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Enzyme activities of monokaryotic and dikaryotic strains of the marine Basidiomycete *Nia vibrissa*

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Abstract

Mono- and dikaryotic isolates of the marine wood-inhabiting Basidiomycete *Nia vibrissa* from various marine zones were investigated in semiquantitative tests for 15 enzymes in redox metabolism (laccase, peroxidase, tyrosinase), carbohydrate metabolism (amylase, cellulase, chitinase, β-glucosidase, laminarinase, pectate transeliminase, xylanase), fat metabolism (lipase), nitrogen metabolism (caseinase, gelatinase, nitrate reductase, urease). All experiments were conducted at 22 °C on agar plates or test tubes with media containing synthetic seawater or deionized water. Most of the strains showed an identical enzyme pattern. Amylase, caseinase, cellulase, gelatinase, laminarinase, lipase, nitrate reductase, peroxidase and xylanase were found in all strains, tyrosinase in none. The production of enzymes did not show any significant differences as regards the nuclear status (mono- or dikaryon) or the biogeographical origin of the strains.

Introduction

Nia vibrissa MOORE and MEYERS (1959), is the only wood degrading marine Gasteromycete. Previous investigations of *Nia vibrissa* dealt primarily with morphology and taxonomy (DOGUET 1967, KOHLMEYER 1963), the few physiological investigations were carried out usually using only one isolate (DOGUET 1968, 1969a, 1969b; for further references see also SCHIMPFHAUSER 1990). In their search for the enzyme pattern of the fungus, LEIGHTLEY and EATON (1979) tested for enzymes involved in both lignin degradation (laccase and tyrosinase) and carbohydrate metabolism (cellulase, mannanase and xylanase). The presence of laccase indicated a white rot type of wood degradation.

In this paper several monokaryotic and dikaryotic strains from several marine biogeographical zones (HUGHES 1974, KOHLMEYER 1983) were compared for the production of 15 enzymes on synthetical seawater and deionized water media.

Material and methods

Biological material: The origin and suppliers of *Nia vibrissa* strains are given in the first paper in this series (SCHIMPFHAUSER and MOLITORIS 1991). Three of the 11 strains were shown by microfluorimetry to be monokaryotic (see SCHIMPFHAUSER and MOLITORIS 1991).

Media, incubation, growth and evaluation: The strains were transferred to 9 cm ϕ petri dishes and grown on a glucose (1 g/l) - peptone (0.5 g/l) - yeast extract (0.1 g/l) medium made up with synthetic seawater (GPYS) or deionized water (GPYI), as described in MOLITORIS and SCHAUMANN (1986). The synthetic seawater was prepared with RILA MARINE MIX (RILA PRODUCTS, Teaneck, N.J., USA). The incubation temperature was 22 °C.

Enzyme tests: For the determination of laccase with syringaldazine (HARKIN and OBST 1973), cellulase with carboxymethylcellulose (TEATHER and WOOD 1982), cellulase with avicel coupled with RBBR (Remazol-brillant-blue-R) (NG and ZEIKUS 1980, SMITH 1977, COLLETT 1984) and β-glucosidase with arbutin (KREISEL and SCHAUER 1987), see ROHRMANN and MOLITORIS (1991). The presence of laminarinase was determined with soluble laminarin in GPY-medium containing 5 % soluble laminarin (prepared after THIEM et al. 1977) by staining the non-degraded laminarin with congo red (TEATHER and WOOD 1982; as modified by ROHRMANN, personal communication). Urease activity (splitting of urea into CO_2 and ammonia) results in a pH increase which is demonstrated by turning red the pH indicator phenol-red (CHRISTENSEN 1946 in KREISEL and SCHAUER 1987). Xylanase activity was shown by precipitating the residual xylan by ethanol (FLANNIGAN 1980). For the remaining enzyme tests (laccase with α-naphthol, laccase with guajacol, laccase with benzidin, peroxidase with benzidin, tyrosinase with p-cresol and glycin, amylase with soluble starch, chitinase with colloidal chitin, laminarinase with insoluble laminarin, pectate transeliminase with pectin from apples, lipase with tween 80 and $CaCl_2$, caseinase with skim milk powder, gelatinase with gelatin and nitrate reductase with $NaNO_3$ and Gries Ilosvaye reagent) see MOLITORIS and SCHAUMANN (1986).

