

J. Natn. Sci. Coun. Sri Lanka 1985 13 (1) :

ISOLATION AND CHARACTERIZATION OF YEASTS OF SOME FRUITS AND FRUIT PRODUCTS OF SRI LANKA

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(Date of receipt : 28 June 1984)

(Date of acceptance : 7 May 1985)

Abstract: Thirty six strains of yeasts isolated from some fruits and fruit products were identified using their biochemical and morphological characteristics. These yeasts belonged to six genera viz: *Candida* (Sixteen strains), *Kloeckera* (nine strains), *Hanseniaspora* (three strains), *Pichia* (four strains), *Saccharomyces* (three strains) and *Torulopsis* (one strain). The most predominant species was *Candida krusei* (Ten strains).

1. Introduction

Microbial spoilage of food is commonly the result of the combined activities of yeasts, moulds and bacteria. However, depending upon the environment, one of these groups of microbes may prevail over the others. Yeasts ordinarily do not compete well in mixed populations, and therefore, cause spoilage only under conditions which are favourable for their growth, but unfavourable for growth of most bacteria.¹ The most important factors which determine the ability of yeasts to compete with moulds and bacteria are the number and types of contaminating yeasts, available nutrients, pH, redox potential and water activity.² Spoilage of fresh fruits and vegetables by yeasts usually result from their fermentative activity rather than degradation of plant tissue with degradative enzymes. However, some yeasts are reported to be capable of producing such enzymes and cause spoilage.³

Sri Lanka produces a variety of tropical fruits, and also, possesses a rapidly developing fruit processing industry. However, a considerable amount of the produce is lost due to post harvest deterioration where microbial attack is suspected to be one of the predominant causes. In the present study an attempt was made to isolate and characterize the yeast flora of some local fruits and fruit products which may play a role in their spoilage.

2. Experimental

Random samples (about 10 each) of fresh ripe fruits (except dates and raisins, which were dried), canned fruits, juices, syrups and cordials were

obtained from retail sellers, canneries and supermarkets of Colombo. These samples included fruits of papaw, grape, date, tomato, passion fruit, lime, pineapple, nelli, raisin, plum, banana, amberella, grapefruit, tangerine, lawulu and mango, and papaw juice, grape-juice, pineapple-juice, nelli-juice, mango-juice and passion fruit cordial (Botanical names of fruits are given in Table 1).

Table 1 — Botanical names of fruits

Papaw	—	<i>Carica papaya</i>
Grapes	—	<i>Vitis vinifera</i>
Dates	—	<i>Phoenix dactylifera</i>
Tomatoes	—	<i>Lycopersicum esculentam</i>
Passion fruit	—	<i>Passiflora edulis</i>
Lime	—	<i>Citrus aurantifolia</i>
Pineapple	—	<i>Ananas sativus</i>
Nelli	—	<i>Philanthus emblica</i>
Raisin	—	<i>Vitis vinifera</i>
Plums	—	<i>Prunus domestica</i>
Plantains	—	<i>Musa sapientum</i>
Ambarella	—	<i>Spondias mangifera</i>
Grape fruit	—	<i>Citrus decumana</i>
Tangerine	—	<i>Citrus nobilis</i>
Lawulu	—	<i>Chrysophylla sp.</i>
Mango	—	<i>Mangifera indica</i>

Yeasts were isolated by plating on a medium which had the following composition in g litre⁻¹ of distilled water : glucose 20.0, yeast extract 3.0, peptone 5.0 and agar 20.0. Plating was done by streaking a loopful from enrichment cultures prepared by inoculating small amounts (about 1–2 g) of the sample into tubes containing broth of the same medium less agar. Cultures were incubated at room temperature for 2–4 days depending on growth. Methods used to study the biochemical and morphological characteristics were same.⁴ The following criteria were used to characterize and identify the isolated yeasts: fermentation pattern, assimilation pattern, morphology and sporulation. Some of these criteria were represented by codes.¹³

3. Results and Discussion

In this study thirty six strains of yeasts were isolated and identified. These thirty six strains of yeasts belonged to the six genera *Candida*, *Kloeckera*, *Hanseniaspora*, *Pichia*, *Saccharomyces* and *Torulopsis*. The predominant species were *Candida krusei* (ten strains) and *Kloeckera apiculata* (seven

strains). The morphological and biochemical characteristics of these strains and their sources of isolation together with their completed identification codes¹³ are given in Table 2.

Candida krusei the most predominantly encountered in this study was isolated on eight occasions from fresh fruits and in two occasions from dried fruits (Date and Raisin). Mrak *et al*⁷ and Miller and Mrak¹² has listed *Candida krusei* as one of the most numerous yeasts in spoiled figs. A strain of *Candida tamarandei* which is synonymous with *Candida krusei* has been isolated from fermenting date.¹⁴ Another species, *Candida guilliermondii* two strains of which were isolated from papaw juice and grape juice in this study has been earlier isolated from fig wasps.¹⁴ *Candida mesenterica* (three strains) and *Candida peliculosa* (one strain) which also were encountered in this study have not been isolated from fruits earlier.

