

curred in spite of the replacement of content of stage 1 due to interaction of remaining mycelium on the agitator shaft and tank walls with the freshly supplied mycelium.

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Cacao Fermentation

V. Yeasts Isolated from Cacao Beans during the Curing Process

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ABSTRACT

MARTELLI, H. L. (University of Brazil, Rio de Janeiro), and H. F. K. DITTMAR. Cacao fermentation. V. Yeasts isolated from cacao beans during the curing process. *Appl. Microbiol.* **9**:370-371. 1961.—Cacao beans must be subjected to fermentation before they are used in making chocolate, and their commercial value is related to a proper procedure. *Saccharomyces rosei*, *Hansenula anomala*, *Pichia fermentans*, *Pichia membranaefaciens*, and *Trichosporon cutaneum* were found in fermenting cacao beans. All species isolated during the investigation grew on cacao pulp, but only *S. rosei*, *H. anomala*, and *P. fermentans* exhibited fermenting capacity on the sugars of cacao pulp. Species of the genus *Saccharomyces* were identified as the agents responsible for the alcoholic phase of the cacao fermentation.

Before being used in making chocolate, cacao beans are subjected to a curing process known as cacao fermentation. The pulp and the beans from ripe fruits are accumulated in large wooden tanks and an alcoholic

fermentation starts, followed later by an acetic phase (Martelli, 1955a). Through this process the temperature of the mass rises to 50 C, the embryo of the bean dies, and autolysis of the cotyledons is induced; then, the polyphenolic substances of the beans are liberated and transformed (Martelli, 1955b, c). The beans become brown and sweet, and upon roasting develop the typical smell of cocoa. Although a proper fermentation is essential to obtain a product of value (Martelli, 1960), existing knowledge about the yeasts participating in the alcoholic phase is very incomplete, and former descriptions of some species isolated from cacao fermentations do not fit into the system of yeast classification in current use. A case in point is the species isolated by Preyer (1913) and described as *Saccharomyces theobroma*, later reclassified by Knapp (1937) as *Torulopsis theobroma*, but no longer recognized by Lodder and Van Rij (1952). A similar situation exists for the species referred to by Ciferri (1931). Martelli (1955d) isolated a yeast from the husk and from the beans in fermentation which undoubtedly participated in the alcoholic process. That organism was reported at the time to represent *T. theobroma*, but was later reclassified as *Saccharomyces carlsbergensis*. In the study reported here, strains of yeast were isolated from several

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fermenting batches and classified according to the system of Lodder and Van Rij (1952). Considerations about its role in the process are also presented.

MATERIALS AND METHODS

Beans² of the Forastero type from Bahia were collected from several batches in different stages of fermentation, others not yet fermented, and some dried and ready for sale. Enrichment cultures were made in molasses-ammonia medium (Martelli, 1955*d*) and in glucose broth and kept at room temperature. The isolations were made in plates of malt-infusion agar, also incubated at room temperature for several days; with frequent observation, all different types of colonies were picked. The pure cultures thus isolated were studied and classified according to Lodder and Van Rij (1952).

RESULTS AND DISCUSSION

1) *Saccharomyces rosei* (Guill.) Lodder and Van Rij. Strain no. 1: from fresh beans. Strain no. 2: from beans in the first day of fermentation. Both strains showed the characters of the prototype.

2) *Hansenula anomala* (Hansen) H. P. Sydow. Strain no. 3: isolated from beans not yet fermented. All the characters of the prototype were present, with a remarkable tendency to produce esters.

3) *Pichia membranaefaciens* Hansen. Strain no. 4: from the material in the fifth day of fermentation. Strain no. 5: from the material in the seventh day of fermentation. Both specimens possessed the characteristics of the prototype.

4) *Pichia fermentans* Lodder. Strain no. 6: isolated from fresh beans, before fermentation started. The fundamental characters were those of the prototype. In the isolated strain, spores in helmet form were always observed; also a whitish, thick, and wrinkled pellicle soon formed. The latter is not a characteristic of the prototype, but it is observed in *Pichia dombrowski* Schetti, reclassified by Lodder and Van Rij (1952) as identical to *P. fermentans*.

5) *Trichosporon cutaneum* (de Beurm et al.) Ota. Strain no. 7: isolated from fermented beans ready for sale. Strain no. 8: from beans in the very late stage of fermentation. Both isolates presented the characteristics of the prototype.

To confirm these results, cultures were made with the isolated strains on a medium obtained from sterilized cacao fruit pulp.² Growth and fermentative capacity were recorded, the latter measured by gas production in a Durham tube. The sugar content of the pulp was adjusted to 10% and the pH to 4.0, and the

² Obtained from the Estação Experimental do Instituto do Cacao da Bahia, Uraçuca, Bahia, Brasil.

TABLE 1. Growth and fermentation of yeasts isolated from cacao fermentation when inoculated into a medium composed of sterilized cacao pulp

Yeasts	Growth (turbidity)	Fermentation (gas production)
<i>Saccharomyces rosei</i>	+	+
<i>Hansenula anomala</i>	+	+
<i>Pichia membranaefaciens</i>	+	-
<i>Pichia fermentans</i>	+	+
<i>Trichosporon cutaneum</i>	+	-

tests were carried on at room temperature for 7 days. The results are shown in Table 1.

All of aforementioned strains grew well in cacao pulp, as could be expected considering its composition, as previously determined by Dittmar (1956). Since glucose is the main sugar present in cacao pulp, the fermentation tests of the laboratory were confirmed in nature. The fermentative species were found in the beans not yet fermented, or in the first day of fermentation, as expected. Because of the prior isolation of *S. carlsbergensis* from beans in the first phase of the fermentation (Martelli, 1955*d*) it is concluded that the alcoholic phase of the cacao fermentation is carried on by fermentative yeasts of the genus *Saccharomyces*.

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