All direct enzyme tests (for explanation see Table 1) and the growth determination were conducted in triplicate; for the indirect enzyme tests 5 plates were used for each enzyme/strain combination. Growth determination and enzyme tests were carried out at weekly intervals for at least 7 weeks.

Results and discussion

The results of the enzyme tests for 15 enzymes in redox, fat, nitrogen and carbohydrate metabolism, both on synthetic (S) and deionized water (I) media, are given in arbitrary units for the monokaryotic and dikaryotic strains of *Nia vibrissa* in Table 1. From Table 1 the following results can be summarized:

Amylase, caseinase, cellulase, gelatinase, laminarinase, lipase, nitrate reductase, peroxidase and xylanase are produced by all strains in at least one test on I- and/or S-medium.

Chitinase, β-glucosidase, laccase, pectate transeliminase and urease are found for about 2/3 of the strains in at least one test on I- and/or S-medium.

Tyrosinase is not produced by any of the strains tested.

Regarding the enzyme activity investigated using several tests (cellulase, laccase and laminarinase), it is evident that for cellulase the results differ only quantitatively, whereas for laccase the results depend on the strain and enzyme tests used. When tested with soluble laminarin, laminarinase was found in 100 % of the strains, when tested with insoluble laminarin, laminarinase was not found in any strain.

No general picture could be formed of the effect of synthetic or deionized water medium on enzyme activity, since some enzymes are produced by a high percentage of strains on seawater medium (chitinase, β-glucosidase, peroxidase, urease), laccase was produced preferentially on deionized water medium (laccase), whereas lipase was found exclusively on deionized water medium. Amylase, caseinase, cellulase, gelatinase, nitrate reductase and xylanase were produced by all strains on both media.

Neither nuclear status nor the biogeographical origin of the strains appeared to have a significant influence on enzyme production.

Redox metabolism

Peroxidase (EC 1.11.1.7.), tyrosinase (EC 1.14.18.1.) and laccase (EC 1.10.3.2.) are involved in wood synthesis and degradation (MOLITORIS 1976 and MOLITORIS 1979) laccase being specific of the white rot type of wood degradation and responsible for the linkage of lignin with cellulose degradation (MOLITORIS 1979). Owing to methodological difficulties, laccase was investigated using different tests. Taking the results together, all strains are able to produce laccase, preferentially on I-medium. This agrees well with the fact that *Nia vibrissa* is a wood-degrading fungus of the white rot type, confirming also the results of LEIGHTLEY and EATON (1979). Laccase production was also specifically found in the peridia of *Nia vibrissa* fruitbodies in the guajacol test (this paper) pointing to the often-discussed role of phenoloxidases in fungal morphogenesis and propagation (MOLITORIS 1976).

The absence of tyrosinase in the p-cresol test agrees with previous results by LEIGHTLEY and EATON (1979) for *Nia vibrissa*. This enzyme was not found in the other wood-degrading marine Basidiomycete, *Halocyphina villosa* (ROHRMANN and MOLITORIS 1986) either, whereas peroxidase was found in our tests in *Nia vibrissa* and previously for *Halocyphina villosa* (ROHRMANN and MOLITORIS 1986).

Carbohydrate metabolism

Since wood is frequently found as a substrate for marine fungi, and since *Nia vibrissa* is regularily found on wood and was shown to produce laccase, which participates in wood degradation, it was to be expected that cellulases and other enzymes participating in the use of cellulose and other carbohydrates as energy source would be found.

