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Colony growth and fruitbody production of monokaryotic and dikaryotic strains of the marine Basidiomycete *Nia vibrissa*

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Abstract

Isolates of the marine wood-inhabiting Basidiomycete *Nia vibrissa* from various marine zones were investigated for nuclear status, colony growth and fructification. Eight of the eleven strains tested by microfluorimetry proved to be dikaryotic, three were monokaryotic. Investigation of colony growth showed better growth at 22 °C than at 18 °C; the addition of wood and incubation under light had no significant effect on growth. The combined influence of temperature (18, 22, 27 °C) and salinity (0, 25, 50, 75, 100, 150, 200, 300 % of natural seawater) was tested and an increase of salinity optimum with increasing incubation temperature (PHOMA-PATTERN) was shown for almost all strains. All dikaryotic strains showed clamp connections. Fructification occurred in both dikaryotic and monokaryotic strains and more frequently on seawater media than on deionized water media. Addition of wood increased fructification only slightly. Generally, the best fructification occurred around 18 °C and in the salinity range with a concentration of between 25 % - 150 % seawater. Light and electron microscopy showed the typical structure of a gasteroid fructification with spores and both with and without peridia.

Introduction

Nia vibrissa MOORE and MEYERS (1959), a wood-degrading cosmopolitan Gasteromycete, is one of only six marine Basidiomycetes. Previous investigations of *Nia vibrissa* dealt primarily with morphology and taxonomy; the few physiological investigations were carried out usually with only one isolate (DOGUET 1968, 1969a, 1969b; for literature see also SCHIMPFHAUSER 1990).

In this paper several monokaryotic and dikaryotic strains from several marine biogeographical zones (HUGHES 1974, KOHLMEYER 1983) were compared for their growth on different substrates, salinities and temperatures, for the appearance of PHOMA-PATTERN and for the formation and type of fruitbodies.

Materials and methods

Biological material: Origin, suppliers and type of *Nia vibrissa* strains are given in

Table 1. Three of the eleven strains were shown by microfluorimetry to be monokaryotic (BRESINSKY et al. 1987).

Table 1. Strains, origin, biogeographical zone and nuclear status of the isolates of *Nia vibrissa*.

Strain	Original[1] designation	Location	Biogeograph. zone	Nuclear status
M154	JK 5071	Virgin Isl. (USA)	tropical	dikaryon
M167	pp 2765	Holland	northern temp.	dikaryon
M168	pp 2767	Holland	northern temp.	dikaryon
M169	pp 2768	Holland	northern temp.	dikaryon
M170	pp 2770	Holland	northern temp.	dikaryon
M171	pp 2772	Holland	northern temp.	dikaryon
M175	ATCC 34606	Rhode Isl. (USA)	northern temp./ subtropical	dikaryon
M21	KMPB 14662 ATCC 34606	Rhode Isl. (USA)	northern temp./ subtropical	dikaryon
M172	pp 1068	Seychelles	tropical	monokaryon
M173	IFO 32088	Japan	northern temp.	monokaryon
M174	IFO 32086	Japan	subtropical	monokaryon

[1] JK = J. Kohlmeyer, USA; pp = E.B.G. Jones, U.K.; ATCC = American Type Culture Collection; KMPB = K. Schaumann, Kulturensammlung mariner Pilze, Bremerhaven, Germany; IFO = A. Nakagiri, Japan.

Media, incubation, growth and evaluation: The strains were transferred and grown in 9 cm Ø petri dishes on glucose (1 g/l) - peptone (0.5 g/l) - yeast extract (0.1 g/l) medium prepared with natural (GPYN) or synthetic (GPYS) seawater and deionized (GPYI) water as described in MOLITORIS and SCHAUMANN (1986). The synthetic seawater was prepared with RILA MARINE MIX (RILA PRODUCTS, Teaneck, N.J., USA). Wood (W) was added in the form of sterilized tooth picks (*Betula* sp.) at the agar surface. Incubation temperatures are indicated in Table 3. Illumination conditions were constant darkness (D) or a cycle of 12h light/12h darkness (L/D). Growth was measured as an increase in colony diameter. All experiments were conducted on triplicate plates and the means are given in the tables.

