ACTA MYCOLOGICA
Vol. 35 (1): 53–60
2000

Differentiation of enzymatic activity of yeasts and yeast-like microorganisms isolated from various environments

ELŻBIETA BOGUSŁAWSKA-WĄS, WALDEMAR DĄBROWSKI, KATARZYNA SKOROPADA and KINGA RÓŻYCKA-KASZTELAN

Department of Food Microbiology, Agricultural University
Kazimierza Królewicza 4, PL-71-550 Szczecin, Poland

Bogusławska-Wąs E., Dąbrowski W., Skoropada K., Różycka-Kasztelan K.: *Differentiation of enzymatic activity of yeasts and yeast-like organisms isolated from various environments.* Acta Mycol. 35 (1): 53–60, 2000.

The aim of study was to determinate enzymatic activity of yeast-like organisms – *Candida lipolytica, Rhodotorula rubra, Trichosporon beigelii, Zygosaccharomyces* sp. – isolated from the Szczecin Lagoon and herring salads. We have shown that lipolytic activity was higher than proteolytic for every strain tested. The lowest activity level was found out for amylolytic hydrolases. The results also demonstrated that yeast-like organisms isolated from the Szczecin Lagoon revealed much higher average enzymatic activity compared to the same species isolated from herring salads, excepting *C. lipolytica.*

Key words: yeasts, yeast-like organisms, enzymes, water, herring salads.

INTRODUCTION

Yeasts and yeast-like organisms colonize environments, which differ widely in physical and chemical parameters. Not only quick growth and development but also the adaptation of an enzymatic apparatus allow them to exploit such different environments. They are considered to be either required and beneficial to natural biochemical processes or responsible for spoiling of food. Although at present yeasts and yeast-like organisms are represented by 700 species, only a few are commonly known (Wolf 1996). Relatively little understanding of natural biochemical abilities of yeasts and yeast-like organisms caused that only few species are used in various branches of science and industry, mostly in biotechnology (Wolf 1996, Kochman 1986). Biotechnology has been increasingly significant for food industry for some time. Recently the range of its applications is

rising in the environmental protection, mainly in sewage treatment and waste bioutilisation. Biotechnological processes are generally based on activity of microorganisms or their enzymes. Enzymes are a group of proteins, which is essential for all living organisms. Their fundamental role is to lower the activation energy required for the chemical reaction to proceed. Enzymes are highly specific to catalysed reaction and particular substrates (Schlegel 1996). In the environment enzymatic activity of microorganisms is significantly modified by its abiotic factors (Kręgiel 1998; Mendoza et al. 1994; Fiedurek and Szczodrak 1994). Accordingly, the primary objective of this work has been to compare the enzymatic characteristics of yeasts isolated from food products and the environment.

MATERIALS AND METHODS

Yeasts and yeast-like organisms: *Candida lipolytica* (Diddens et Lodder), *Rhodotorula rubra* (Lodder), *Trichosporon beigelii* (Vuillemin) and *Zygosaccharomyces* sp. (Kluyver et van Niel) isolated from the Szczecin Lagoon waters and herring salads were examined.

The Szczecin Lagoon and the Straits of Piana, Świna and Dziwna comprise the secondary estuary. The main tributary of the Szczecin Lagoon is the Odra river which supplies 97% of waters, the rest is contributed by other tributaries (Robakiewicz 1993). The Odra waters flowing through the industrialised river-basin carry a considerable amount of municipal, industrial and agricultural sewage. The sewage reaches the Szczecin Lagoon hardly treated. The additional source of municipal and industrial waste is sewage from the north part of the lagoon.

Herring salads are popular products on the market. They consist of pickled filleted herring, cream sauce, pickled cucumbers, salt, sugar, vinegar and a preservative – sodium benzoate (E 211).

Details of isolation and identification of yeasts from the Szczecin Lagoon were described previously (Dąbrowski et al. 1998). Herring salad samples of 25 g were diluted in 225 ml of dilution liquid (sodium chloride 0.85%, peptone 0.1%) and homogenized. Then decimal dilutions were prepared and directly inoculated on solid medium supplemented with an antibiotic (PN–ISO 7954). Isolated strains were identified according to Lodder (1971) and Kockova-Kratochvilova (1990) key. Tests ID 32C (bioMerieux) were used to confirm the species and genus classification.

Evaluation of hydrolase activity was carried out with API ZYM kit (bioMerieux). The analysis was based on the enzyme set using chromatogenic substrates (Table 1).