Furthermore, all strains tested produce amylase (EC 3.2.1.1.), cellulase (mainly EC 3.2.1.4. and EC 3.2.1.91) and xylanase (EC 3.2.1.8.) on both media. β-Glucosidase (EC 3.2.1.21), laminarinase (EC 3.2.1.6.) and pectate transeliminase (EC 4.2.2.2.) were found in most of the strains and media tested, the latter enzyme, however, only in small amounts. Chitinase (EC 3.2.1.14.), the enzyme degrading chitin, a substrate on which *Nia vibrissa* is not found, was produced to a much lesser degree. Cellulase and xylanase were also found by LEIGHTLEY and EATON (1979) in *Nia vibrissa*. A similar set of enzymes as described here for

Table 1 (Part 1). Enzyme activities of 3 monokaryotic and 8 dikaryotic strains of *Nia vibrissa* on synthetic seawater and deionized water media.

Enzyme activity [1]													
Enzyme (Substrate)	M[2]	Dikaryons								Monokaryons			% of[3] active strain
		M154	M167	M168	M169	M170	M171	M175	M21	M172	M173	M174	
REDOX METABOLISM													
Laccase/d[4] (-Naphthol)	I	+++	+	+l	0	+++	(+)	+++	+	0	++	+++	82%
	S	++	(+)l	0	0	0	0	0	0	0	-	++	27%
Laccase/d (Guajacol)	I	+++	+l	-	-	++	++	++	-	-	-	+++	54%
	S	++	-	-	-	+	++	-	-	+	-	++	45%
Laccase/i[5] (Benzidine)	I	++	-	++	+++l	++	+++	-	-	-	-	+++	54%
	S	-	-	-	-	-	-	-	-	-	-	+	9%
Laccase/i (Syring-aldazin)	I	++	-	-	-	++	+++	+++	(+)	-	-	++	54%
	S	-	-	-	-	-	-	-	-	-	-	-	0%
Peroxidase/i (Benzidin)	I	+++	-	-	(+)e	-	-	(+)	(+)l	(+)	(+)	-	54%
	S	+++	+	+	(+)l	+l	+	++l	+l	++	++l	+l	100%
Tyrosinase/i (p-Cresol)	I	-	-	-	-	-	-	-	-	-	-	-	0%
	S	-	-	-	-	-	-	-	-	-	-	-	0%
CARBOHYDRATE METABOLISM													
Amylase/d (Soluble Starch)	I	+	++	+	+	+	+	++	+	++	++	+	100%
	S	++	++	+++	+++	++	++	+++	++	+++	+++	++	100%
Cellulase/i (CMC[6])	I	++	+++	+++	+++	++	+++	++	++	++	++	++	100%
	S	++	++	++	++	++	++	++	++	++	++	++	100%
Cellulase/d (Avicell +RBBR)	I	++	++	++	++	++	+l	+++	++	+++	++	++	100%
	S	++	++	++	++	+++	++	+++	+	++	+l	++	100%
Chitinase/d (Colloidal Chitin)	I	-	-	-	-	-	-	-	-	-	++l	++l	18%
	S	+	+	(+)	++	-	-	+	+	-	+++	+++	72%
ß-Glucosidase /d (Arbutin)	I	(+)l	-	+++	(+)l	+++	++	-	-	++	-	+++	64%
	S	+	+l	++	+++	++	++	+l	++	+l	-	+++	91%
Laminarinase /d (Insoluble Laminarin)	I	-	-	-	-	-	-	-	-	-	-	-	0%
	S	-	-	-	-	-	-	-	-	-	-	-	0%
Laminarinase /i (Soluble Laminarin)	I	++	++	++	++	+	++	+	++	++	++	+	100%
	S	+	++	++	++	++	+++	+	+	-	+	++	91%

Table 1 (Part 2).

