Vitis **14**, 43—47 (1975)

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The development of non-foaming yeast strains for wine-making

by

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Die Gewinnung nicht schäumender Hefestämme für die Weinbereitung

Zusammenfassung. — Nicht schäumende Hefen für die Weinbereitung können mit Hilfe des „froth flotation"-Verfahrens unter Verwendung von Glasröhren zur Prüfung des Tresterhutes leicht aus Reinkulturen gewonnen werden. Schwebefähigkeit und Hutbildung von 5 Parentalstämmen und 10 Mutanten wurden bestimmt. Der derzeitige Wissensstand über die Schaumbildung durch die Zellwandstruktur der Hefen sowie durch die Zusammensetzung des Gärsubstrates wird kurz diskutiert.

Introduction

Pure culture yeast strains are being used in all wine producing countries. Unlike the brewing industry, where much more emphasis is placed on strains with particular characteristics, the wine industry does not make full use of the potential of different yeast strains as yet (Radler 1973). Although a number of species of the genus *Saccharomyces* with an enormous variety of winemaking characteristics is known, mostly strains are employed, which do not influence the varietal character of a wine.

Besides many favourable properties, pure culture yeasts may still have some undesirable characteristics, some of which may only become apparent with increasing quality standards of the final product or when rigid economic calculations of fermentation processes are applied.

Froth-head formation is one such undesirable property, which is particularly evident at an early stage of grape juice fermentations. A large number of yeast cells is included in the froth and it appears that these cells adhere to the surface of the CO_2 bubbles originating from the breakdown of glucose and contribute to stabilising the froth-head. Up to 5% of the capacity of a fermentation tank is normally reserved to prevent such froth from spilling over. From a practical point of view, frothless yeast strains are quite advantageous since a tank could be used to its full capacity.

In this paper we describe the development of non-foaming yeast strains for winemaking.

Materials and Methods

1. Frothing procedure

The frothing procedure has been described in detail by Nunokawa *et al.* (1971). Fundamental to this method is the ability of a yeast cell to adhere to gas bubbles. By bubbling compressed air through a yeast culture fluid all cells are removed by the froth except those mutants lacking this ability. The residual cell suspension is grown in a fresh medium and after one day again subjected to frothing. In a

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series of such selection procedures, until removal of cells with the froth is no longer observed, the non-foaming mutant is concentrated to a maximum level. This culture, usually obtained after ten frothings, is then plated for single cell isolates which can be further characterised. To facilitate frothing a non-ionic detergent, polyoxyethylene-(9)-nonylphenol ether (0.04 mg/ml), is added to the yeast suspension. The air flow is maintained at 100 ml/min and frothing is stopped after 3—5 minutes, as soon as 20 ml of foam is collected.

Flotability, the removal rate of cells by the froth, is expressed by the Concentration Index (C.I.) and the Separation Index (S.I.) according to NUNOKAWA *et al.* (1971).

C.I. expresses the concentration of the yeast suspension and is calculated as the optical density of the collected yeast suspension in the cylinder/optical density of the original suspension. S.I. shows the degree to which yeast cells have been removed by the foam from the original solution. It is calculated as the optical density of the collected yeast suspension (froth) multiplied by 20/optical density of the residual suspension multiplied by 60.

2. Head-formation tubes

In our study the froth-head formation of many yeast strains was assessed in a reasonably short time by using "head formation tubes" (HARRIS and WATSON 1971). These glass tubes, 40 cm × 1.0 cm diameter and containing 15 ml of sterile grape juice, were inoculated with the strains under investigation. Head foaming yeasts produced a clearly defined yeast ring on the glass surface just above the liquid, whereas non-foaming yeasts gave a yeast free froth which collapses with no yeast ring after one day of fermentation.

3. Yeast strains

Five strains of *Saccharomyces cerevisiae* of the Ruakura collection were used. R92 and R93 are pure culture yeasts widely used in the wine industry. R104 is a non-foaming yeast, which was isolated at Geisenheim, Germany, but is a high sulphite forming strain (ESCHENBRUCH 1974). R107 and R108 are newly isolated strains which are being assessed for their winemaking properties. The yeasts were grown at 25 °C in batch cultures on a rotary shaker (250 rpm) for 24 h or longer using a medium containing $(NH_4)_2SO_4$, 2g; KH_2PO_4, 1g; $MgSO_4 \cdot 7H_2O$, 0.3g; KCl, 0.5g; $CaCl_2 \cdot 2H_2O$, 0.4g; glucose, 40g; yeast extract (Difco), 5g; dist. H_2O, 1l. The cells were washed once in fresh medium, suspended again in the same substrate and the density of this suspension was adjusted to 660mμ O.D. of between 0.300 and 0.400 using 1 cm cells. It was shown that the flotability increased as the cell population increased, but the influence was negligible between O.D. of 0.300 and 0.500 (NUNOKAWA *et al. 1971).*

