

Nature and occurrence of yeasts in Haryana grapes and wines

by

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The study of yeast flora of grapes is important from the enological point of view, particularly where natural fermentation is practised. Microbiologists have paid considerable attention to the naturally occurring yeasts of grapes and wines, e. g. MRAK and McCLUNG (1940), DOMERCQ (1956, 1957), TOLEDO *et al.* (1959) to the yeast flora of grapes of California, France and Brazil respectively, and VAUGHN (1956), VERONA and FLORENZANO (1956), JOSLYN and AMERINE (1964) and AMERINE and KUNKEE (1968) to various other aspects of grapes, musts and wines.

This paper reports on the nature and occurrence of yeast flora of Haryana grapes and their sequential appearance in musts and wines prepared therefrom.

Materials and Methods

Seven different varieties of grapes widely grown in Haryana were selected, viz. Beauty seedless, Perlette A, Perlette B, Pearl of Csaba, Anab-e-Shahi, Selection-seven and Bhokri. Except for Perlette B and Bhokri, obtained through the courtesy of Mr. LAVAN SINGH from his farm 14 km away, the others were drawn from the farms of the University. The yeasts were isolated by enrichment culture procedures normally employed for the purposes and were taxonomically characterized by the methods of LODDER and KREGER-VAN RIJ (1967).

Results and Discussion

More than one hundred isolates of yeasts were made from juice, must (at different stages of fermentation) and wine prepared from the above named grape varieties. The isolates represented 14 species of 6 different genera, i. e. *Saccharomyces*, *Schizosaccharomyces*, *Endomycopsis*, *Debaryomyces*, *Candida* and *Rhodotorula*. Table 1 shows the species encountered during this investigation and the stage at which they were isolated. Clearly, the sporogenous yeasts were dominant as 11 of these strains were sporogenous while only 3 belonged to the asporogenous group. The predominance of sporogenous yeasts in nature has been reported by CIFERRI (1941) and DOMERCQ (1956).

Among the sporogenous yeasts *Saccharomyces* was the most frequently encountered during the fermentation and all but one of its species were identified as *S. cerevisiae*, *S. cerevisiae* var. *ellipsoideus*, *S. steineri*, *S. carlsbergensis* and *S. mellis*. They outnumbered all others in their frequency of occurrence in juice, must as well as wine. MRAK and McCLUNG in 1940 and very recently MINÁRIK and NAGYOVA (1966) had also noticed *Saccharomyces* and apiculate yeasts as dominant in grapes and in musts during early stages of fermentation, whereas DOMERCQ (1957) encountered more abundantly in the French vineyards of Bordeaux region *Kloeckera api-*

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Yeast flora of grapes, musts and wines

Sr. Nr.	Name of grape variety	Juice	Must	During fermentation	From wine	From mat
1.	Beauty seedless	<i>Candida pulcherrima</i>	<i>Rhodotorula glutinis</i>	<i>Saccharomyces cerevisiae</i> var. <i>ellipsoideus</i>	<i>Saccharomyces cerevisiae</i>	<i>Endomycopsis fibuliger</i> var. <i>bispora</i>
2.	Perlette A	<i>Candida pulcherrima</i>	<i>Saccharomyces carlsbergensis</i>	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	<i>Endomycopsis fibuliger</i>
3.	Perlette B	<i>Candida guilliermondii</i>	<i>Schizosaccharomyces pombe</i>	<i>Debaryomyces vini</i>	<i>Saccharomyces cerevisiae</i>	<i>Endomycopsis fibuliger</i>
4.	Pearl of Csaba	<i>Candida pulcherrima</i>	<i>Saccharomyces carlsbergensis</i>	<i>Saccharomyces steineri</i>	<i>Saccharomyces cerevisiae</i>	<i>Endomycopsis fibuliger</i>
5.	Anab-e-Shahi	<i>Candida pulcherrima</i>	<i>Saccharomyces carlsbergensis</i>	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	<i>Endomycopsis fibuliger</i>
6.	Selection-seven	<i>Candida pulcherrima</i>	<i>Saccharomyces mellis</i>	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	<i>Endomycopsis fibuliger</i>
7.	Bhokri	<i>Candida pulcherrima</i>	<i>Schizosaccharomyces pombe</i>	<i>Saccharomyces</i> sp. unidentified	<i>Saccharomyces</i> sp. unidentified	<i>Endomycopsis fibuliger</i> var. <i>monospora</i>

culata and *S. cerevisiae*.