PHOMA-PATTERN: Growth on agar plates was determined after 10 days at different temperatures (10, 18, 22, 27 and 30 °C) each at different salinities (0, 25, 50, 75, 100, 150, 200 and 300 % of natural seawater). Plotting the salinity growth optima (ordinate) against the incubation temperature resulted in an upwards directed curve in the case of PHOMA-PATTERN (RITCHIE 1957), indicating an increased salinity optimum with increasing incubation temperature.

Reproductive structures: Formation and quantity of reproductive structures were determined from the experiments on growth (up to six months) and PHOMA-PATTERN (up to four months). The reproductive structures were documented using light microscopy (Zeiss photomacroscope in vivo and Zeiss Axiomat after semi-thin sectioning) and scanning electron microscopy (Zeiss DSM 950) as described in SCHIMPFHAUSER (1990).

Results and discussion

Colony growth: *Nia vibrissa* grew on GPY-agar plates as a fine white mycelium of medium density. Colonies often showed zonation and sometimes sectorformation. Sclerotia (hard and white, usually spherical and of various dimensions) were found regularly, regardless the incubation conditions. The growth of monokaryotic strains resembled that of dikaryotic strains. In most cases natural seawater resulted in a somewhat better colony growth, while illumination slightly retarded it. Addition of wood in combination with deionized water or synthetic seawater also retarded colony growth slightly, whereas the addition of wood in combination with natural seawater slightly stimulated it. Colony growth was better at 22 °C than at 18 °C. The optimal salinity for colony growth was generally between 50 % and 75 % of natural seawater.

PHOMA-PATTERN: Optimal growth temperature was between 22 °C and 27 °C with the exception of the tropical strain M154 with a higher growth optimum of 30 °C. The optimal salinity was between 50 % and 75 % natural seawater with no differences between monokaryons and dikaryons or isolates of different biogeographical zones. Of the eleven strains investigated, seven showed definitely PHOMA-PATTERN (Fig. 1), three originated from subtropical zones (M174, M175 and M21) and one from the tropical zone (M172). Two strains showed only a slight PHOMA-PATTERN (M154, M173) whereas two others from northern temperate zones (M168, M170) showed none. Furthermore, PHOMA-PATTERN was observed in both monokaryotic (M172) and dikaryotic (M167, M169, M171, M175, M21) strains.

Fruitbody production: As is evident from Table 2, clamp connections occurred only in dikaryotic strains. The strains M170, M171, M21, M173 and M174 did not produce primordia or fruitbodies. All other strains formed primordia after 3-6 weeks of incubation, from which mature fruitbodies with spores developed. Only strain M154 did not produce fruitbodies for at least 10 weeks. Two different types of fruitbodies were observed: Type A - hairy fruitbodies, the gleba mass being covered by a peridium with protruding hyphae (Plate 1a, 1b, 1c) showing bifurcate tips (Plate 1d). Type B - smooth fruitbodies without peridium and without protruding hyphae (Plate 1e, 1f).

As Table 3 shows, illumination did not enhance fructification but reduced the number of fruitbodies, as did the medium prepared with deionized water (GPYI). The addition of wood (W) stimulated fruitbody formation. Fructification was generally better at 18 °C than at 22 °C and 27 °C. The best results were found at salinities between 50 % and 100 % of natural seawater whereas at 0 % very few and at 200 % and 300 % no fruitbodies were produced at all (Table 4).

