Advanced Journal of Microbiology Research Vol. 2010
Available online at http://internationalscholarsjournals.org/journal/ajmr
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Full Length Research Paper

Heat resistance of genus *Byssochlamys* isolated from bottled raphia palm wine

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Accepted 14 March, 2010

Bottled *raphia* palm wine was cultured in a laboratory medium amended with 0.5% acetic acid (pH 4.8). Two cultures of identified heat resistant yeast (HRY) and one unidentified yeast species were isolated. Cultures of the isolates grown on potato dextrose agar for 10 days at 26°C, survived pasteurization temperature at 80°C for 20 min and 85°C for 15 min. Of these HRY identified were *Byssochlamys nivea*, *Byssochlamys zollerniae* and one unidentified yeast species. To determine the source of contamination, fresh un-pasteurized *Raphia* palm wine was cultured. Result revealed that, colonies of the three HRY were higher compared with the pasteurized *Raphia* palm wine. Frequencies of occurrence at 80°C, 85°C and in un-pasterurized raphia palm wine were *B. nivea* 15.2, 6.1 and 24.2%; *B. zollerniae* 6.1, 3.0 and 12% and yeast species 9.1, 6.1 and 18.2% respectively. The thermal destruction time were *B. nivea* 90°C for 15 min, *B. zollerniae* 90°C for 5 min and yeast species 90°C for 10 min. The result indicates they are acid tolerant and thermophilic yeasts with *B. nivea* having the highest frequency of occurrence.

Key words: Culture, pasteurization, spoilage, identification, thermophilic.

INTRODUCTION

Raphia hookeri is the most economically important plant among the eight raphia species indigenous to Nigeria (Okolo, 2008; Otedoh, 1978). The exploitation of *Raphia* for the sap (palm wine) and other products of socio-economic importance such as pissava, fibre, oil edible grubs, poles, thatch etc are mainly from the wild (Udom, 2000). Wine is tapped from the panel which consists of the base of short spear leaves and the apical emerging terminal inflorescence axis (Tuley, 1965). The wine is rich in vitamins, carbohydrates and yeast (Obahiagbon, 2007).

Filamentous fungi are morphologically complex micro-organisms exhibiting different structural forms through out their life cycles (Adrio and Demain, 2003). The life cycles of filamentous fungi starts and ends in the form of spores. In submerged cultures, these fungi have different morphological forms ranging from dispersal of mycelial filaments to densely mycelial masses as pellets (Xu and Yank 2007). Microorganisms are an important part of our environment and are a principal cause of food spoilage. When food is contaminated by harmful microorganism, the products can cause severe human food-borne diseases, either due to the organisms themselves or the toxins released by them (Laplace-Buihe et al., 1993) . The presence of these microorganisms in the products, even at low concentration may severely affect their quality (Laplace-Buihe et al., 1993).

Fruit juices contain various concentration of sucrose, which constitutes a very important component of the medium for the growth of fungi (Palou et al., 1998). Microbial spoilage is a serious problem for the food industry as fungal contamination can occur during processing as well as handling of the end products. Since yeast can generally resist extreme conditions better than bacteria, they are often found in products with low pH and in those containing preservatives (Macrae et al., 1993) . Especially yeast spoilage has increased in recent years as a result of lower doses of preservatives and milder preservation