Kloeckera apiculata, of which seven strains were isolated from fruits in this study, has been earlier reported⁸ to cause softening in strawberries. This also has been reported as the only yeast isolated from blackcurrents picked at the end of the harvesting period.³ Also, *Kloeckera apiculata* has been reported much earlier under the synonym *Saccharomyces apiculatus* as an organism widely distributed in fruits and orchards.¹⁰ Next to *Saccharomyces cerevisiae* this species has been reported to be the most prominent in wine grapes.⁹ Another species *Kloeckera africana*, isolated from Lawulu (two strains) has earlier been isolated from figs.⁹

Saccharomyces cerevisiae which is generally a common contaminant in many food products was encountered only once in this study (one strain from Tomato sauce). Two other species, *Saccharomyces exiguus* and *Saccharomyces italicus* a strain each of which was isolated from nelli juice and mango juice respectively has earlier been reported as a contaminant in grape must.⁵

Among the other yeasts encountered in this study which have been reported earlier are *Hanseniaspora valbyeansis*⁶ (wine grape) and *Pichia fermentans*¹ (orange juice). None of the other yeasts (*Pichia polymorpha*, *Pichia kuriavzevii* and *Torulopsis inconspicua*) have been reported as contaminants of fruits earlier.

Table 2 - Identification of Yeasts from Fruits & Food Products

Fermentation Code ^a	Assimilation		Sugar Code ^a	Cell shape	Morphology				Completed Code ^a	Strain
	Ethanol	Nitrate			Ascospore	Ps	My	Pe		
GA	+	0	4	short-ovoid, ovoid	-	+	-	+	2	<i>Candida guilliermondii</i> (Pj1, Gj1)
D	-	0	1	Lemon-shaped, ovoid or elongate	-	-	-	-	BB	<i>Kloeckera apiculata</i> (p1, Pf1, Pa1, Pap1, Paj1, Pla1, Ta1)
D	-	0	1	Oval, apiculate or elongate	Hat-shape or ovoid	-	-	-	BB	<i>Hanseniaspora valbyensis</i> (P1, M1, T1)
D	+	0	1	Cylindrical or ovoid	-	+	-	+	2	<i>Candida krusei</i> (D1, M1, Ti, P1, G1, Pf1, L1, R1, Am1, Grp1)
O	+	0	ISM	Ovoid, elongated	-	+	+	+	2	<i>Candida mesenterica</i> (G1, L1, Mj1)
D	+	0	5	Oval, cylindrical	Spherical	+	-	+	2	<i>Pichia polymorpha</i> (D1)
D	+	0	1	Oval, long oval	Spherical	+	-	+	2	<i>Pichia kudriavzevii</i> (T1, P1)
GMA	-	0	4	Spheroidal, subglobose or cylindrical	Spheroidal	+	-	-	1	<i>Saccharomyces cerevisiae</i> (Ta1)
D	+	0	1	Ovoid	-	+	-	-	1	<i>Torulopsis inconspicua</i> (Pfc1)
GMA	+	N	4	Ovoid, Ellipsoidal	-	+	-	+	2	<i>Candida pelliculosa</i> (Pap1)
GA	+	0	3	Spheroidal, ellipsoidal	Spheroidal	-	-	+	1	<i>Saccharomyces exiguus</i> (Nj1)
D	-	0	ISM	Ovoid, Elongated	-	+	-	-	BB	<i>Kloeckera africana</i> (La2)
GMS	+	0	GMS	Spheroidal, subglobose	Spheroidal	+	-	-	2B	<i>Saccharomyces italicus</i> (Mj1)
D	+	0	1	Oval to long-oval	Spherical	+	-	+	2	<i>Pichia fermentans</i> (G1)

Abbreviations: Ps, Pseudomycelium; My, Mycelium; Pe, Pellicle; Pj, Papaw juice; P, Papaw; G, Grapes; Gy, Grape juice; D, Dates; T, Tomato; Ts, Tomato source; Pf, Passion fruit; Pfc, Passion fruit cordial; L, Lime; Pa, Pineapple; Pap, Pineapple pulp; Paj, Pineapple juice; Nj, Nelli juice; R, Raisin; Pl, Plums; Pla, Plantains; Am, Ambarella; Grp, Grape fruit; Ta, Tangerine; La, Lawulu; M, Mango; Mj, Mango juice; Gl, Glucose; Ga, galactose; Su, Sucrose; Ma, Maltose; Ra, Raffinose; Eth, Ethanol; KNO₃, Potassium nitrate.

Code^a: (Based on method by Beach et al)

Sugar Fermentation:

O - : No. Fermentation; D - : Gl; Su, Ma, Ra; Ms - : Gl, Su, Ma; A - : Gl, Su, Ra; GA - : Gl, Ga, Su; GMA - : Gl, Ga, Su, Ma, Ra; GMS - : Gl, Ga, Su, Ma

Nitrate Assimilation:

N - : Assimilation; O - : No assimilation

Sugar Assimilation:

ISM - : Gl, Su, Ma, Ra, 1 - : Gl only; 3 - : Gl, Ga, Su, Ra; 4 - : Gl, Ga, Su, Ma, Ra

Morphology Code:

1 - : Multilateral budding, No pseudomycelium, No pellicle; 2 - : Multilateral budding, with pseudomycelium with pellicle; 2B - : Multilateral budding with pseudomycelium, No pellicle; BB - : Bipolar budding.

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