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Use of Chromosome Profile and PCR Pattern for Characterization of *Saccharomyces cerevisiae* from Fermented Maize Dough

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Abstract

Saccharomyces cerevisiae strains have been shown to be involved in spontaneous fermentation of maize dough used in the production of *Kenkey* in Ghana. Several isolates have been characterized with the purpose to investigate their similarity as regards to their chromosome profiles and polymerase chain reaction (PCR) patterns. In previous studies Pulse Field Gel Electrophoresis (PFGE) was performed. All isolates investigated had 16 chromosomes with size distribution patterns typical for *Sacch. cerevisiae*. For several of the isolates chromosome polymorphism was evident. The present work comprising an additional number of isolates has confirmed the polymorphism showing that several strains are involved in the fermentation process. The strains investigated could be grouped into clusters based on their chromosome profiles and PCR patterns. The two methods both had a high discriminating power and were able to differentiate the isolates at a subspecies level.

Keywords: Chromosome profile; PCR pattern; *Saccharomyces cerevisiae*; maize fermentation; *Kenkey*

Introduction

Yeasts are well known for their beneficial contribution to the fermentation of alcoholic beverages, bread and other fermented products. In Ghana fermented maize dough forms part of the main diets of the people in the southern coastal belt. The fermentation process of this dough has been fully studied and apart from the main fermenting organism *Lactobacillus fermentum* yeasts such as *Saccharomyces cerevisiae* and *Candida krusei* are found to be dominant (Halm *et al.* 1993, Jespersen *et al.* 1994). The part these yeasts play in the maize dough fermentation cannot be disregarded and consequently rapid and accurate identification

of *Sacch. cerevisiae* and *C. krusei* in fermented dough is essential.

Traditionally yeasts are identified by morphological and physiological criteria. (Casey *et al.* 1990, Querol *et al.* 1992). However, these methods are generally laborious and time consuming as well as having low discrimination power when subspecies identification is required. Moreover, these methods some times provide doubtful identification, hence limiting their application. Methods based on molecular biology techniques have been developed (Schwartz and Cantor, 1984; Smith and Cantor, 1987; Querol *et al.* 1992). Among such techniques, DNA fingerprinting, electrophoretic karotyping and restriction fragment length polymorphism have shown to be helpful (Vezinhet *et al.* 1990; Lavellée *et al.* 1994).

More recently, a PCR technique has been developed in which by means of 2 primers δ1 and δ 2 (oligonucleotide homologous with delta sequences of the Ty transposon) fingerprinting were generated from several yeasts strains. (Bidenne *et al.* 1992, Ness *et al.* 1993, Gainvors *et al.* 1994, Lavellée *et al.* 1994). Pulse field gel electrophoresis (PFGE) as reported by Schwartz and Cantor (1984) has made it possible to separate most part of the chromosomes of *Sacch. cerevisiae* and other yeasts. The PFGE technique makes it possible also to determine or detect chromosomal polymorphism within strains of *Sacch. cerevisiae* hence allowing for strain characterization (Vezinhet *et al.* 1990).

This paper presents preliminary investigation in the use of DNA based techniques such as Pulse Field Gel Electrophoresis and Polymerase Chain Reaction (PCR) for the characterization of *Sacch. cerevisiae* isolates from fermented maize dough.The objectives of this study were to establish a DNA based method for characterization of yeasts isolated from fermented maize dough which could form a basis for identification to species and subspecies level and ultimately to create an information pool on the characteristic features comprising genetical, physiological and technological properties of industrial important yeasts isolated from fermented maize products.

Materials and methods

Cultures

Fifteen isolates of *Sacch. cerevisiae* previously isolated and identified by conventional method (Jespersen *et al.* 1994) and reference strains from Centraalbureau voor Schimelcultures (CBS), Netherlands were used in this study. The list of strains are shown in Table 1. Cultures were grown on YGP broth/agar containing per litre 10 g yeast extract (Difco 27179), 40 g glucose (Merck 8342) and 20 g bactopeptone (Difco18170) pH= 5.6, incubated at 25°C for 48 h-72 h.