MOORE and MEYERS (1959) in their first paper on *Nia vibrissa* described this marine wood-degrading fungus and classified it as a Deuteromycete within the

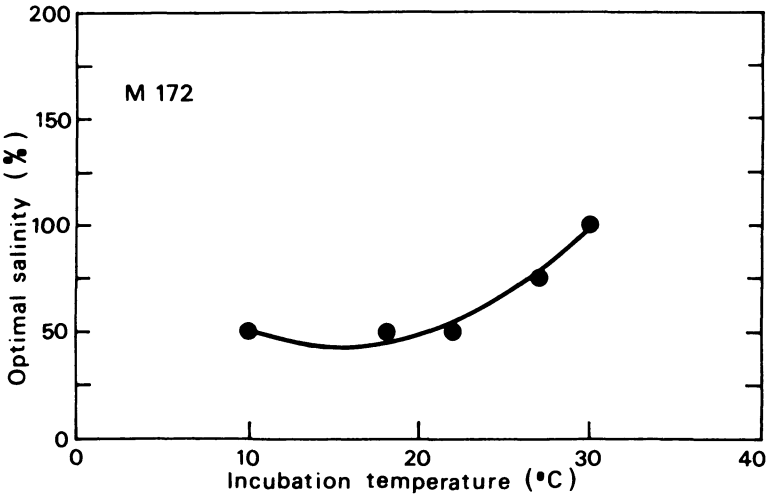

Fig. 1. PHOMA-PATTERN in the monokaryotic strain M172 of *Nia vibrissa*.

Table 2. Formation of clamp connections and reproductive structures in monokaryotic and dikaryotic strains of *Nia vibrissa*.

Strain	Clamps	Sclerotia	Primordia	Fruitbody	Spores
Dikaryons					
M154	+	+	+	o	+
M167	+	+	+	o/*	+
M168	+	+	+	o/*	+
M169	+	+	+	o/*	+
M170	+	+	-	-	-
M171	+	+	-	-	-
M175	+	+	+	o/*	+
M21	+	+	-	-	-
Monokaryons					
M172	-	+	+	o/*	+
M173	-	+	-	-	-
M174	-	+	-	-	-

+ = present; - = absent; o = fruitbody without hairy peridium; * = fruitbody with hairy peridium.

Thalassiomycetes. DOGUET investigated this fungus further in 1967 and reclassified it as the only marine Gasteromycete, belonging to the Melanogastrales (DOGUET 1969a, KOHLMEYER and KOHLMEYER 1979).

Table 3. Frequency of fruitbody production of *Nia vibrissa* strains depending on temperature, light, water quality and addition of wood (experiments on colony growth; incubation time: 4 months).

		Fruitbodies								
		18°C D[2]			22°C D			22°C L/D		
M[1]	strain	single	few	many	single	few	many	single	few	many
I	M154									
	M167									
	M168									
	M169									
	M175	■			■					
	M172	■	■							
I+W	M154									
	M167							■		
	M168	■	■							
	M169	■	■		■			■		
	M175	■			■	■		■		
	M172				■	■		■	■	
S	M154									
	M167	■	■		■	■		■		
	M168	■	■		■					
	M169	■	■	■	■	■		■	■	
	M175									
	M172	■	■		■	■		■	■	
S+W	M154	■						■		
	M167	■	■	■	■	■		■	■	
	M168	■	■		■	■		■	■	■
	M169	■	■	■	■	■		■	■	■
	M175				■					
	M172	■			■	■		■		
N	M154									
	M167	■	■		■	■		■		
	M168	■								
	M169	■	■	■	■			■		
	M175									
	M172	■	■		■	■		■	■	
N+W	M154				■			■		
	M167	■	■	■	■			■		
	M168	■	■		■	■		■	■	
	M169	■	■	■	■	■		■		
	M175	■								
	M172				■			■		

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[1] M = Medium: I = deionized water; S = synthetic seawater; N = natural seawater; W = wood.

[2] Illumination: D = constant darkness; L/D = cycle of 12h light/12h darkness.

Table 4. Frequency of fruitbody production of *Nia vibrissa* strains depending on temperature and salinity (experiments on PHOMA-PATTERN; medium: GPYS; illumination: constant darkness; incubation time: 6 months).

