A C T A A L I M E N T A R I A P O L O N I C A

Vol. XII (XXXVI), No. 2 1986

MAGDALENA WŁODARCZYK

CHARACTERISTIC OF YEASTS FROM SPONTANEOUSLY FERMENTING BREAD STARTERS

Institute of Fermentation Technology and Microbiology, Łódź
Technical University

Key words: yeast, fermenting bread startes, Saccharomyces cerevisiae, Saccharomyces exiguus, Torulopsis candida, Candida krusei.

Yeast microflora in spontaneously fermenting bread starters was studied. It was found that in starters from industrial bakeries the dominant yeast, accounting for about 99% of all yeasts, was *Saccharomyces cerevisiae*. In starters from small private-owned bakeries about 40% of the yeasts were of different species; among those there were identified *Saccharomyces exiguus*, *Torulopsis candida* and *Candida krusei*.

In the technology of rye and rye-wheat bread the preparation of dough is preceded by a series of starters, renewed and activated by additions of new flour and water quantities [8, 23]. The fermentation of starters in industrial conditions, most often by the three-phase method, takes place with the simultaneous presence of lactic acid bacteria and yeasts. Their physiological activity as well as the character of their coexistence determine the course of the traditionally spontaneous fermentation.

Numerous reports about the microflora of bread starters [4, 7, 9-13, 15, 17-22] indicate the considerable differentiation of microorganisms coexisting in these media. The local peculiarities of the quantitative and qualitative arrangements of lactic acid bacteria and yeasts is significant. The genera and species as well as the mutual relations between both physiological groups [22] depend to a large extent on the quality of raw materials and on the conditions of fermentation.

The present research concerned the quantitative and qualitative analysis of yeasts active in spontaneously fermenting bread starters from industrial and from small private-owned bakeries. The yeast strains representative for these media were analysed taxonomically.

MATERIAL AND METHODS

The bread starters came from four private-owned bakeries using the five-phase fermentation cycle (I-IV P) and from three industrial bakeries (I-III I) in which the three-phase cycle was used. In all cases the studies were performed in the so called acid phase in which the flour content was 50%, fermentation temperature was 28°C, and the acidity after the end of fermentation ranged from 8.6 to 10.0 ml 0.1 N NaOH/10 g in starters from industrial bakeries, and from 11.0 to 13.5 ml 0.1 N NaOH/10 g in those from private bakeries.

Microbiological analysis was performed by the method of plate inoculation [1]. The following was determined: total number of yeasts in the conventional medium (malt wort with 10° Blg extract content); the number of yeasts which were not inhibited in their growth by cycloheximide (actidione), hereinafter called wild yeasts, in the conventional medium and in YPG culture medium [2]. Actidione concentration was 75 μg/ml. The media were solidified with 1.5% agar addition. The plates were incubated at 28°C for 48 h. The results were given in numbers of individuals capable of growth (CFU) per 1 kg of starter.

TAXONOMICAL ANALYSIS OF ISOLATED YEAST STRAINS

Pure yeast cultures containing 68 strains isolated from starters obtained from private-owned bakeries, and 55 strains from industrial starters were identified on the basis of results of diagnostic tests [6].

RESULTS AND DISCUSSION

The analysed starter samples, both those from the private and from the industrial bakeries, had correct organoleptic properties. Observed in direct microscope preparations were short bacteria bacilli and yeast cells morphologically resembling *Saccharomyces cerevisiae*. These constituted 30-50% of microorganisms in the field of vision. The quantitative results concerning the studied microorganisms are given in Table 1.

The starters from industrial bakeries (I-III I) were found to exhibit a practically constant level of *Saccharomyces cerevisiae* ($7.9\text{-}9.72 \times 10^{10}$ CFU/kg), and yeasts immune to cyclohexamide accounted for a mere 0.02-0.08% of the entire number of these microorganisms. This means a $1.06\text{-}6.40 \times 10^3$-fold predominance of the *Saccharomyces cerevisiae* population over the wild yeasts. In starters from private bakeries the number of *Saccharomyces cerevisiae* cells differed, ranging from 0.21 to 6.25×10^{10} CFU/kg. The amount of wild yeasts was considerable

(37-40% of all yeasts). Relatively speaking, in all the analysed starters from private bakeries, there were 1.5-1.7 cells of *Saccharomyces cerevisiae* for each cell of wild yeasts.