Table 1
The enzymes and their abbreviations

Catalog number	Abbreviation	Enzyme
3.1.1.1.	Est	Esterase (C4)
3.1.1.2.	El	Esterase lipase (C8)
3.1.1.3.	Lip	Lipase (C14)
3.4.1.1.	Leu	Leucine arylamidase
3.1.4	Val	Valine arylamidase
3.1.4	Cys	Cystine arylamidase
3.4.21.4.	Try	Trypsin
3.4.21.1.	Chy	Chymotrypsin
3.1.4.	Ph	Naphtol-As-BI-phosphohydrolase
3.2.1.22.	α Ga	α-galaktosidase
3.2.1.23.	β Ga	β-galaktosidase
3.2.1.31.	β Gk	β-glucuronidase
3.2.1.20.	α Gl	α-glucosidase
3.2.1.21.	β Gl	β-glucosidase
3.2.1.29.	Nac	N-acetyl-β-glukosamidase
3.2.1.24.	α Ma	α-mannonidase
3.2.1.38.	α Fu	α-fucosidase

RESULTS

The analysis of the average enzymatic activity of strains isolated from the Szczecin Lagoon and herring salads has shown that strains of *Candida lipolytica* and *Rhodotorula rubra* share a similar ability to break the substrate down in these environments (Table 2). Both microorganisms also display much higher enzymatic activity than *Trichosporon beigelii* and *Zygosaccharomyces* sp. The lowest enzymatic activity refers to the hydrolases of *T. beigelii* (Table 3).

Table 2
Average enzymatic activity of yeasts and yeast-like organisms

Strain	Environment	Average enzymatic activity nmol*
Candida lipolytica	salad lagoon	7.6 7.9
Rhodotorula rubra	salad lagoon	6.4 9.5
Trichosporon beigelii	salad lagoon	1.4 4.9
Zygosaccharomyces sp.	salad lagoon	2.2 6.7

* nmol of substrates broken down

It is interesting that strains of *Rhodotorula rubra*, *Zygosaccharomyces* sp. and *Trichosporon beigelli* are more active in the aquatic environment than in food medium. Only enzymes of *Candida lipolytica* strains isolated from herring salads and water have shown similar activity (Table 2).

The results of analysis demonstrate that all examined microorganisms show the highest lipolytic activity. However, strains isolated from the environment display higher activity of esterase and lipase than those isolated from salads. Reduced differentiation of the activity in both environments refers to esterase lipase (Table 3).

The isolated strains express different particular preferences for substrates used for the study of their proteolytic activity. Salad isolates of *Candida lipolytica* and *Rhodotorula rubra* show much higher ability to hydrolyse leucine than the rest of strains (Table 3). Besides, they do not express any other proteolytic activity with the exception of *Candida lipolytica*. It displays low activity of valine arylamidase, which together with leucine arylamidase

Table 3
Enzymatic activities of strains isolated from the Szczecin Lagoon

Enzyme	*Candida lipolytica*		*Rhodotorula rubra*		*Trichosporon beigelii*		*Zygosaccharomyces* sp.	
	salad [nmol]*	lagoon [nmol]	salad [nmol]	lagoon [nmol]	salad [nmol]	lagoon [nmol]	salad [nmol]	lagoon [nmol]
Est	20.0	31.3	15.0	35.0	5.0	20.0	16.0	40.0
El	20.0	31.3	15.0	25.0	5.0	5.0	9.0	10.0
Lip	0.0	21.3	5.0	20.0	0.0	20.0	0.0	5.0
Leu	40.0	8.8	40.0	11.3	5.0	5.0	9.0	5.0
Val	5.0	0.0	8.0	5.0	0.0	5.0	0.0	1.7
Cys	0.0	25.0	1.5	35.0	0.0	20.0	0.0	33.3
Try	0.0	3.8	0.0	20.0	0.0	5.0	0.0	10.0
Chy	0.0	1.3	0.0	9.6	0.0	5.0	0.0	5.0
Ph	40.0	0.0	20.0	0.0	5.0	0.0	2.4	0.0
α-Ga	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
β-Ga	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
β-Gk	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
α-Gl	0.0	0.0	0.0	1.3	5.0	0.0	2.0	1.7
β-Gl	5.0	12.5	6.0	2.0	0.0	0.0	0.0	1.7
Nac	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7
α-Ma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
α-Fu	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*nmol of substrates broken down

was also found in *Rhodotorula rubra* (Table 3). A wider range of activity of proteolytic enzymes is shown by the environmental microorganisms. Cystine seems to be the most effective protease in examined yeasts and yeast-like organisms. The ability to hydrolase peptides was much lower among the rest of proteases.

