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Edmund Strzelczyk, Wojciech Donderski, Wiesława Lewosz

Występowanie mikroorganizmów zdolnych do rozkładania organicznych połączeń fosforu w dwu typach osadów dennych eutroficznego jeziora Jeziorak

Streszczenie

Przeprowadzono badania nad zdolnością do rozkładania organicznych połączeń fosforu (dwufosforan fenolftaleiny, glicerofosfat, fityna, lecytyna, DNA i RNA) przez drobnoustroje wyodrębnione z 2 typów osadów dennych jeziora eutroficznego.

Stwierdzono, że osady płaszczyste zawierają więcej drobnoustrojów zdolnych do atakowania różnych połączeń organicznych fosforu niż osady typu „dy". Procentowy udział bakterii rozkładających organiczne połączenia fosforu wśród ogólnej mikroflory zmieniał się w różnych porach roku.

Wśród zbadanych drobnoustrojów najwięcej szczepów rozkładało dwufosforan fenolftaleiny, glicerofosfat i DNA. Mniej drobnoustrojów atakowało lecytynę i RNA a żaden z zbadanych szczepów nie rozkładał soli wapniowej kwasu fitynowego.

Dwufosforan fenolftaleiny hydrolizowały głównie drobnoustroje należące do rodzajów *Nocardia* i *Arthrobacter-Corynebacterium* a glicerofosfat — należące do rodzajów *Bacillus* i *Nocardia*. Pozostałe połączenia fosforowe atakowane były głównie przez Gram ujemne nieprzetrwalnikujące pałeczki oraz przez drobnoustroje należące do rodzajów *Bacillus* oraz *Arthrobacter-Corynebacterium* i *Nocardia*.

Authors' address:

Doc. Dr Edmund Strzelczyk,
Zakład Mikrobiologii, Instytut Biologii,
Uniwersytet Mikołaja Kopernika,
Toruń, ul. Sienkiewicza 30/32, Polska.

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Jadwiga Jakubowska

Some Biochemical Features of Flocculent and non-Flocculent Yeast Used in the Top Beer Brewery in Grodzisk Wlkp

From the Institute of Technology of Fermentation and Microbiology, Technical University, Łódź, Poland

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Summary

Two kinds of top brewer's yeasts, belonging to *Saccharomyces cerevisiae*, flocculent and non-flocculent types, were isolated from bottled beer. The pure cultures showed the same morphological properties as those described by Szmelich (1963, 1964), who has used them in beer production in top brewery in Grodzisk Wlkp. Both types of yeasts behaved alike: the same sugars and sources of nitrogen being able to assimilate. Both types adapted to anaerobic fermentation conditions were low respiring. Nevertheless for both types distinct differences in the rate of oxydative decarboxylation in glucose, using Warburg's technique, were observed.

Introduction

The selection of strains suitable for certain type of beer production offers many difficulties from the methodological point of view. The yeasts living in special technological conditions are influenced by several factors of biochemical, physical and biological origin, being responsible for the technological value of growing population and their behaviour in the first and second stage of fermentation (Rainbow, 1966; Ingram, 1969; Suomalainen, 1969).

The majority of investigations dealing with the phenomenon of flocculation of botton- and of top brewer's yeast are concentrated on the type of flocculence and the attenuating power (Gilliland, 1955; Holm, Nøhr and Thorne, 1953; Eddy and Phil, 1958; Hough, 1959; Szmelich, 1964). Development of yeast science and practice, based on cytology and biochemistry of the yeast cell, revealed the importance of phenotypic and genetic variation. The mitochondrial structure and function and changes in electron transport system, being responsible for respiration and fermentative ability of yeasts populations, must be taken into consideration with respect to the technological brewing conditions (Masschelein *et al*, 1963; Yotsuyanagi, 1962; Ingram, 1955, 1969; Suomalainen, 1969 l. c.).

