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Isolation and Characterization of Microorganisms Present in Coconut Water from Coconut Mills Producing Desiccated Coconut Powder

S. Umesha and B. Narayanaswamy

Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bangalore-560 065

Abstract

An investigation was carried out to isolate and characterize microorganisms in desiccated coconut water from coconut mills. The coconut water samples were collected at two different stages, one at nut breaking stage (coconut water) and other at final / end stage (residual coconut milk). The results revealed that residual coconut milk had highest microbial population such as bacteria (15.11 x 10^5 cfu / ml), yeast (15.33 x 10^4 cfu / ml), *Rhizobium* sp. (4.33 x 10^5 cfu/ml), *Azotobacter* sp. (9.67 x 10^5 cfu/ml), *Pseudomonas* sp. (10.33 x 10^5 cfu/ml), PSB (8.67 x 10^5 cfu/ml), *Escherichia coli* (6.33 x 10^5 cfu/ml) and *Salmonella* sp. (7.67 x 10^5 cfu/ml) as compared to coconut water.

The Desiccated Coconut (DC) industry is one of the major export oriented food processing industries in India. It consists of around 266 DC units, with an average capacity varying from 10,000 to 50,000 nuts/ day. Karnataka consists of around 45-50 DC units mainly located in coconut growing areas. The DC industries produce lot of waste water, including 1500 to 2000 liters of coconut water, 7000 to 8000 liters of chlorinated water/washed water and about 800 to 1000 liters of pasteurized water during the processing is left out as an effluent from desiccated powder producing industries having a capacity of 1000 kg/day (Anonymous, 1993).

Coconut water is traditionally used as a growth supplement in plant tissue culture / micropropagation and a best medium for microbial growth. The wide applications of coconut water can be justified by its unique chemical composition of sugars, vitamins, minerals, amino acids and phytohormones (Sathiyavimal *et al*., 2014). Coconut water is biologically sterile when it is in drupe and is free from microorganisms. In desiccated coconut industry, the initial contaminants might have been introduced during the handling of de-shelled coconuts, extraction of the coconut milk, the water used in the extraction, the utensils that come in contact with the grated coconut milk, coconut shell, the air and the handlers (Priyanthi, 1997). Hence, to considering all these factors for isolation of microorganisms from coconut water obtained from desiccated coconut mills.

In most of these industries**,** eight to nine months old matured coconuts were used for the production of DC powder. The coconut water and residual coconut milk samples were collected at two different stages from Maruthi desiccated coconut mills at Kaidal gate, Tiptur taluk, Tumkur district.

Stage 1. Coconut water- this water was collected at the time of nut breaking. It contains only pure coconut water (Fig.1).

Stage 2. Residual coconut milk – this was collected after de-shelled coconut pieces were washed. This milk contains- Coconut water, de-shelled coconut milk and water used for washing the de-shelled coconut pieces (Fig.2).

Fig. 1. Coconut water discharged

Fig. 2. Residual coconut milk discharged

TABLE I

Isolation of microorganisms from coconut water obtained from desiccated coconut mills

Samples	General microorganisms				Beneficial microorganisms				Harmful microorganisms			
	Bacterial isolates 10^5 Cfu/ml				Yeast 10^4 Cfu/ml	Actinomycetes 10^3 Cfu/ml	*Rhizobium* sp. 10^5 Cfu/ml	*Azotobacter* sp. 10^5 Cfu/ml	*Pseudomonas* sp. 10^5 Cfu/ml	PSB 10^5 Cfu/ml	*Escherichia coli* 10^5 Cfu/ml	*Salmonella* sp. 10^5 Cfu/ml
	1	2	3	Mean								
Coconut water	8.67	11.33	9.67	9.89	10.00	8.33	2.67	3.33	7.33	5.00	2.67	3.33
Residual coconut milk	14.33	18.33	12.67	15.11	15.33	10.33	4.33	9.67	10.33	8.67	6.33	7.671

TABLE II

Morphological and Biochemical characteristics of isolates

Morphological	Isolate 1	Isolate 2	Isolate 3	*Rhizobium* sp.	*Azotobacter* sp.	*Pseudomonas* sp.	PSB	*Escherichia coli*	*Salmonella* sp.
Colony morphology	Circular, Glistening, Cream	Circular, Dry, Whitish	Circular, Smooth, Whitish	Circular, Smooth, Whitish pink	Circular, Glistening, Whitish mucoid	Circular, Smooth,Creamy whitish	Circular, Smooth, Whitish	Circular, Smooth, Metallic sheen	Circular, Smooth, Black
Elevation	Convex	Flat	Convex	Raised	Convex	Raised	Raised	Flat	Flat
Opacity	Translucent	Opaque	Opaque	Opaque	Translucent	Opaque	Opaque	Opaque	Opaque
Cell shape	Coccus	Rod	Coccus	Rod	Coccus	Rod	Rod	Rod	Rod
Gram staining	+	+	-	-	-	-	+	-	-
Catalase	+	+	+	+	+	+	-	+	+
Indole	+	+	+	+	+	-	-	+	-
Methyl red	+	-	+	-	+	-	-	+	+
Vogesproskauer	-	+	-	-	-	-	-	-	-
Citrate test	-	-	-	-	+	+	-	-	+