Results and Discussion

Generally, a high froth forming strain has a high flotability with (C.I.) and (S.I.) values of 3.0 or more. Non-frothing strains have a low flotability with (C.I.) values less than 2.0 and (S.I.) values less than 1.0. The Table lists these two indexes for five parent strains and ten mutants. R92 and R93 clearly show high flotability and also a very prominent yeast ring when fermenting grape juice in head formation tubes. Surprisingly, R107 and R108 have to be regarded as non-foaming yeasts, since their (C.I.) and (S.I.) values are very low and they do not exhibit any stable yeast ring when tested for head formation. Strain R104, regarded as a wild non-foaming yeast, belongs to the same group, its (C.I.) and (S.I.) as well as no head formation define it as a non-foaming yeast. The mutants of R92 and R93 show a

Table

Flotability and head-formation of pure culture yeasts and their non-foaming mutants. The data represent mean values of three replicates
Schwebefähigkeit und Hutbildung von Kulturhefen und ihren nicht schäumenden Mutanten. Die Zahlen sind Durchschnittswerte aus drei Wiederholungen

Yeast Strain	Flotability C. I.	Flotability S. I.	Head formation after two days[1])
R92	2.7	5.2	+
92.11.1	0.9	0.3	—
.11.5	0.7	0.4	—
.19.4	0.7	0.2	—
.19.6	1.2	0.6	—
R93	2.7	3.2	+
93. 9.1	1.3	0.4	—
. 9.3	1.0	0.4	—
R107	0.8	0.3	—
107.9.5	0.8	0.3	—
.9.22	0.6		—
R108	1.1	0.4	—
108.9.3	1.1	0.3	—
.9.6	1.0	0.4	—
R104	1.2	0.4	—

[1]) + Clearly defined yeast ring.
— no yeast ring.

clearly improved flotability, whereas those of R107 and R108 do not differ from their parent strains.

Most of the information on non-foaming yeasts has so far been collected from investigations into saké yeasts (Ouchi and Akiyama 1971; Nunokawa and Ouchi 1972) and brewers' yeasts (Harris and Watson 1971; Stewart *et al.* 1973). There seem to be no morphological and physiological differences between parental strains and their non-foaming mutants (Ouchi and Nunokawa 1973). Neither did the cell wall composition of such strains differ (Ouchi, Takahashi *et al.* 1973). Genetical experiments showed that all hybrids between wild-type and non-foaming strains formed a high froth head. Hence the wild type allele is dominant over the non-foaming allele (Kasahara *et al.* 1974). Only when physico-chemical properties of cell surfaces were analysed, methods were found to distinguish between foaming and non-foaming yeasts (Nunokawa *et al.* 1971; Ouchi and Nunokawa 1973; Nagai *et al.* 1972).

Yeast cell walls consist of several distinct layers. The outermost layer is composed essentially of mannan-protein and the inner one is mainly glucan-protein, which is bounded by the innermost layer, the lipid rich plasma membrane. The detailed structure, however, may vary from one yeast to another, reflecting the different surface characteristics of each strain.

By determining the electrophoretic mobilities of various strains of *S. cerevisiae* and *S. carlsbergensis* three types of charged groups on the cell surface, depending on the pH, were reported (Eddy and Rudin 1958). Subsequently Ouchi and Nunokawa (1973) demonstrated that the non-foaming mutants differ from their parent strains in their pattern of pH-mobility in electrophoresis.

Furthermore, parent strains seem to have more hydrophobic groups on the cell surface (Ouchi and Nunokawa 1973). By measuring changes in flotability it was

suggested that the hydrophobic substances were some specific residues of protein and that the reason why mutant cells show a more hydrophilic character was that the hydrophobic substances would be masked by phosphomannan.

Inferring that parent strains can only be distinguished from the non-foaming mutants by assessing their physico-chemical properties, such mutants should be obtained from pure culture wine yeasts, the physiological properties of which are fully established. Using the froth flotation method non-foaming yeasts can easily be selected from pure culture strains.

As far as foaming caused by the yeast strain is concerned, this problem can be solved successfully. There remains, however, the foam formation independent from the yeast, but caused by the composition of the fermenting substrate as so far has been shown for nitrogenous constituents of beer only (CLAPPERTON 1971 a, b, BISHOP *et al.* 1974) with the intriguing aspect of secretion of peptides, amino acids and polysaccharides during fermentation of wort by *S. carlsbergensis* (CLAPPERTON 1971 c). To date no information is available to our knowledge as regards the influence of different protein and polysaccharide fractions of must on foaming, although some data have been published on soluble proteins and polysaccharides in musts and wines (SOMERS and ZIEMELIS 1973, RADOLA and RICHTER 1972, FEUILLAT *et al.* 1972, NANITASHVILI *et al.* 1972, ZINCHENKO and ALEKSEICHUK 1973). Tests using non-foaming yeasts on a commercial basis are under way.

Summary

Non-foaming yeasts for winemaking can be obtained easily from pure culture strains by using the froth flotation method and head formation tubes. Flotability and head formation of five parental strains and ten mutants are assessed. Current knowledge of foaming caused by the yeast cell wall structure as well as by the composition of the fermenting substrate is discussed briefly.

Acknowledgment

The skilful technical assistance of Mr. B. M. FISHER is much appreciated.

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Eingegangen am 29. 1. 1975

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