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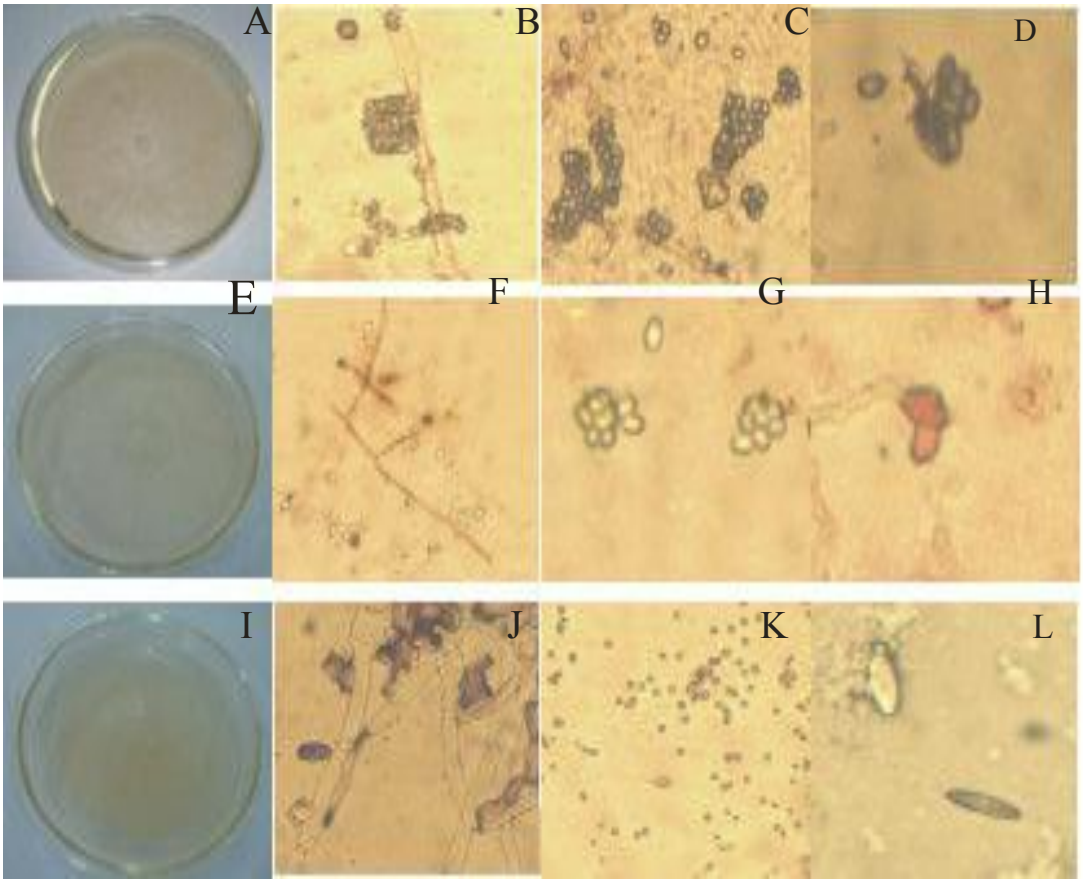

Plate 1 (a-l). Light photograph and microscope of *Byssochlamys* grown on PDA 10 days after Incubation.(a) Pure culture of *Byssochlamys nivea*; (b) Conidiophore (c). Conidia (d) Ascospores. (Conidia-scale bars = 10 m). (e) Pure culture of *Byssochlamys zollerniae*; F. Conidiophore; G. Conidia; (h) Ascospores. (Conidia-scale bars = 10 m). (i) Pure culture of Yeast species; (j) Conidiophore; (k) Conidia; L. Ascospores.

preservation processes required for higher standards of food quality (Beuchat, 1989). Spices, herbs and plants are essential oils added to foods primarily as flavoring agents have been shown to possess a broad range of antimicrobial activities (Palou et al., 2002).

The genus *Byssochlamys* contains two economically important species, *Byssochlamys nivea* and *Byssochlamys fulva*. Both species cause spoilage of processed fruit products and are among the most commonly encountered fungi associated with spoilage of heat processed fruits in countries worldwide (Tournas, 1994). *Byssochlamys* species produce ascospores which are heat resistant and survive considerable periods of heat above 85°C (Beuchat and Rice, 1979; Splittstoesser, 1987). In addition to their heat resistance, *Byssochlamys* species can grow under very low oxygen tension (Taniwaki, 1995) and can form pectinolytic enzymes. The combination of these three physiological characteristics makes *Byssochlamys* species very important spoilage fungi in pasteurized and canned fruits. *Byssochlamys* has a *Paecilomyces* anamorph (Samson et al., 2009). Patulin, a toxic secondary metabolite can be produced by *B. nivea* and *B. fulva*, as well as several species of *Penicillium* and *Aspergillus* (Jackson and Dombrink-Kurtzman, 2006). The objective of this study was to isolate and identify yeasts causing spoilage of the pasteurized *Raphia* palm wine with the view to determine their thermal destruction temperatures (Figure 1).

MATERIALS AND METHODS

Experiment

Different bottled *Raphia* palm wines (BRPW) were collected as samples from four different locations in Edo state of Nigeria. Eight out of the twenty-two BRPW stored for four months from each of the four locations were spoiled. The medium was constituted according to Chu and Chang (1973). Different aqueous dilutions (10^{-2} , 10^{-3} and 10^{-4}) of the suspension were applied onto plates and 20 ml of melted medium at around 50°C was added. After gently rotating, the plates were incubated at 26°C for 10 days. Isolated colonies of yeasts were transferred from mixed cultures of the plates onto respective agar plates (Waksman, 1961) and incubated aerobically

Table 1. Yeasts isolated from pasteurized and un-pasteurized raphia palm wine and their frequencies of occurrence.