Table 1: Yeast strains used in the study

Strains	Origin
Saccharomyces cerevisiae isolates: 20-1-4, 26-1-E, 26-1-0, 26-1-10, 26-1-9, 26-1-8, 26-1-11, 26-1-3, 26-1-6, 26-1-5, 26-1-7, 17A3-5, I1, N4, V4	Fermented maize dough
Saccharomyces cerevisiae	CBS*400, CBS*1171, KVL 1001**
Candida tropicalis	CBS*94
Candida kefyr	CBS*834
Candida krusei	CBS*573

* CBS: Centraalbureau voor Schimmelcultures, Netherlands.
** Bakery yeast

Sample preparation for PFGE analysis

Strains of *Sacch. cerevisiae* were grown for 48 h at 30 °C in 100 ml YGP broth after two successive transfers in 10 ml YGP. The culture was centrifuged at 4,000 x g for 10 min, the sediment was washed with 8 ml buffer (1.2 M sorbitol, 10 mM Tris-HCL, 10 mM $CaCl_2$) and resuspended in 6 ml buffer C. The yeast cell wall was hydrolyzed with 200 µl of 5 mg/ml zymolase solution (Seikaguru Corporation 120493, Japan) in 50 % buffer C and 50 % glycerol and incubated at 37 °C for 1 h. One ml of spheroplast suspension was mixed vigorously with 1.5 % low melting point agarose in 10.3 % sucrose in TES buffer (10 mM Tris-HCL, 100 mM NaCl, 1 mM ETDA). The mixture was then poured into a moulding chamber and kept at 4 °C for 20 min to harden. The agarose plugs were removed and incubated overnight at 45 °C in 1.5 ml protease solution (50 µg/ml pronase E, 500 mM EDTA, 1 % laurylsarcosine) to digest proteinaceous substances. The plugs were rinsed 3 times in 20 ml TE buffer (10 mM Tris-HCL, 10 mM EDTA) at 50 °C for 1 h. Prepared plugs were stored at 4 °C in TE buffer until the PFGE gels were run as described below.

Sample preparation and PCR analysis

For PCR work, yeast strains were streaked onto YGP agar and incubated for 72 h at 25 °C. One ml sterile Milli Q water (Milli-Q Plus Ultra pure Water System Millipore, Molsheim, France) were used to suspend cultures on the agar plates. Cell suspension from plates were transferred to 1.5 ml Eppendorf tubes. Cells were then counted using Thoma´s Counting Chamber and counts adjusted to 10^8 cells per ml. One hundred µl of cells were first denatured in Thermal Cycler (GeneAmp PCR System, 2400 Perkin Elmer, California, USA) at 95 °C for 10 minutes and thereafter cooled to 4 °C. The supernatant was used as the source of template for the PCR reaction. Each sample was (50 µl total volume) amplified in a reaction mixture containing 200 µM dNTP (Promega, Madison, WI. USA), 1 µM each of primers 1 and 2 (1: 5' CAA AAT TCA CCT ATA/TTC TCA 3', 2: 5' GTG GAT TTT TAT TCC AAC A 3'), 25 mM $MgCl_2$ (Promega A351H) and PCR buffer recommended by the manufacturer. The DNA (supernatant) was added last to prevent cross contamination. Samples were denatured at 95°C for 5 min and amplification was carried out in a Thermal Cycler (Gene amp PCR system 2400, Perkin Elmer,) according to the following protocol modified from Gainvors *et al.* (1994): 95 °C, 30 s; 45 °C, 30 s; 72 °C, 2 min; for 34 cycles and terminated at 72 °C, 7 mins and thereafter cooled to 4 °C. Gel electrophoresis of PCR-products was carried out as described below.

Gel electrophoresis

PCR gels were run by applying 20 µl sample to a submerged horizontal slab gel (GNA-200 Gel Apparatus, Pharmacia LKB Biotechnology AB, Uppsala, Sweden) 3 % NuSieve agarose (Medinova, Copenhagen, Denmark) at 130 Volts for 1.5 h and 300 mA at ambient temperature using TBE buffer (Tris-base 54 g, Boric Acid 27.5 g, EDTA 3.72 g/litre). Φ X174/Hae III (Promega G176A) was used as marker. The gel was stained with 1 mg ethidium bromide (Sigma E-1510) per litre TBE buffer for 20 min and destained twice in milliQ water.

