Yeasts Isolated from Sugar Cane and Its Juice During the Production of Aguardente de Cana

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Sugar cane plantations have been expanding over a large area of the world for many centuries. During that time, sugar and several types of liquor, particularly rum, have been manufactured in huge quantities. In Brazil, about 300 million L of aguardente de cana are produced annually (Anon., 1953) by distilling fermented cane juice.

Although the natural yeast flora of sugar cane juice may contribute to that of sugar and may play an important role in manufacturing liquors made from sugar cane products, very little work has been published on this point. Ashby (1909) isolated several yeast cultures from sugar cane juice. He referred to some of these cultures as oval budding yeasts and to the others as fission yeasts. Different types of yeasts were isolated by workers from cane molasses, cane sugar, and syrup (Owen, 1949).

The work reported here was carried out to obtain a better idea of the natural yeast flora of sugar cane and its fresh and fermented juice. Moreover, certain yeast cultures could be selected in order to study their effect, when used as pure starters, on the production of aguardente de cana which is the Brazilian national liquor.

Materials and Methods

Samples were collected from five different factories located near Piracicaba in the state of São Paulo, and from six experiments carried out in the pilot plant at the Institute of Enzyme Technology in Piracicaba. Samples of cane juice were gathered at the crusher, from the canals leading from the crusher to the fermenting tanks, and from the fermentors during the fermentation. Two samples were taken from the fermenting juice: one near the top and the other near the bottom of the fermentor. Several samples of fresh water used for washing canes were also collected.

Direct streaking on malt agar of 10° Balling was used for isolation. Plates were kept at room temperature for several days, with frequent observations for picking different colonies. Isolates were purified by successive transfers to new media. The pure cultures were classified according to the procedures of Lodder and van Rij (1952).

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Results and Discussion

A total of 139 yeast cultures were isolated from 77 samples of washing water and cane juice collected from various factories and experiments. Table 1 shows the number of samples collected from various sources and the number of genera and species of yeasts isolated.

Generally, one or two types of colonies were noticed on each plate as may be seen by comparing the number of isolates with the number of samples. In the case of cane juice collected from the distributing canals, three or four different types of colonies were picked from each sample.

The occurrence of only one or two different types of colonies in each sample of fermenting juice may be attributed to the predominance of a certain species during the fermentation process. This view is supported by the fact that only five species in three genera were isolated from 33 samples of cane juice taken from the fermentors.

The fresh cane juice collected directly from the crusher or from the distributing canals showed a heterogenous yeast flora. Six different genera and 14 species were identified from samples collected from each source, although the number of samples taken from the canals was much less than that taken from

TABLE 1

Number of samples and number of isolates, genera, and species of yeasts obtained from various sources

Source of Samples	No. of Samples	No. of Yeasts Isolated	No. of Species Represented	No. of Genera Represented
Washing water				
Commercial plant	1	2	1	1
Pilot plant	10	14	7	4
Fresh juice—crusher				
Commercial plant	4	9	8	6
Pilot plant	20	38	10	5
Fresh juice—canals				
Commercial plant	9	32	14	6
Pilot plant	—	—	—	—
Fermentor—top				
Commercial plant	5	6	2	1
Pilot plant	9	13	2	1
Fermentor—bottom				
Commercial plant	15	16	5	3
Pilot plant	4	9	1	1

under the crusher. Each sample of juice taken from the distributing canals represented a larger quantity of juice than that taken directly from under the crusher. This may be the reason for obtaining more types of different colonies from each of the former samples than from the latter.

The occurrence of yeast species according to place and source of isolation is presented in table 2. The 139 yeast isolates belong to nine genera and to 26 species. Of these nine genera, *Saccharomyces*, *Candida*, *Pichia* and *Torulopsis* were more frequent than the others on the cane plant and in fresh juice. However, only *Saccharomyces*, *Candida*, and *Schizosaccharomyces* were isolated from fermenting juice. Failure to isolate *Pichia* and *Torulopsis* from fermenting juice may be due to the domination of the other genera which are strong fermenters, whereas *Pichia* and *Torulopsis* yeasts are nonfermenters or comparatively weak fermenters.

One or very few isolates of *Endomyces*, *Hansenula*, *Kloeckera*, and *Saccharomycodes* were obtained from fresh juice.

As shown in table 2, the most common yeast species are *Saccharomyces carlsbergensis* var. *alcoholophila*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida krusei*, *Torulopsis stellata*, *Candida guilliermondii*, *Pichia fermentans*, *Candida intermedia* var. *ethanophila* and *Schizosaccharomyces pombe*. Most of the remaining 17 species were isolated only once or twice. The heterogenous yeast flora of fresh cane juice may be due to the presence of soil particles which are commonly present on the canes. Many of these species have been isolated from soil by Bouthilet (1951), Lund (1954), and Capriotti (1955).