The lowest enzymatic ability was found in amylolytic hydrolases. Only β-glucosidase was indicated in the environmental and salad strains of *Candida lipolytica*, *Rhodotorula rubra* and also in *Zygosaccharomyces* sp. isolated from the environment (Table 3).

DISCUSSION

Strains from two exceedingly different environments were examined in this study. Herring salads as a growth matrix show the high concentration of nutrients. They consist of mayonnaise – a semisolid water-oil emulsion, salted herring – a main source of proteins, and vegetables – a source of carbohydrates. Low pH found in salads was not optimal for yeasts and yeast-like organisms but enabled them to grow (Kockova-Kratochvilova 1990). Besides, water activity (a_w) was reduced. Salads were also supplemented with the preservative – sodium benzoate to limit growth of bacteria and fungi. Although it is often used in food production its antimicrobial effectiveness may be questioned. To summarise it, salads are characterised by rich concentration of nutrients, low pH and presence of an inhibitor.

Even though polluted by municipal sewage, waters of the Szczecin Lagoon lack nutrients. Their pH is higher (7.0–8.6) (Bogusławska 1999) and the presence of inhibitor may be discussed. It is distinctly possible that there are no inhibitors produced by other organisms in water, due to interspecific competition. Yeasts and yeast-like organisms, which produce a killer toxin provides an excellent example of it (Schmitt et al. 1996). It is suggested to be a natural regulator of quantity and a possible competition mechanism. Other notable examples are blue-green algae of the genus *Microcystis* causing monospecific blooms, which release toxin intermediates (Piesik 1992). Though their influence on other microorganisms has not been described yet, it should not be excluded (Bogusławska-Wąs and Dąbrowski in press).

Results have shown that enzymatic activity of strains of the same species isolated from such various environments differ remarkably. Isolated strains were cultured and stored in the same medium thus possible phenotypic and metabolic differences caused by the environmental conditions and stress were eliminated. Yeasts and yeast-like organisms from the Szczecin Lagoon presented higher enzymatic activity (Table 2) and spectrum (Table 3) than those isolated from herring salads. These differences were probably

caused by the variety and reduced level of nutrients in the Szczecin Lagoon in comparision to herring salads. Strains, which show higher enzymatic activity and differentiation, are promoted in such conditions. The environmental microorganisms must be ready to exploit every available nutrient molecule immediately because secreted enzyme molecules may be quickly eliminated in the aquatic environment, for instance by dilution. Microorganisms developing in nutrient–rich medium (herring salads) do not require high enzymatic efficiency. Even a relatively small amount of enzyme released to such environment provides cells with sufficient concentration of nutrients due to the substrate breakdown.

Strains of high lipolytic activity are promoted by both environments (Table 1). Lipids are a group of food substrates, which has the biggest energetic value. A known fact is that enzymes involved in breaking the lipids down act only on a fat-water boundary surface. In natural conditions food sources exploited by fungi are usually dispersed in water solutions (eg. milk, vacuoles within the cytoplasm of plant cells) (M ü l l e r and L o e f f l e r 1987). The presence of lipids in emulgated form like in cream sauce of herring salads helps to break them down. It may be the reason why lipids were a main source of energy for strains isolated from salads. Accumulation of lipids in the aquatic environments is regulated mainly by plant and animal productivity and the level of pollution. The Szczecin Lagoon is a reservoir at which municipal and industrial sewage from a city with a population of nearly half a million is dumped, thus its waters provide a suitable nutritional environment.

The conditions in the Szczecin Lagoon are also profitable to strains with proteolytic activity. But it does not refer to every examined strain and enzyme. Salad strains did not show any activity of tripsin and chymotripsin while the environmental strains were of average activity (Table 3). Just the reverse applies to naftol-AS-BI-phosphohydrolase, the activity of which was expressed only by salad strains on average level (Table 3). The results in question seem to indicate that strains from various environments are equipped with different enzyme sets.

Undoubtedly the lowest level of activity refers to enzymes, which are responsible for the carbohydrate breakdown (Table 3). It may be forced by presence of highly calorific lipids in both environments.

Enzymatic activity observed in isolated strains could be different in natural conditions due to various physical and chemical conditions and the concentration of substrates. pH is generally a limitation factor. Most lipolytic, proteolytic and amylolitic fungi enzymes have an optimum pH close to neutral or slightly alkaline (M ü l l e r and L o e f f l e r 1987). pH decreases away from the optimum can affect proteolytic enzymes adversely and cause their inhibition or even loss of catalytic activity (M o l s k a 1988). It is the result of interaction with the active centre of the enzyme and its denaturation

(P a l u c h 1973). Activity of amylolytic enzymes can be remarkably affected not only by pH but also by temperature (W i l l s o n and I n g l e d e w 1982). Low temperatures reduce activity of hydrolases which are responsible for the carbohydrate breakdown (K r ę g i e l 1998; S t o b i ń s k a et al. 1997).