PFGE gels were run by applying a small piece of plugs of each sample to submerged horizontal 1.0% agarose (Pharmacia LKB Biotechnology AB) slab gel. Gel electrophoresis was performed with Electrophoresis power supply -EPS3500, GN Controller and GeneNavigator, all Pharmacia LKB Biotechnology AB at 10 °C under the following running conditions: 165 V 14 h 90 s; 165 V 12 h 105 s; 165 V 14 h 120 s. TBE buffer as stated above was used and changed after 24 h run time. Yeast DNA markers (Pharmacia LKB Biotechnology AB 27-4520-01) were used for determination of chromosome size. The gel was stained with 1 mg ethidium bromide (Sigma E-1510) per litre TBE buffer for 1 h and rinsed twice with milliQ water for 5 min.

All the gels were visualized at 302 nm with UV transilluminator (Pharmacia LKB Biotechnology AB) and photographed with a land camera (Polariod MPE).

Interpretation of bands and numerical analysis

Band patterns and photo-negatives were scanned and data collected by using LKB 2400 GelScan XL program (Pharmacia LKB Biotechnology AB) then normalized and further processed by use of GelCompar 3.0 program (Applied Maths, KortrijK, Belgium). Cluster analysis was generated using the GelCompar 3.0 program.

Results

Fig. 1 shows in duplicate the chromosome profile of some of the different isolates of *Sacch. cerevisiae* from fermented maize dough. All the isolates showed the typical number of bands with size distribution typical for *Sacch. cerevisiae* chromosomes ranging from 200 kb - 1900 kb. However, chromosome length polymorphism (CLP) is evident, especially between 945 kb and 1900 kb.

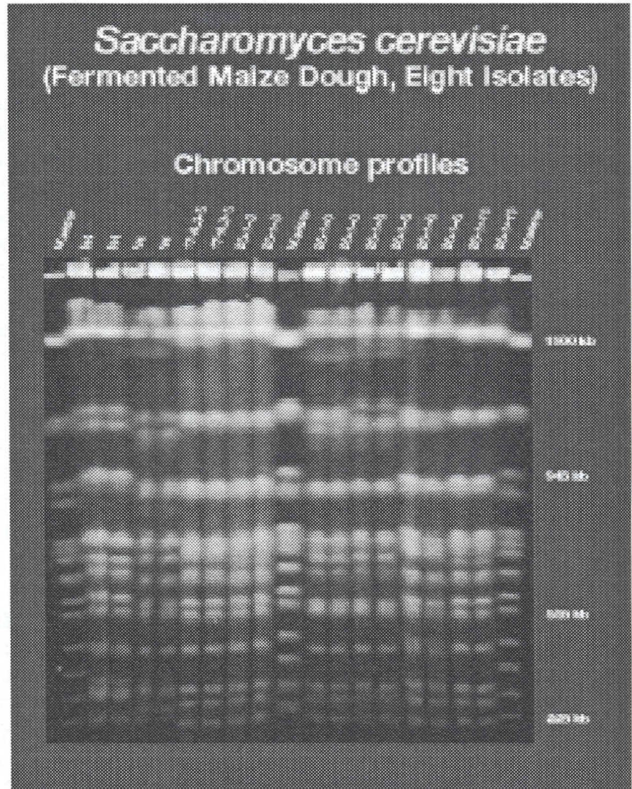

Fig. 1:
Chromosomal profiles of different strains of *Saccharomyces cerevisiae* from fermented maize dough

Fig. 2a shows the profiles of PCR products after amplification of DNA from different isolates of *Sacch. cerevisiae.* For comparison, strains of *Candida krusei* and other reference strains are shown (Fig. 2b). For *Sacch. cerevisiae* the primers generated considerable number of bands (Fig. 2a). The sizes of PCR product ranged from 72 bp to less than 873 bp. The *Sacch. cerevisiae* strains could be differentiated on the basis of „amplified sequence polymorphism", whereas the *C. krusei* strains did not generate many bands (results not shown), indicating the unacceptability of these primers for *C. krusei* characterization.

