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Biodiversity of *Saccharomyces cerevisiae* isolated from a survey of *pito* production sites in various parts of Ghana

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Abstract

Biodiversity among *Saccharomyces cerevisiae* predominating the spontaneous fermentation of Dagarti *pito* in Ghana was assessed. Two hundred and forty-nine isolates obtained from samples of dried yeast taken from commercial *pito* production sites in eight geographical regions of Ghana were characterized phenotypically by colony and cell morphology as well as carbohydrate assimilation profiling. Yeast populations ranged between 10^6 and 10^8 cfug^{-1}. Ninety-nine percent of the isolates (247) investigated showed macro-and micro morphological characteristics typical of *S. cerevisiae.* Of these, 72% (179) had assimilation profiles similar to *S. cerevisiae* while 28% (68) had assimilation profiles atypical of *S. cerevisiae* or any other member of the *Saccharomyces sensu stricto* complex. Amplification of the region spanning the two intergenic transcribed spacers (ITS) and the 5.8S ribosomal gene (ITS1-5.8S rDNA-ITS2), followed by restriction analysis, as well as determination of chromosome length polymorphism by pulsed field gel electrophoresis (PFGE) of 25 representative isolates strongly indicated that all belonged to *S. cerevisiae,* notwithstanding the phenotypic differences. Sequencing of the mitochondrial cytochrome-*c* oxidase II gene (*COX 2*) and the actin-encoding gene (*ACT1*) of four isolates, confirmed their close relatedness to *S. cerevisiae*, particularly to the type strain CBS1171 (98.7%), as well as other members of the *Saccharomyces sensu stricto* complex. Twenty isolates selected from eight geographical regions of Ghana and investigated for their technological properties, showed different patterns of growth and flocculation but otherwise similar technological characteristica. Most of the isolates produced *pito* having sensory attributes, which compared favourably with commercially produced *pito.*

Keywords: *Pito*; Ghana; *Saccharomyces cerevisiae*; Biodiversity; Phenotyping; Genotyping

Introduction

Pito, a traditional alcoholic beverage prepared from guinea corn (*Sorghum vulgare*), is common to the people of Nigeria, Ghana and Togo [7,8]. In Ghana, *pito*

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brewing is traditionally associated with women in the northern parts of the country, but migration has led to its production throughout the whole country [18]. *Pito* is golden-yellow to dark-brown in colour with taste varying from slightly sweet to very sour and contains lactic acid, unfermented sugars, amino acids and 2–3% alcohol (v/v), as well as vitamins and proteins [2,8]. Since *pito* is closely related to *kaffir* (sorghum) beer on which extensive nutritional studies have been conducted [1,12,15] it would be expected that *pito* is a good source of B vitamins, including thiamine, folic acid, riboflavin, and nicotinic acid [9,14].

Pito production includes a 12–36 h spontaneous mixed fermentation involving lactic acid bacteria and yeasts [18,21]. Bacteria of the genera *Lactobacillus* and *Leuconostoc* are the major contributors to the acidity of *pito* during the initial souring stage [18]. Most of the acid produced is lactic acid with only traces of acetic and formic acid being present [18]. Demuyakor and Ohta [7] reported yeasts associated with alcoholic fermentation of Konkomba and Nandom *pito* in the northern regions of Ghana as *Saccharomyces cerevisiae* (33%), *Kluyveromyces* spp. (23%), *Candida* spp. (17%) and members of six other genera. Sefa-Dedeh et al. [19] also reported the isolation and characterization of 21 yeast strains belonging to seven genera from Dagarti *pito* produced in the Greater Accra Region of Ghana. These comprised eight *S. cerevisiae*, four *Candida tropicalis*, three *Torulaspora delbrueckii*, two *Kloeckera apiculata*, two Pichia *anomala*, one *Schizosaccharomyces pombe* and one *Kluyveromyces africanus*. These results, while agreeing on the dominance of *S. cerevisiae*, seem to suggest some locality-dependent diversity in yeast populations involved in alcoholic fermentation of *pito* in Ghana. Sanni [15] and Sanni and Lonner [16] attributed the diversity of the associated yeast microflora of traditional alcoholic beverages including *pito,* in sub-Saharan Africa, to the spontaneous nature of the fermentation, sources, and types of ingredients used. This assertion was countered by the findings of our previous study [21], which highlighted the almost exclusive occurrence of *S. cerevisiae* strains in yeast associated with production of Dagarti *pito* and *dolo* from northern Ghana and neighboring Burkina Faso, respectively. Previous studies on *pito* yeast covered just one or two regions of Ghana [2,7,17,18]. Characterization of *pito* yeast from all the regions of Ghana seems not to be reported and no link seems to be established between predominant yeast and *pito* quality.

In the present study we have characterized taxonomically, the predominant yeast associated with Dagarti *pito* production within eight geographical regions of Ghana and investigated technological properties of representative strains, including sensory attributes of *pito*, to evaluate their potential for starter culture development common to Ghana.

Materials and methods

Origin of isolates

Samples of dried yeast were obtained from randomly identified commercial Dagarti *pito* producers at Tamale-Nyankpala (Northern Ghana, two production sites, samples denoted T); Monaco-Kumasi (Ashanti Region, samples denoted M); Accra (Greater-Accra Region, samples denoted AC); Cape Coast (Central Region, samples denoted CC); Takoradi (Western Region, samples denoted TK); Sunyani (Brong Ahafo Region, samples denoted SY); Ho (Volta Region, samples denoted HO); and Suhum (Eastern Region, samples denoted SH). One gram of each sample was crushed aseptically in a mortar, suspended in sterile saline peptone water (0.1% bactopeptone [Oxoid, Hampshire, England], 0.8% NaCl [Merck, Darmstadt, Germany]), pH 5.6 and incubated at 30 °C for 90 min. From 10-fold serial dilutions of saline, 0.1 ml portion was surface-spread onto MYGP agar (3 g malt extract [Oxoid]), 3 g yeast extract [Oxoid], 5 g bactopeptone [Oxoid], 10 g glucose [Merck] and 20 g bactoagar [Oxoid] per litre distilled water, at final pH of 5.6 ± 0.1), supplemented with 100 mg of chloramphenicol (Oxoid) and 50 mg of chlortetracycline hydrochloride (Sigma, St. Louis, MO, USA). After incubation at 30 °C for 3–5 days, colony-forming units (CFU) were enumerated and 25 colonies were randomly selected from plates with distinct colonies, recultivated in MYGP broth at 30 °C for 2 days and purified on MYGP agar (without antibiotics). Isolates from a *pito* production site in Tamale (samples denoted A) previously identified as strains of *S. cerevisiae* [21], were also included.

Phenotyping of isolates

Colony characteristics i.e., size, colour, elevation, shape, texture, margin and surface type were determined for all isolates. Phase contrast microscopy was employed to determine cell shape, size, type of budding and aggregation. The ability of isolates to assimilate various carbon sources was assessed using the API ID 32 C Kit (Biomerieux SA, Marcy L'Etoile, France), guided by the manufacturer's instructions.

ITS-PCR RFLP

Forty-two representative isolates and the *S. cerevisiae* type strain CBS 1171 were genotyped by amplification of their ITS1-5.8S-ITS2 regions followed by restriction analysis with *Hae*III. The isolates were pre-grown and analysed as previously described [21].