The taxonomic evaluation (Table 2) of isolates from starters from industrial bakeries confirmed the results of the quantitative analysis. Out of the 55 strains only one was classified as *Saccharomyces inusitatus* (less

Table 1. Yeast level in bread starters

Determination	Starters from private bakeries				Starters from industrial bakeries		
	I R	II R	III R	IV R	I P	II P	III P
Total number of yeasts a (CFU × $\times 10^{10}$/kg)	10.4	0.35	1.32	1.11	8.33	7.96	9.72
Yeasts with growth uninhibited by cyclohexamide b (CFU × 10^8/kg)	415.0	14.3	48.7	43.0	0.63	0.75	0.15
Saccharomyces cerevisiae (CFU × $\times 10^{10}$/kg) (a-b)	6.25	0.21	0.83	0.68	8.32	7.95	9.71
$\frac{a-b}{b}$	1.50	1.47	1.70	1.58	1.23 $\times 10^3$	1.06 $\times 10^3$	6.4 $\times 10^3$

than 2% of the studied cultures). The starters from private-owned bakeries contained a wider variety of yeast strains. Out of the 68 diagnosed cultures, *Saccharomyces cerevisiae* constituted 54% of the isolates, *Saccharomyces exiguus* — 25%, *Torulopsis candida* — 12%, and *Candida krusei* — 9% (Table 2).

The observations appear to confirm the studies of Spicher et al. [14], and the results are also similar to those obtained by Sugihara et al. [18] and Ng [7]. Worth stressing is the presence of *Saccharomyces exiguus* in starters from private bakeries; the importance of this strain for the proper maturing of starters is pointed out by Spicher et al. [15] and Kozmina [5]. The value of the strain is due to its heat resistivity, the acidity of the medium and the lack of ability to ferment maltose. The properties of *Saccharomyces exiguus* show it to play an important role in the multi-component biocenose of bread starters.

The observed different yeast microflora composition of starters from industrial and from private-owned bakeries indicate that the genus and species of the strains in the medium depend on the method of fermentation. In the industrial technology with more rapid starter maturing and large additions of pressed yeasts, the dominance of *Saccharomyces cerevisiae* is very frequent [8, 10, 22]. In private bakeries which supplied the

Table 2. Diagnostics of pure yeast cultures isolated from bread starters

Starters providing pure cultures	No. of strains	Growth		arbutin decomposition	pseudomycelium	sporulation	Sugars fermentation						Sugars assimilation					Diagnostic qualification
		nitrate as N source	ethanol as C source				glucose	galactose	saccharose	maltose	lactose	raffinose	glucose	galactose	saccharose	maltose	lactose	
From industrial bakeries	54	−	+ (weak)	−	−	+	+	+	+	+	−	$+\frac{1}{3}$	+	+	+	+	−	*Saccharomyces cerevisiae*
	1	−	−	−	−	+	+	−	+	+	−	$+\frac{2}{3}$	+	−	+	+	−	*Saccharomyces inusitatua*
From private bakeries	37	−	+	−	−	+	+	+	+	+	−	$+\frac{1}{3}$	+	+	+	+	−	*Saccharomyces cerevisiae*
	17	−	−	−	−	+	+	+	+	−	−	$+\frac{1}{3}$	+	+	+	−	−	*Saccharomyces exiguus*
	8	−	+	+	−	−	+	−	−	−	−	−	+	+	+	+	+	*Torulopsis candida*
	6	−	+	−	+	−	+	−	−	−	−	−	+	+	+	+	+	*Candida krusei*

starters, fermentation was performed by the five-phase method with no additions of commercial yeasts.

Earlier studies of bacterial microflora active in starters from industrial bakeries [22] demonstrated that also the group of lactobacilli was represented by a smaller number of species than in starters from private owned bakeries. The lactic acid bacteria-to-yeast ratio was unfavourable: the predominance of the latter in industrial starters was often of the order of 10^3 [22]. The experiments of Stegeman et al. [16], Sugihara et al. [17], as well as the studies of Jegorowa et al. [3] and Spicher et al. [12, 13] reveal that the taste, aroma, crumb structure and shelf life of bread are all improved by a more aboundat and diversified starter microflora, both as regards lactic acid bacteria and the yeasts coexisting with them.

CONCLUSIONS

1. In bread starters from industrial bakeries yeasts were represented by practically one species, *Saccharomyces cerevisiae*; the strains which proved resistant to the growth inhibitor cyclohexamide amounted to a mere 0.02-0.08% of the total microflora population.

2. The starters from small private-owned bakeries contained a wider variety of yeasts. In addition to *Saccharomyces cerevisiae* inhibited by cyclohexamine, there were also present *Saccharomyces exiguus*, *Torulopsis candida* and *Candida krusei* constituting about 40% of all the yeasts.