		Fruitbodies								
		18°C			22°C			27°C		
M[1])	strain	single	few	many	single	few	many	single	few	many
0%	M154									
	M167									
	M168									
	M169	■								
	M175									
	M172	■	■	■	■					
25%	M154									
	M167	■	■	■	■					
	M168	■	■							
	M169	■	■		■			■		
	M175	■								
	M172	■			■					
50%	M154									
	M167	■	■	■	■					
	M168	■	■	■	■			■		
	M169	■	■		■	■		■		
	M175									
	M172	■	■	■	■	■				
75%	M154									
	M167	■	■							
	M168	■	■		■					
	M169	■	■		■	■				
	M175									
	M172	■	■	■	■	■		■		
100%	M154									
	M167	■	■	■						
	M168	■	■	■	■					
	M169	■	■		■	■		■	■	
	M175									
	M172	■	■		■	■				
150%	M154									
	M167	■	■							
	M168	■	■	■	■					
	M169	■	■							
	M175									
	M172	■	■	■	■	■		■	■	

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[1] M = Medium; salinity varying from 0 % to 150 % natural seawater concentration.

The first physiological investigations on *Nia vibrissa* were conducted by DOGUET (1968, 1969a and 1969b) using isolates from one location of the northern temperate biogeographic zone. This is in contrast with our work in which we investigated several mono- and dikaryotic isolates from various biogeographical zones.

Colony growth: As already reported by JONES and IRVINE (1971) and confirmed by our findings *Nia vibrissa* grows very slowly under laboratory conditions with the exception that some tropical isolates (e.g. M154) show faster growth if the incubation temperature is increased.

DOGUET (1968) reported better growth in total darkness than in a cycle of 12h light/12h darkness, which we could confirm since our cultures also showed better growth in constant darkness than in alternating 12h light/12h darkness.

The observed slight inhibition of colony growth caused by the addition of wood might be a merely mechanical effect. According to DOGUET (1969b) optimal growth temperature for the cosmopolitan *Nia vibrissa* is between 15 °C and 25 °C with a lower limit of 4 °C (reversible inhibition) and an upper limit of 35° C (irreversible inhibition). In our experiments, based on a wider selection of strains, this could be confirmed in principle, however with an extension of the optimum temperature to 27 °C. This is comparable with another wood-degrading marine Basidiomycete, ***Halocyphina villosa***, from tropical and subtropical zones showing best colony growth between 22 °C and 27 °C (ROHRMANN and MOLITORIS 1986) whereas the wood-degrading marine Basidiomycete ***Digitatispora marina*** from temperate zones showed best growth at 15 °C (DOGUET 1964).

PHOMA-PATTERN: JENNINGS (1982) reported optimal growth for some higher marine fungi at a salinity of 100 % of natural seawater but stated that in most cases growth is better at lower salinities, particularly for marine Basidiomycetes which in this respect are more sensitive than Asco- and Deuteromycetes. This is in agreement with our observation that in none of the *Nia vibrissa* strains tested the optimal salinity was higher than 100 % natural seawater, regardless of incubation temperature. At medium incubation temperatures, the salinity optima

Plate 1.