The top fermentation brewery, situated in Grodzisk, near Poznań, has been known since the XIIIth century. Grodzisk yeast, of exceptionally low attenuating power and early flocculating, had been used until the end of XIXth century

(Warschauer, 1893: Schönfeld, 1938). The highly appreciated "Grodzisk beer" possesses characteristic taste and flavour due to the specific technology and to the type of brewer's yeasts. Extensive studies on the characteristics of those strains, in order to assure typical beer production, were made by Schmelich (1963, 1964). According to his invention the desired effect, from technological point of view was achieved, when a mixture of pure culture, consisting of flocculent and non-flocculating strains in a given proportion, was used. Both types ought to be propagated separately and mixed in the fermentation vat in a ratio 1 : 2. During the fermentation period distinct predominance of the non-flocculating type occurs. The "Grodzisk beer" corresponds to a genuine Graetzer Beer. The second stage of fermentation achieved in bottles assured the intense saturation of beer with natural carbon dioxide, typical taste and foam formation. Some analytical data characterizing the Grodzisk bottled beer (Jakubowska, 1971) in comparison with the mild ale (Hopkins and Krause, 1951) are presented in Table I. Low alcohol and extract content and very high saturation with CO_2 are characteristic for this type of beer.

Table I

Some data characterizing the finished bottled beer

Estimation	Grodzisk Beer (1964*—1970)	Mild ale (Hopkins, 1951)
alcohol % by weight	2.22—2.29	3.3 —3.5
extract %	2.81—2.89	3.4 —3.8
acid as lactic a. %	0.11—0.106	0.11—0.13
pH	4.1 —4.3	
CO_2% by weight	0.69—0.70	0.4 —0.5
colour ml N/10 iodine	0.55—0.60	

* Małecka, 1965

In present study the occurrence of both kinds of top yeasts in bottled beer was investigated. The isolated pure cultures identified after Szmelich (1964), were characterized with respect to their sedimentation and agglomeration properties, the use of several sugars by growing and resting cells, the oxydative respiration and fermentation were taken into account as well.

Experimental

Methods

In order to define the content of both types of yeasts present in the beer, several flasks obtained from the Grodzisk top brewery, produced in 1964—71, were examined. The samples of sediment, taken out of the bottles, were investigated by plate method. The yeasts were isolated from the typical colonies of *Saccharomyces cerevisiae*, growing in Petri dishes in 2% agar-malt at 25°, after 4 days of incubation. According to the previous statement made by Szmelich (1964 l.c.), the structure of colonies allowed to distinguish the flocculent from the non-flocculent type.

Several colonies of both types were transferred on wort, 10° Blg. After 4 days of cultivation at 25° the behaviour of yeasts population was examined, namely: the type of sediment, occurrence of chains or aggregates, the clarification of the liquid.

The sporulation ability was tested on acetate medium (Harrigan and Cancell, 1966). Diagnostics and identification were carried out according to yeasts taxonomy (Lodder and Kreger v. Rij, 1952). The ability to assimilate several sugars and some sources of nitrogen were estimated on agar plates by auxanographical method (Barnett and Ingram, 1955).

The measurements of gaseous exchange were executed by Warburg routine method (Umbreit, Burris, Stauffer, 1964). The cells suspensions were harvested from the stationary phase of growth after 48 hr of cultivation at 25° in wort, 10° Blg. The washed yeast cells, centrifuged at 2,000 rpm for 3 minutes, obtained in homogenous suspensions in citrate buffer (pH 4.85), contained 2—4 mg of cells dry mass per 1 ml. The reaction mixture in Warburg's flask contained: 1.5 ml of yeast suspension, 0.6 ml of substrate in an 0.1% aqueous solution. The incubation was carried out at 30° by continuous shaking equal to 120 rev/min, the deflection being about 5 cm. The output of CO_2 and the uptake of O_2 in the air, as gas phase, were measured every 15 minutes within 2 hr. The average results, with difference not exceeding 5% in parallel measurements, were accepted.

Results and Discussion

The microscopical examination of colonies and of yeasts populations grown in wort allowed to prove some distinct differences between the yeasts present in bottled beer. As is shown in Table II both types, representing top yeast used in the Grodzisk beer production, were found, their ratio being maintained in proportion 3 : 1, non-flocculating type always predominating.