Enzyme (Substrate)	M[2]	Enzyme activity [1]											% of[3] active strain
		Dikaryons								Monokaryons			
		M154	M167	M168	M169	M170	M171	M175	M21	M172	M173	M174	
CARBOHYDRATE METABOLISM													
Pectate[7] transelimin. /i (Pektin A)	I	(+)l	(+)	(+)	+	(+)	-	(+)	(+)	(+)	(+)	(+)	91%
	S	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Xylanase/i (Xylan)	I	(+)e	++	++	+	+	++	+	+	+	+	+	100%
	S	(+)	++	+	+	+	+	(+)	+	+	+	+	100%
FAT METABOLISM													
Lipase/d (Tween 80)	I	+l	++	+	+	+l	+l	++	++	++	+++l	++l	100%
	S	-	-	-	-	-	-	-	-	-	-	-	0%
NITROGEN METABOLISM													
Caseinase/d (Skim-milk -powder)	I	+++	++	++	++	+++	+++	+++	+++	+++	+++	+++	100%
	S	++	++l	++l	++l	++	++	+	++	(+)l	++	+++	100%
Gelatinase/i (Gelatin)	I	++	+++	++	++	++	++	++	++	++	++	+	100%
	S	++	++	+++	+++	++	+++	+++	+++	++	+++	++	100%
Nitrate reductase/i ($NaNO_3$)	I	+	(+)	+	(+)	(+)	(+)	(+)	(+)	+	(+)	(+)l	100%
	S	(+)	(+)l	(+)	(+)	(+)l	(+)	(+)	(+)l	(+)	(+)	(+)l	100%
Urease/d (Urea)	I	+++	(+)l	(+)l	(+)l	-	-	-	-	++	+++	-	54%
	S	+++	(+)l	(+)l	(+)l	-	+++	(+)l	-	+l	++	+l	82%

1) Enzyme activity given as highest activity within the test period, in arbitrary units: -; (+); +; ++; +++; n.d. = not determined; 0 = enzyme activity lacking because of no growth; e = early activity, only in the first incubation week; l = late activity after the 3rd incubation week;
2) M = Medium; I = Deionized water; S = synthetic seawater;
3) Percentage of strains showing the respective enzyme activity in at least one of the tests;
4) d = direct test; substrate and detecting reagent contained in the medium before inoculation;
5) i = indirect test; substrate and/or detecting reagent is added after the incubation period;
6) CMC = Carboxymethylcellulose sodium;
7) Pectate transelim. = Pectate transeliminase.

Nia vibrissa could be shown for the carbohydrate metabolism of *Halocyphina villosa* (ROHRMANN and MOLITORIS 1986).

Fat metabolism

Lipase (EC 3.1.1.3.) enables the fungus to use fat from various substrates as its energy source. This enzyme was found (at least on I-medium) in all strains tested. This corresponds with the results of NERUD et al. (1982) who found lipase in 15 of the 16 wood-degrading terrestrial Basidiomycetes and also with GESSNER (1979) who found it in 20 higher marine fungi from salt marshes. ROHRMANN and MOLITORIS (1986) showed the presence of lipase also in the wood-degrading marine Basidiomycete *Halocyphina villosa*.

Nitrogen metabolism

Protein-hydrolysing enzymes (caseinase (EC 3.4.) and gelatinase (EC 3.4.)) were found in large amounts in all *Nia vibrissa* strains and on all media tested, which corresponds with the results of ROHRMANN and MOLITORIS (1986) for *Halocyphina villosa* and the results of PISANO et al. (1964), who found gelatinase in 13 out of 14 marine fungi.

Nitrate reductase was found (BRESINSKY and SCHNEIDER 1975) to be an enzyme of systematic significance in terrestrial Basidiomycetes. For marine fungi it was shown by MOLITORIS and SCHAUMANN (1986) that this enzyme was present in all marine fungi tested so far and might therefore be a typical characteristic for the marine group of fungi. Since nitrogen often constitutes a limiting factor in fungal growth, the production of nitrate reductase was discussed (MOLITORIS and SCHAUMANN 1986) as a positive selective advantage to fulfill the nitrogen requirement by using the nitrogen of the nitrate present in seawater. This view is confirmed by the results of RAU and MOLITORIS (1991). The presence of nitrate reductase in all strains of *Nia vibrissa* and in both media in the present investigation fits well into this picture.

Summing up the results, it may be stated that generally the enzymes found in *Nia vibrissa* agree well with expectations correlated with the natural substrates and living conditions of this marine Basidiomycete.

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