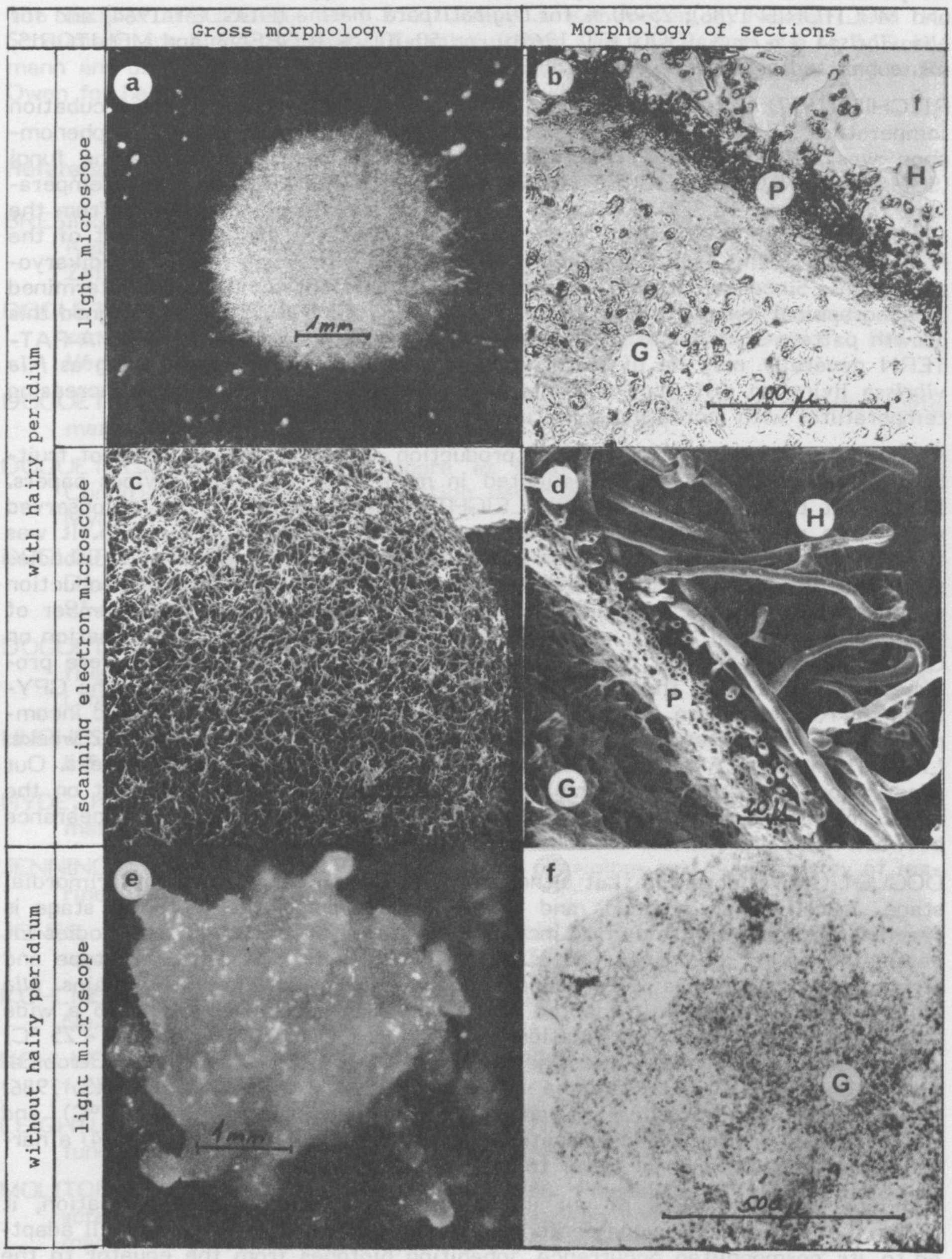
a) Gross morphology of "hairy" fruitbody of *Nia vibrissa*, strain M172, in the photomacroscope (12 w incubation, 22 °C, S-medium)
b) Semi-thin section of "hairy" fruitbody of *Nia vibrissa*, strain M172, under a light microscope (16 w incubation, 22 °C, S-medium)
c) Gross morphology of "hairy" fruitbody of *Nia vibrissa*, strain M172, in scanning electron microscope (9 w incubation, 22 °C, S-medium, freeze-dried)
d) Freeze-fractured section of "hairy" fruitbody of *Nia vibrissa*, strain M172, in scanning electron microscope (9 w incubation, 22 °C, S-medium)
e) Gross morphology of "naked" fruitbody of *Nia vibrissa*, strain M169, in the photomacroscope (8 w incubation, 22 °C, S-medium)
f) Semi-thin section of "naked" fruitbody of *Nia vibrissa*, strain M172, in light microscope (16 w incubation, 22 °C, S-medium)