Table II

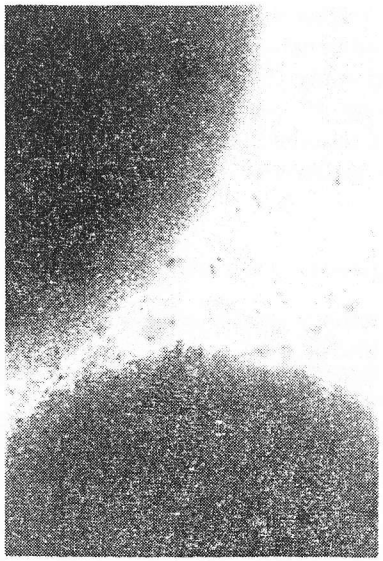
Non-flocculating and flocculent types of yeasts estimated in finished bottled Grodzisk Beer

Samples of beer produced in 1964—71	Number of colonies of *Sacch. cerevisiae* grown in Petri dishes		
	nf*	f*	Ratio nf:f
1	48	15	3.2:1
2	29	10	2.9:1
3	42	20	2.1:1
4	49	14	3.5:1
5	31	8	3.8:1
6	25	9	2.8:1
7	37	12	3.0:1

* non-flocculent type B (nf) and flocculent type A (f) after Kocková et al (1970)

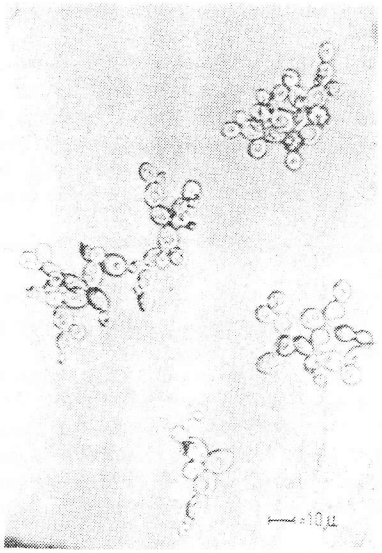
The proper structure of colonies visible on Phot. 1 corresponds to those described by Kocková *et al* (1970) and revealed by Szmelich (1964 l.c.). By microscopical examination (125x) the outline of colonies showed characteristic differences, namely: smooth and regular margin, typical for flocculent yeasts, type A according to Kocková. For non-flocculating type the colonies were irregular and branched.

Several colonies of both types were transferred in wort, 10°Blg, their behaviour being observed during cultivation at 25°. After 22 hr the cells of the non-flocculating



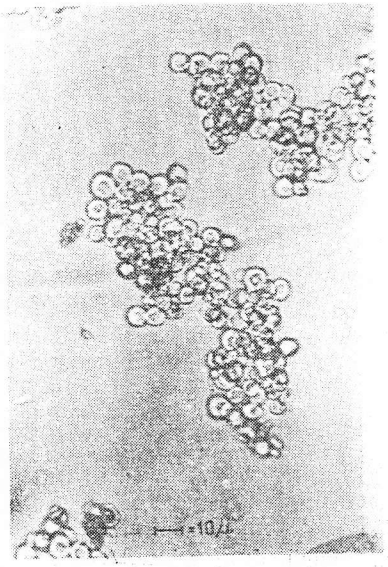
Phot. 1. Outlines of 2 colonies: smooth (flocculent), branched (non-flocculent one); (125x)

type were dispersed in the liquid, strong fermentation and big foam were observed. After 4 days the liquid was clarified. By shaking, the cells were quickly rising up from the sediment, then slowly settling. Pairs or short chains of cells (size 3—5x5—12 μ) have been microscopically distinguished (Phot. 2).



Phot. 2. Non-flocculent strain (PL) 48 hr in malt wort

The cells of flocculating strains were quickly settling down, forming compact sediment. Flocks consisting of large aggregates of matures identical in size cells (3—6x4—10 μ) are shown on Phot. 3.



Phot. 3. Flocculent strain (K_1) 48 hr in malt wort

The non flocculating type, rich in glycogen, easy sporulated. Within 3 days on acetate medium up to 25% of cells contained 1—3 ascospores.

Both types of yeasts behaved alike: the same sugars were assimilated and fermented, viz., glucose, galactose, fructose, maltose, sucrose and raffinose 1/3 Melibiose and lactose were not utilized. Peptone as source of nitrogen was most favourable for growth, in presence of nitrate and ammonium sulfate weak growth was observed.