T°C	Yeast contaminants	Pasteurized mean number of yeast colonies	% Frequencies
80°C – 20 min	*B. nivea*	5	15.2
	B. zollerniae	2	6.1
	Yeast species	3	9.1
85°C – 15 min	*B. nivea*	2	6.1
	B. zollerniae	1	3.0
	Yeast species	2	6.1
90°C-15 min	*B. nivea*	-	-
	B. zollerniae	-	-
	Yeast species	-	-
Unpasteurized	*B. nivea*	8	24.2
	B. zollerniae	4	12.1
	Yeast species	6	18.2

at 26°C for 10 days. Each BRPW sample was treated with four plates for each aqueous dilution, totaling twelve plates for each sample. Their mean colonies and frequencies of occurrence were determined according to Omamor (2007). The first yeast isolate with the highest frequency of occurrence was sent to CABI identification services Surrey United Kingdom for morphological and molecular identifications, the second yeast isolate was identified according to Samson et al. (2009) while the third yeast isolate was left unidentified.

Morphological characterization

Purified isolates of yeasts were identified to the generic level by comparing their morphology of spore-bearing hyphae with the entire conidiophores and structure of ascospores with the *Byssochlamys* morphologies as described by Samson et al. (2009). This was done by using the cover-slip method in which an individual culture was transferred to the base of cover slips buried in potato dextrose agar (PDA) (Duarte and Archer, 2003) for photomicrographs. They were incubated for ten days at 26°C ± 2. Structures of conidiophores and ascospores were visually estimated by using a motic microscope attached to a motic digital camera connected to a computer. The experiment was repeated three times.

RESULTS AND DISCUSSION

B. nivea, *Byssochlamys zollerniae* and one unidentified yeast species were isolated from both pasteurized and un-pasteurized *Raphia* palm wine. *B. nivea* with the highest frequencies of occurrence at 80, 85°C and in un-pasteurized *Raphia* palm wine (Table 1), which was sent to CABI identification services Surrey United Kingdom for morphological and molecular identification, was identified as *B. nivea* Wasting IMI No. 396923. Both *B. nivea, B. zollerniae* and the yeast species survived pasteurization temperatures at 80°C for 20 min and 85°C for 15 min. However, *B. nivea* at 90°C for 15 min, *B. zollerniae* at 90°C for 5 min and Yeast species at 90°C for 10 min were eliminated. Their colonies spread averagely on PDA at 26°C and covering the petri plates within 10 days (Plates 1a, e and i). Frequencies of occurrence at 80, 85°C and in un-pasterurized *Raphia* palm wine were *B. nivea* 15.2, 6.1 and 24.2; *B. zollerniae* 6.1, 3.0 and 12, and yeast species 9.1, 6.1 and 18.2 respectively (Table 1).

The genus *Byssochlamys* is morphologically well-defined and characterized by ascomata in which crosiers and globose asci are formed with ellipsoidal ascospores. The ascomatal initials consist of swollen antheridia and coiled ascogonia. Using light micrograph, the conidiophores, conidia and ascospores were seen. The conidium measured 3.1 - 4.3 × 2.6 - 3.2 µm (Plate 1c) and was characterized with smooth wall. The conidium of *B. zollerniae* measured 3.1 - 3.8 × 2.5 - 3.2 µm (Plate 1g).

The introduction of *B. nivea*, *B. zollerniae* and the yeast species into the bottled and pasteurized *Raphia* palm wine might have been due to inadequate pasteurization, contaminants from the host palm, sub-standard condition of the palm wine tapping panel and the bottling unit. This was supported by Odutayo et al. (2004).

The thermal destruction temperature at 90°C for 15 min in this study will most likely result in loss of desirable fresh flavor, vitamins, carbohydrates and other nutritional substances, although food scientists and the food industry are searching for novel methods that may destroy undesired microorganisms with less adverse effects on product quality (Rosenthal and Silva, 1997). Thermally pasteurized fruit juices are often characterized by a loss of desirable fresh flavor characteristics (Butz and Tauscher, 2002). The thermal pasteurization employed in this study has eliminated the acid tolerant and thermophilic yeasts causing spoilage of the *Raphia* palm wine, thus increase the shelve life and reduce the

superior quality. This agrees with Butz and Tauscher (2002) reports that un-pasteurized products perceived by customers are to be of superior quality but its shelf life is very limited.

The present study on morphological identification and features of *B. nivea* confirms the presence of ascospores. It is responsible for the spoilage of bottled *Raphia* palm wine. This agrees with Beuchat and Rice (1979), reported that *Byssochlamys* spp. Produce ascospores which are heat resistant and survive considerable periods of heat above 85°C. The same was supported by Splittstoesser (1987). In addition to their heat resistance, *Byssochlamys* species can grow under very low oxygen tensions (Taniwaki, 1995) and can form pectinolytic enzymes. The combination of these three physiological characteristics makes *Byssochlamys* species very important spoilage of pasteurized and canned fruits (Samson et al., 2009).

Pasteurization temperature for fresh palm wine is between 70 - 80°C. At this temperature *B. nivea* is still viable. There must be alternative means to ensure that, these yeasts are eradicated in-order to ensure good quality *Raphia* palm wine with the reduction of thermal destruction time. This study did not investigate the product quality after the thermal destruction time.

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