Abbreviations:

G = gleba with spores
P = peridium
H = hooked hyphes of the peridium

LM = light microscopy
SEM = scanning electron microscopy
w = weeks

Plate 1. Morphology and anatomy of fruitbodies of *Nia vibrissa*.



for growth were lower in the case of some other wood-degrading marine Basidiomycetes tested so far: namely 50-100 % for *Halocyphina villosa* (ROHRMANN and MOLITORIS 1986); 25-90 % for *Digitatispora marina* (DOGUET 1964) and for *Nia vibrissa* 15-75 % (DOGUET 1969b), or 50-100 % (LORENZ and MOLITORIS, ms. submitted) and (SCHIMPFHAUSER 1990).

RITCHIE (1957) called an increased salinity tolerance with increasing incubation temperature "PHOMA-PATTERN" according to the fungus in which this phenomenon was first observed. This was confirmed for several other marine fungi (SCHAUMANN 1974). DOGUET (1969b) found PHOMA-PATTERN in the temperature range from 10 °C - 30 °C for a dikaryotic isolate of *Nia vibrissa* from the temperate zone. The present paper shows PHOMA-PATTERN for most of the *Nia vibrissa* strains investigated with no differences between mono- and dikaryotic strains. Since our strains are derived from different temperature-determined biogeographical zones, this is in conflict with RITCHIE (1957) who assigned this growth pattern to tropical and subtropical isolates. Nevertheless, PHOMA-PATTERN generally may be of selective advantage for marine strains such as *Nia vibrissa* living on driftwood and intertidal wood and being exposed to increasing temperatures with increasing salinities, at least for a short time.

Fruitbody production: The fruitbody production and types and structure of fruitbodies in *Nia vibrissa* were investigated in more detail than in previous papers. In contrast to DOGUET (1968) and LEIGHTLEY and EATON (1979), we observed wood as a natural substrate was not necessary to induce fructification. It was found, however, that the addition of wood enhances the number of fruitbodies produced. Similarily, we proved that light does not prevent fruitbody production as claimed by DOGUET (1968), although darkness does enhance the number of fruitbodies produced. HYDE et al. (1987) did not observe fruitbody formation on GPY-medium whereas in our experiments at least some fruitbodies were produced, the number of them being increased when starch was added to the GPY-medium. Regarding the time of fructification, DOGUET (1969a) observed incomplete fruitbodies after only 4-8 weeks and complete fruitbodies after 12 weeks. LEIGHTLEY and EATON (1979) found the first fruitbodies after 10 weeks. Our observations show that the time of fructification is strongly dependent on the isolate of *Nia vibrissa* investigated with no difference in the time of appearance between hairy and hairless fruitbodies.

DOGUET (1969a) observed that although fructification can stop at the primordial stage, fructification proceeds and spores are formed if the protocyst stage is reached. We can confirme this, including the observation that all fruitbodies investigated so far contained spores. No differences regarding number, type and structure of fruitbodies were found between mono- and dikaryotic strains. *Nia vibrissa* seems to be well adapted to its universal occurrence owing to a wide temperature range for fructification as shown by DOGUET (1969b) (15 - 25 °C) and as confirmed by our investigation (18 - 27 °C). In contrast, the tropical marine Basidiomycete *Halocyphina villosa* (ROHRMANN and MOLITORIS 1986) shows a narrower fructification range at higher temperatures (22 - 27 °C) and the temperate marine Basidiomycete *Digitatispora marina* (DOGUET 1964) a narrow fructification range at lower temperatures (15 - 20 °C).

Summing up the results of our investigation of growth and fructification, it seems that the marine wood-degrading Basidiomycete *Nia vibrissa* is well adapted to its cosmopolitan occurrence, inhabiting biotopes from the equator to the arctic zone.

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