The resting cells suspensions tested by manometric technique, in presence of several sugars, were very low respiring (Fig. 1). This fact was in agreement with Chin's (1950) observations on non-aerated top brewer's yeast. The endogenuous respiration, illustrated in Fig. 2 showed that non-flocculent strain possesses some interior reserves, being responsible for the increasing gaseous exchange during two hours of experiments, whereas in case of the flocculating strain indistinct changes were observed. In presence of several sugars the carbon dioxide output always predominated the oxygen uptake (Fig. 3a, b). Higher respiration coefficient characterized the non-flocculating cells.

The present investigations let us to conclusion that the type of metabolism examined by Warburg's technique should be included in testing the properties of industrial strains (Lafon, 1956; Fichter and Phenninger, 1962; Haboucha, Masschelein, Devreux, 1959). In such experiments, however, the phase of

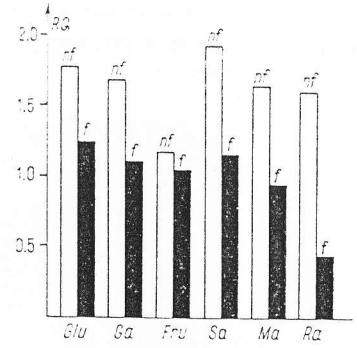


Fig. 1. Respiratory quotient of both strains of *Saccharomyces cerevisiae* in glucose, galactose, fructose, sucrose, maltose and raffinose

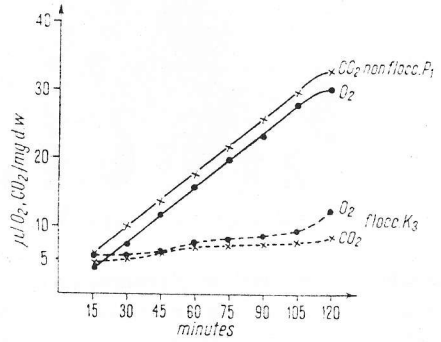


Fig. 2. Endogenuous respiration of both strains of *Saccharomyces cerevisiae*

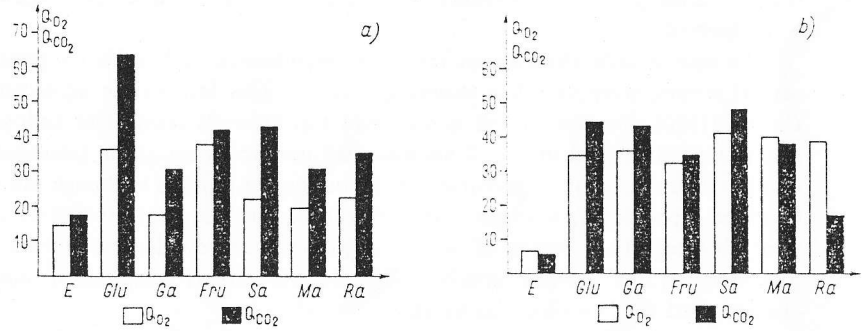


Fig. 3. Oxydative sugars consumption by both strains; *a*) non-flocculent (strain P1) *b*) flocculent one (strain K_1)

growth must be respected. The both types of top brewer's yeasts were stable in technological conditions keeping their morphological and physiological features. It is evident that some differences between non-flocculating and flocculent types concerning the rate of several sugars consumption and the dynamics of fermentation process are connected with the cell membrane permeability its structure and physico-chemical properties.

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Jadwiga Jakubowska

Niektóre cechy biochemiczne kłaczkujących i pylistych drożdży stosowanych w górnej fermentacji piwa w Grodzisku Wlkp

Streszczenie

Drożdże pyliste i kłaczkujące fermentacji górnej, stosowane w produkcji piwa grodziskiego w stosunku 2:1, utrzymują się w tym stosunku w toku procesu fermentacyjnego. Wykazano to na podstawie stosunku wymienionych drożdży w analizie piwa butelkowanego, który wynosił 2—3:1.

Cechy morfologiczne odpowiadały opisanym przez Szmelicha (1964), a także taksonomicznie typowi A i B *Saccharomyces cerevisiae* Hansen według Kockovej i *wsp.* (1970). Oba typy nie wykazywały różnic w uzdolnieniach do asymilacji cukrów oraz źródeł azotu, natomiast pewne różnice zaznaczały się w intensywności oksydatywnej dekarboksylacji glukozy mierzonej metodą Warburga.

Author's address:
Prof. dr J. Jakubowska,
Technical University,
Łódź, Gdańska 166, Poland