Although the first eight species were isolated frequently from the fresh cane juice, only two of them were prevalent in fermenting juice. These two species, *S. carlsbergensis* var. *alcoholophila* and *S. cerevisiae*, were isolated equally from both top and bottom samples from the fermentors. *S. pombe* was isolated only from samples taken near the bottom of the fermentor and at only two of the five factories. The predominance of these three species in fermenting juice may be attributed to their comparatively strong fermentative

TABLE 2

Yeasts isolated from different sources

Identification of Yeasts Isolated	Frequency of Isolation								
	Washing water		Fresh juice—crusher		Fresh juice—canals	Fermentor—top		Fermentor—bottom	
	Commercial plant	Pilot plant	Commercial plant	Pilot plant	Commercial plant	Commercial plant	Pilot plant	Commercial plant	Pilot plant
Candida guilliermondii		2		3	2				
Candida intermedia var. *ethanophila*				4	1				
Candida krusei	2	2			6			1	
Candida mycoderma				2					
Candida parapsilosis var. *intermedia*		2			1				
Candida tropicalis								1	
Candida saccharum n. sp.			1						
Endomyces magnusii					1				
Hansenula anomala			1						
Kloeckera apiculata			2	1	1				
Pichia fermentans		2		4					
Pichia membranaefaciens				11	1				
Saccharomyces acidifaciens					1				
Saccharomyces carlsbergensis var. *alcoholophila* n. var.		4		4	5	1	10	2	9
Saccharomyces cerevisiae			1	1	5	5	3	7	
Saccharomyces chevalieri			1						
Saccharomyces marxianus			1		1				
Saccharomyces microellipsodes					4				
Saccharomyces rosei					1				
Saccharomycodes ludwigii					2				
Schizosaccharomyces pombe								5	
Torulopsis glabrata					1				
Torulopsis globosa		1							
Torulopsis stellata			1	7					
Torulopsis stellata var. *cambresieri*				1					
Torulopsis saccharum n. sp.		1							

power, nutritional requirements, or tolerance to higher temperatures.

All but two of the isolates could be identified by the system of Lodder and van Rij (1952). Since these two isolates differ in their characteristics from the known, published species, they are described here as new species and listed in table 2 under the following names:

Candida saccharum n. sp. Growth in malt extract: after 3 days at room temperature cells oval to spherical, 2.2 to 5.5 by 2.2 to 5.5 μ; single or in pairs or short chains. Thin, smooth, and whitish pellicle; ascending ring and a sediment are formed. Streak cultures on malt agar: whitish colored, surface smooth and glistening, soft structure, low convex cross-section, border entire with pseudomycelium. Slide cultures on potato dextrose agar: pseudomycelium not well developed; blastospores arranged in short chains. Fermentation positive for glucose, sucrose, maltose (latent and weak) and raffinose (1/3 only). Galactose and lactose not fermented. Assimilates glucose, galactose, sucrose, maltose, and ethanol but not lactose. Nitrate assimilated. Splits arbutin.

Torulopsis saccharum n. sp. Growth in malt extract: after 3 days at room temperature cells short oval to spherical, 1.8 to 3.6 by 1.8 to 4.5 μ; usually in small clusters. A ring and sediment form gradually, but no pellicle. Streak culture on malt agar: white to cream colored, surface smooth and glistening, soft texture, cross-section low convex, border entire. No pseudomycelium on potato dextrose agar slides. Fermentation absent. Assimilates glucose and ethanol but not galactose, sucrose, maltose, and lactose. Assimilation of nitrate—positive. Does not split arbutin.

The remainder of the isolates fit well into the designated species as described by Lodder and van Rij (1952), except those belonging to *S. carlsbergensis* and *C. intermedia*. They differ from the original descriptions in their ability to utilize ethanol as a sole source of carbon. Verona and Toledo (1954) have described a new yeast variety designated *Candida intermedia* var. *ethanophila*, which resembles *C. intermedia* but assimilates ethanol. Therefore, isolates belonging to *C. intermedia* were placed under this new variety. Isolates of *S. carlsbergensis* capable of utilizing ethanol as the sole carbon source have been grouped in a new variety designated *S. carlsbergensis* var. *alcoholophila*.

Moreover, two isolates which were originally classified as *Candida melibiosi* have been placed with *C. guilliermondii* because of the findings of Wickerham and Burton (1954).

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Summary

One hundred and thirty-nine yeast cultures were isolated from 77 samples collected from the washing water of sugar canes and from fresh and fermenting cane juice during the production of aguardente de cana in Brazil. Although 26 species were identified, only eight were predominant in fresh cane juice; namely *Saccharomyces carlsbergensis* var. *alcoholophila* (n. var), *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida krusei*, *Torulopsis stellata*, *Candida guilliermondii*, *Pichia fermentans*, and *Candida intermedia* var. *ethanophila*. Most of the other species were isolated only once or twice.

Only three species were predominant in the fermenting juice: *Saccharomyces carlsbergensis* var. *alcoholophila*, *S. cerevisiae*, and *Schizosaccharomyces pombe*.

Two new yeast species and a new variety were isolated and described.

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