growth measurement and production of inocula. *Mycologia 54*: 521–535.

Tischler, W. 1976. *Einführung in die Ökologie*. Gustav Fischer Verlag, Stuttgart, New York.

West, R. C. 1956. Mangrove swamps of the Pacific coast of Colombia. *Ann. Ass. Am. Geogr. 46*: 98–121.