Despite that, our results show that different environmental conditions select and promote strains of yeasts and yeast-like organisms belonging to the same species which have different enzymatic spectrum and activity.

REFERENCES

B o g u s ł a w s k a-W ą s E. 1999. Analiza mikroflory wód i osadów dennych Zalewu Szczecińskiego w aspekcie występowania drożdży i grzybów drożdżodobnych. Praca doktorska, AR w Szczecinie.

B o g u s ł a w s k a-W ą s E., D ą b r o w s k i W. (in press). Seasonal changes of yeasts and yeast-like organisms in Szczecin Lagoon waters.

D ą b r o w s k i W., B o g u s ł a w s k a-W ą s E., D a c z k o w s k a-K o z o n E. 1998. Analysis of the Szczecin Lagoon waters mikoflora. Acta Mycol. 33 (1): 101–108.

F i e d u r e k J., S z c z o d r a k J. 1994. Selection of strain, culture conditions and extraction procedures for optimum production of beta-galactosidase from *Kluyveromyces fragilis*. Acta Microbiol. Pol. 43 (1): 57–65.

K o c h m a n J. 1986. Zarys mikologii dla fitopatologów. SGGW, Warszawa.

K o c k o v a-K r a t o c h v i l o v a A. 1990. Yeasts and yeast-like organisms. VCH Publishers, NY.

K r ę g i e l D. 1998. Amylazy drożdży *Schwanniomyces* sp. Postępy Mikrobiol. 37 (1): 57–72.

L o d d e r J. 1971. The yeasts. North-Holland Publishing Company. Amsterdam, London.

M e n d o z a I., R u b i o F., R o d r i g u e z-N a v a r r o A., P a r d o J. M. 1994. The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. J. Biol. Chemistry 269 (12): 8792–8796.

M o l s k a I. 1988. Zarys mikrobiologii mleczarskiej. PWRiL, Warszawa.

M ü l l e r E., L o e f f l e r W. 1987. Zarys mikologii. PWRiL, Warszawa.

P a l u c h J. 1973. Mikrobiologia wód. PWN, Warszawa.

P i e s i k Z. 1992. Możliwości biologicznej rekultywacji Zalewu Szczecińskiego. Rocznik Nauk. Szczecin 7: 23–26.

P N-89/A – 86730. Ryby i przetwory rybne. Badania mikrobiologiczne.

R o b a k i e w i c z W. 1993. Monografia. Warunki hydrochemiczne Zalewu Szczecińskiego i cieśnin łączących Zalew z Zatoką Pomorską. IBW PAN, Warszawa.

S c h l e g e l H. G. 1996. Mikrobiologia ogólna. PWN, Warszawa.

S c h m i t t M. J., K l a v e h n P., S c h o e n i n g L., T i p p e r D. J. 1996. Cell studies on the mode of action of yeast K28 killer toxin. Microbiol. 142 (9): 2655–2662.

S t o b i ń s k a H., D r e w i c z E., K r ę g i e l D., O b e r m a n H. 1997. Próby transformacji cechy killerowej drożdży *Saccharomyces cerevisiae* do amylolitycznego szczepu *Schwanniomyces occidentalis*. Biotechnol. 1 (36): 159–166.

W i l l s o n J. J., I n g l e d e w W. M. 1982. Isolation and characterisation of *Schwanniomyces alluvius* amylolitic enzymes. Appl. Environ. Microbiol. 44.

W o l f K. 1996. Nonconventional Yeasts in Biotechnology. Springer–Verlag.

Zróżnicowanie aktywności enzymatycznej drożdży i grzybów drożdżopodobnych wyizolowanych z różnych środowisk

Streszczenie

Przedmiotem badań była aktywność enzymatyczna drożdżaków – *Candida lipolytica, Rhodotorula rubra, Trichosporon beigelii* i drożdży – *Zygosaccharomyces* sp., wyizolowanych z Zalewu Szczecińskiego i sałatek śledziowych. Wszystkie analizowane szczepy charakteryzowała wysoka aktywność lipolityczna i znacznie niższa proteolityczna. Najniższy poziom aktywności stwierdzono dla hydrolaz amylolitycznych. Ustalono, że drożdże i grzyby drożdżopodobne wyizolowane z Zalewu Szczecińskiego wykazują znacznie wyższą średnią aktywność hydrolityczną niż te same gatunki pozyskane z sałatek śledziowych. Wyjątek stanowiła *C. lipolytica.*