MARQUES (1963—1964) reported the isolation of fourteen strains of wine yeasts from port-producing districts of Portugal and found them to be of the *Saccharomyces* species. TOLEDO *et al.* (1959) also found in fermenting musts *S. carlsbergensis* and *S. cerevisiae* var. *ellipsoideus* as much as 82 per cent of the isolates. The occurrence of *S. cerevisiae*, *S. cerevisiae* var. *ellipsoideus* and *S. carlsbergensis* was not unusual; but *S. steineri* found by us had not been isolated so frequently by others, except by DOMERCQ (1957) who reported on its occurrence in French vineyards. *S. mellis* was encountered by us in the must of Selection-seven only but it may be useful in the production of desert wine because it is unable to ferment sugars except dextrose.

The unidentified yeast was isolated from must of Bhokri. Like other *Saccharomyces*, it had round and oval shaped cells and resembled *S. cerevisiae* var. *ellipsoideus* in its fermentation characteristics except that it was unable to ferment maltose.

Only one species of yeast belonging to genus *Debaryomyces*, i. e. *D. vini* was isolated from must of the variety Perlette B. TOLEDO *et al.* (1959) reported the presence of *Debaryomyces* species in fermenting musts. According to TABUCHI *et al.* (1969) this yeast could produce citric acid from glucose in shake cultures and thus bring about an increase in the total acidity of wine. The species seems to be of some use in case of grapes having low acidity, provided it fulfils other requirements of a wine yeast.

Schizosaccharomyces pombe was isolated from musts of Perlette B and Bhokri. The occurrence of this yeast on grapes or grape must has not been previously reported, except by BHARDWAJ (1970) in the musts of Haryana grapes. *Schizosaccharomyces* yeasts are known to ferment and transform malic acid into alcohol (PEYNAUD *et al.* 1964). BENDA and SCHMITT (1969) in fact tested some strains of *S. pombe* for their influence on the decomposition of malic acid and development of the organoleptic quality of wine and reported that in many cases musts inoculated with strains of *Schizosaccharomyces* produced wines of superior quality.

Three different strains *Endomycopsis fibuliger*, *E. fibuliger* var. *monospora* and *E. fibuliger* var. *bispora* were identified. These yeasts were present in all the wines and found to be responsible for the formation of 'mat', i. e. pellicle of yeast growth, on their surface. The presence of this yeast in grapes, musts or wines has not been reported, so far as is known, in other countries. But in contrast, this yeast was found to be prevalent in Haryana grapes. These yeasts are undesirable as they can utilize ethanol as a source of carbon.

Two species of genus *Candida* i. e. *C. pulcherrima* and *C. guilliermondii* were isolated during the present studies. *C. pulcherrima* was found to be associated with grapes of almost all varieties while *C. guilliermondii* was isolated from juice of variety Perlette B only. The occurrence of species of genus *Candida* in French musts has been reported by DOMERCQ (1956). The presence of *C. pulcherrima* in must has also been reported by MINÁRIK and NAGYOVA (1966). They found that there was a typical association of *C. pulcherrima* and *Kloeckera apiculata* in freshly crushed musts. But the absence of *K. apiculata* was noteworthy as none of the apiculate yeasts were found to be present. *C. guilliermondii* has been isolated by TAKEDA and TSUKAHARA (1969) from grape mashes. Since some of these yeasts, like *Endomycopsis* sp., can utilize alcohol as a source of carbon, their presence in wine is undesirable.

One pigmented yeast *Rhodotorula glutinis* was isolated from must of Beauty seedless. MAVLANI and GULYAMOVA (1968), while studying the asporogenic yeasts isolated out of 186 grapes and vinous substrates from different wine making regions of Uzbekistan, found that *Rhodotorula* was one of the most widespread forms.

Sequential occurrence

In general, the sequence of yeasts is *Candida* species in juice followed by *Saccharomyces* and *Schizosaccharomyces* in musts and mostly by *Saccharomyces* during fermentation. Apparently, fermentation of must is initiated by different species of *Candida* invariably present in the juice; *Schizosaccharomyces pombe*, *R. glutinis* and *S. carlsbergensis* then become active, say at the middle of the fermentation process, and during the later, perhaps critical, stage the genus *Saccharomyces* takes over to complete the more active period of fermentation and the *Sacch.* spp. remain till the end of fermentation. Only after the fermentation is over do the mat forming yeasts like the *Endomycopsis* species make their appearance.

The pattern of yeast flora and the sequence of yeast spontaneously fermenting red wine mashes in Czechoslovakia as reported by MINÁRIK (1964) were different in that the fermentation was initiated by some *K. apiculata* and *C. pulcherrima* and continued by *S. vini* and *S. oviformis*. MINÁRIK and NAGYOVÁ (1966) again confirmed the typical association of *K. apiculata* and *C. pulcherrima* in the freshly crushed musts and of *S. cerevisiae* var. *ellipsoideus* and *S. oviformis* in the fermenting must, as well as the role of the former yeasts in the initiation of fermentation and that of the latter in the completion of fermentation. But no *K. apiculata* and *S. oviformis* have so far been isolated by the present or other authors in the fermentation of wines.