LITERATURE

1. Burbianka M., Pliszka A.: Mikrobiologia żywności, PZWL, Warszawa 1977.
2. Jacob H.: Genetics 1965, 52, 75.
3. Jegorowa A. G., Kazanskaja Ł. N.: NTI, Cintipiszczeprom., Moskwa 1963.
4. Kline L., Sugihara T. F.: Appl. Microbiol., 1971, **21**, 459.
5. Kozmina N. P.: Piszcz. Prom., Moskwa 1978.
6. Lodder J.: The Yeasts, a Taxonomic Study, North-Holland Publ. Co., Amsterdam-London 1971.
7. Ng H.: Appl. Environ. Microbiol., 1976, **31**, 395.
8. Pyler E. J.: Baking: Science and Technology, vol. 2, Siebel Publ. Co., Chicago 1973.
9. Saunders R. M., Ng H., Kline L.: Cereal Chem., 1972, **49**, 86.
10. Schultz A.: Bakers Digest 1966, **40** (4), 77.
11. Spicher G., Stephan H.: Zbl. Bakt. Parasit. Infekt. und Hyg., Abt. II, 1966, **120**, 685.
12. Spicher G., Schollhammer R.: Getreide, Mehl und Brot 1977, **8**, 215.
13. Spicher G., Schröder R.: Getreide, Mehl und Brot 1978, **32**, 295.
14. Spicher G., Schröder R., Schröllhammer K.: Z. Lebensm. Unters. Forsch., 1979, **169**, 77.
15. Spicher G., Schröder R.: Z. Lebensm. Unters. Forsch., 1980, **170**, 119.
16. Stegeman J., Rohrlich M.: Brot und Gebäck 1958, **12**, 65.

17. Sugihara T. F., Kline L., Mc Cready L. B.: Bakers Digest 1970, **44**, (2), 51.
18. Sugihara T. F., Kline L., Miller M. W.: Appl. Microbiol., 1971, **21**, 456.
19. Ticha J.: Młynsko-Pekarensky Prumysl 1977, **7**, 224.
20. Włodarczyk M.: Abstr. II. Int. Conference Rye and Triticale — 80, Poznań 1980.
21. Włodarczyk M.: Zeszyty Naukowe, Politechnika Łódzka 1984, nr 452. Scientific Papers, 61.
22. Włodarczyk M.: Acta Alim. Pol., 1985, **11** (3), 345.
23. Wojcieszak P.: Fermentacja ciasta na drożdżach i zakwasie. PWRiL Warszawa 1956.
24. Wojcieszak P., Opuszyńska H.: Roczn. Techn. Chemii Żywn., 1964, **10**, 19.

Manuscript received: March 1984.
Author address: 90-924 Łódź, Stefanowskiego 4/10.

M. Włodarczyk

CHARAKTERYSTYKA DROŻDŻY WYSTĘPUJĄCYCH W SPONTANICZNIE FERMENTUJĄCYCH ZAKWASACH PIEKARSKICH

Instytut Technologii Fermentacji i Mikrobiologii, Politechnika, Łódź

Streszczenie

. Badano stan ilościowy i jakościowy mikroflory drożdżowej zasiedlającej spontanicznie fermentujące zakwasy chlebowe. W zakwasach z piekarń przemysłowych dominowały szczepy *Saccharomyces cerevisiae*. Stanowiły one 99,9% ogólnej liczby drobnoustrojów analizowanej grupy fizjologicznej. W środowiskach z piekarń rzemieślniczych drożdże, których wzrost nie był inhibowany cykloheximidem (drożdże dzikie) stanowiły ok. 40% ogólnej liczby tych drobnoustrojów (tabela 1). W tej sytuacji relacja *Saccharomyces cerevisiae:* drożdże dzikie była jak 1,5-1,7:1, podczas, gdy w zakwasach z produkcji przemysłowej chleba wyrażała się stosunkiem $1{,}1 \times 10^3$-$6{,}5 \times 10^3$:1.

Na podstawie analizy taksonomicznej (tabela 2) wyizolowanych kultur zidentyfikowano w zakwasach z piekarń przemysłowych *Saccharomyces cerevisiae* (98% izolatów) i *Saccharomyces inusutatus* (1,8%); w żurach z piekarń rzemieślniczych na 68 wyodrębnionych szczepów składały się *Saccharomyces cerevisiae* (54% izolatów), *Saccharomyces exiguus* (25%), *Torulopsis candida* (12%) i *Candida krusei* (9%).