Note: '+' - positive, '-' – negative

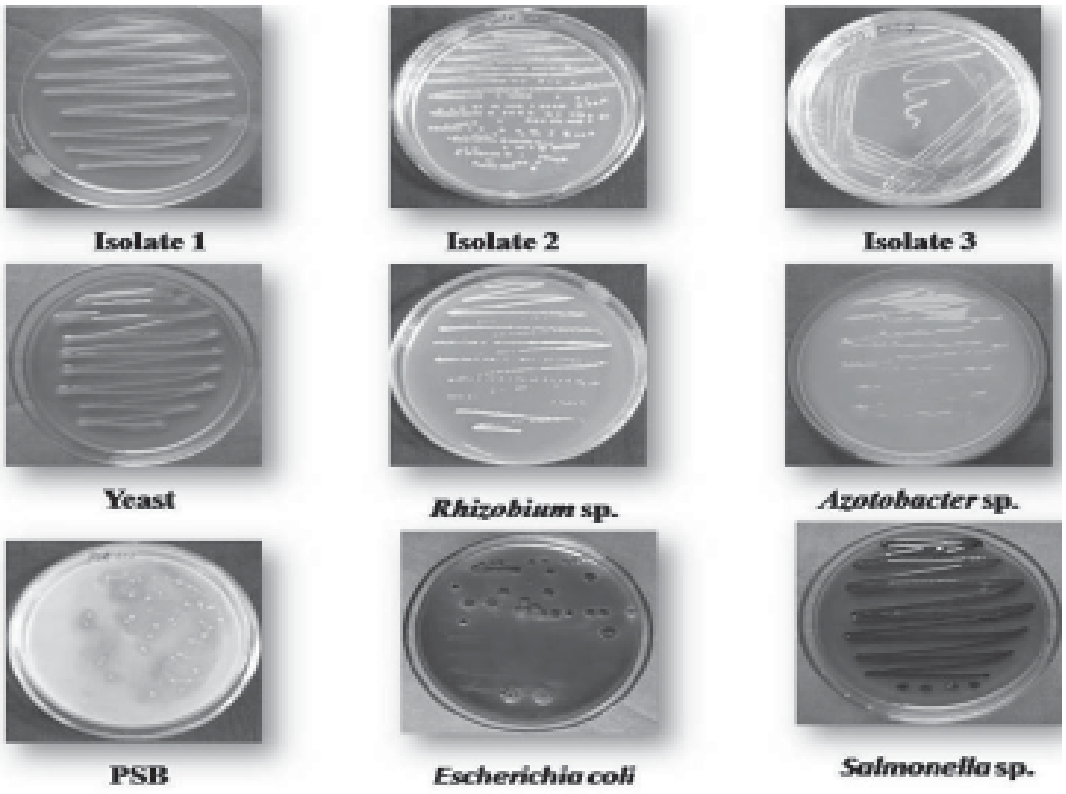

PLATE 1: Isolated colonies from Desiccated coconut mills coconut water

The fresh samples of coconut water and residual coconut milk were collected in sterilized plastic containers and brought to the laboratory to study the microorganisms in these water samples. The enumeration and isolation of microbes from DC mills coconut water was carried out by using serial dilution and plate count method (Bunt and Rovira, 1955). In this study, Nutrient agar (for bacteria), Potato dextrose agar (for fungi/yeast), Kuster's agar (actinomycetes), Yeast extract mannital agar with congo red (for *Rhizobium*), Asbhy's mannital agar (for *Azotobacter*), Pikovskaya's medium (for PSB), King's B medium (for *Pseudomonas*), Eosin methylene blue agar (for *E. coli*) and Bismuth sulphite agar (for *Salmonella*) media were used. The inoculated plates were kept for incubation at $30^0C \pm 1^0C$ for a week time except *E. coli* plates. The *E. coli* plates were incubated at 37 ^0C for two days and recorded the emerged colonies. Further, isolated colonies were purified and streaked with respective media (Plate I). All isolates were characterized by their colony morphology and biochemical tests were performed (Aneja, 2003).

The data presented in Table I indicate that the residual coconut milk has highest microbial population as compared to coconut water. This result explained that, the reaped nuts collected on the ground or from coconut garden or some nuts that were severely damaged during reaping or transport, permitted the seepage of coconut water, an ideal carrier of most organisms. (Nandana and Werellagama, 2001). The initial contaminants must have been introduced during the washing of de- shelled coconut pieces. The water used in the washing, the utensils that came in contact with the grated coconut milk, coconut shell, the air and the handlers are the possible sources of microorganisms. (Priyanthi,1997).

The isolated strains were examined and characterized by performing/taking up morphological as well as biochemical tests (Table II). Most of the isolated colonies were circular, smooth, convex, whitish, opaque and rod shape. Majority of them showed catalase, indole test positive and gram negative reaction (Mohsin, 2014). Further, these DC mills coconut water

was used as foliar spray to know the phyllosphere, rhizosphere microorganisms and yield of Gherkin and Chrysanthemum. As it contains plant growth promoting substances like auxin, cytokinins and gibberellins and essential mineral contents.

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