Summary

More than one hundred isolates of yeasts were taken from juice, must (at different stages of fermentation) and wine prepared from seven different grape varieties grown in Haryana. The isolates fell into 6 genera and 14 species.

Saccharomyces yeasts were the most frequently encountered species during the fermentation, and all but one were identified as *S. cerevisiae*, *S. cerevisiae* var. *ellipsoideus*, *S. steineri*, *S. carlsbergensis* and *S. mellis*. *Schizosaccharomyces pombe*, *Debaryomyces vini*, *Candida pulcherrima*, *C. guilliermondii*, *Endomycopsis fibuliger*, *E. fibuliger* var. *monospora* and *E. fibuliger* var. *bispora* were the other strains isolated.

The occurrence of *Schizosaccharomyces pombe* and strains of *Endomycopsis fibuliger* is of interest in this connection when it is considered that *Kloeckera apiculata* and *Saccharomyces oviformis* have been significantly absent in Haryana grapes and musts.

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Literature Cited

- AMERINE, M. A. and KUNKEE, R. E., 1968: Microbiology of wine making. *Ann. Rev. Microbiol.* **22**, 323—358.
- BENDA, I. und SCHMITT, A., 1969: Untersuchungen zum Säureabbau im Most durch verschiedene Hefestämme aus der Gattung *Schizosaccharomyces*. *Weinberg u. Keller* **16**, 71—83.
- BHARDWAJ, S. K., 1970: Studies on wines produced from different varieties of grapes grown in Haryana. M. Sc. Thesis. Haryana Agricult. Univ., Hissar.
- CIPERRI, R., 1941: Diffusione e ciclo dei lieviti in natura. In: GAROGLIO, P. G. (Ed.): *Trattato di Enologia*. **11**, 310—325 (Firenze).

- DOMERCO, S., 1956: Etude et classification des levures de vin de la Gironde. Ph. D. Thesis. Univ. Bordeaux
- —, 1957: The study and classification of the wine yeasts of the Gironde. Ann. Technol. Agric. (Paris) 6, 5—58; 139—183.
- JOSLYN, M. A. and AMERINE, M. A., 1964: Dessert, appetizer and related flavored wines. Univ. of California, Div. Agricult. Sci. (Berkeley), 12, 483 pp.
- LODDER, J. and KREGER-VAN RIIJ, N. J. W., 1967: The yeasts. A taxonomic study. North. Holland Publ. Co. (Amsterdam).
- MARQUES GOMES, J. V. and VAZ, DE OLIVIERA, M. M. F., 1963-64: Anais Inst. Vinho. Porto. 20, 51—107. [Cited from: Ann. Rev. Microbiol. 22 (1968)].
- MAVLANI, M. I. and GULYAMOVA, N., 1968: Characteristics of asporogenic yeast like organisms. Uzb. Biol. Zh. 12 (1,) 16—19.
- MIÑARIK, E., 1964: Beitrag zur Hefeflora gärender Rotweinmaischen. Vitis 4, 368—372.
- — und NAGYOVA, M., 1966: Mikroflora von Mosten und Weinen im Weinbaugebiet von Nitra und im Donauraum. Pokroky vo Vinohradníckom a Vinárskom Výskume (Bratislava), 277—305.
- MRÁK, E. M. and MCCLUNG, L. S., 1940: Yeasts occurring on grapes and in grape products in California. J. Bacteriol. 40, 395—407.
- PEYNAUD, E. et SUDRAUD, P., 1964: Utilisation de l'effet dés-acidifiant des *Schizosaccharomyces* et vinification de raisins acides. Ann. Technol. Agric. (Paris) 13, 309—328.
- TABUCHI, T., TANAKA, M., and ABE, M., 1968: Studies on organic acid fermentation in yeasts. Examination of yeasts for their ability of producing citric acid. Nippon Nogei Kagaku Kaishi. 42, 440—443.
- TAKEDA, M. and TSUKAHARA, T., 1969: Identification of yeasts isolated from various fruit mashes. Hakko Kogaku Zasshi 47 (7), 399—407.
- TOLEDO, O., TEIXERA, C. G., e VERONA, O., 1959: Vantaggi della associazione di lieviti nella fermentazione vinaria. Riduzione dell'acidi volatile nei vini. Ann. Microbiol. 9, 22—34.
- VAUGHN, R. H., 1955: Bacterial spoilage of wines with special reference to California conditions. Advan. Food Res. 6, 67—108.
- VERONA, O. e FLORENZANO, G., 1956: Microbiologica applicata all'industria enologica. Ed. Agric. (Bologna), 191 pp.

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