The cluster analyses resulting into dendrograms for both PFGE and PCR are shown on Fig.´s 3 and 4. Both dendrograms show two main clusters emerging at a similarity of 80 % for the PCR profiles and 30 % for the chromosome profiles. In the PCR dendrograms cluster 1 and 2 consist of two sub-clusters each closely related at 91.5 % and 93 % respectively. Isolates 26-

1-6 and 26-1-7 are closely related, while 26-1-11 and 26-1-10 are closely related both in their PCR product profile and their chromosomal profiles. In addition isolates 26-1-0 and 26-1-5 also show similarity in both their chromosomal and PCR profiles. The rest of the strains did not correlate significantly between their PCR and chromosome profiles.

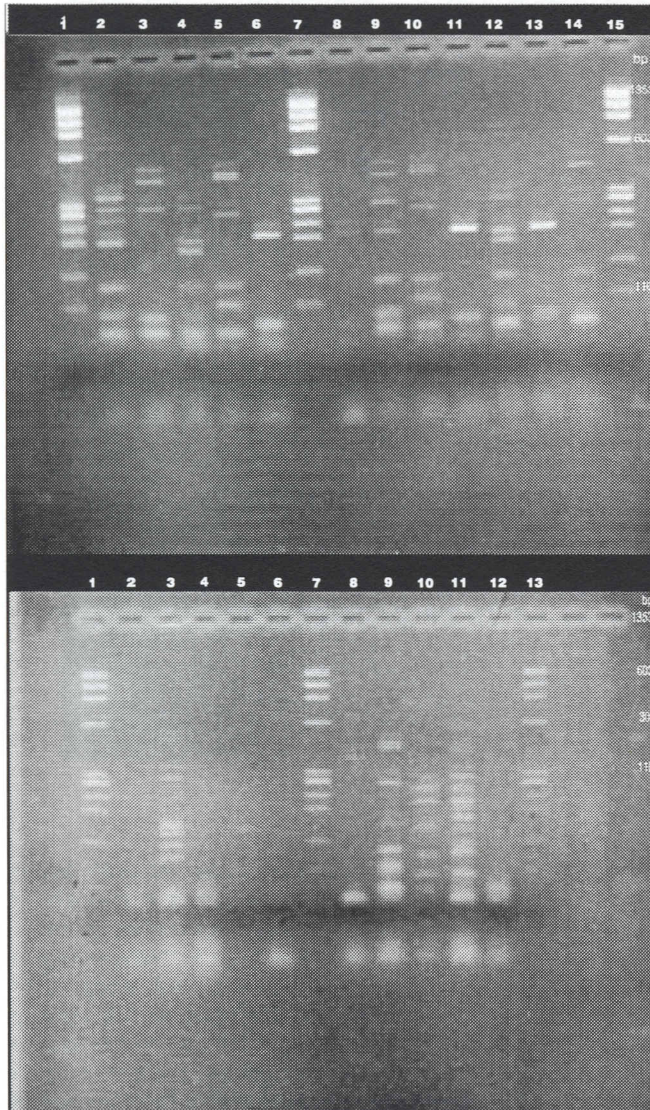

Fig. 2a:
PCR profiles of different strains of *Saccharomyces cerevisiae* from fermented maize dough
Lanes 1, 7 & 15: maker (ΦX174/ Hae III)
Lane 2: 20-1-4,
Lane 3: 26-1-E
Lane 4: 26-1-0
Lane 5: 26-1-10
Lane 6: 26-1-9
Lane 8: 26-1-8
Lane 9: 26-1-11
Lane 10: 26-1-3
Lane 11: 26-1-6
Lane 12: 26-1-5
Lane 13: 26-1-7
Lane 14: 17A3-5

Fig. 2b:
PCR profiles of yeast isolates from fermented maize dough and some reference strains
Lanes 1,7,& 13: maker (ΦX174/ Hae III)
Lane 2: *Candida tropicalis*
Lane 3: *Saccharomyces cerevisiae* (CBS 400)
Lane 4: *Candida kefyr*
Lane 5: *Saccharomyces cerevisiae* (CBS 1171)
Lane 6: *Candida krusei*
Lane 8: *Saccharomyces cerevisiae* (KVL 1001)
Lanes 9, 10 & 11: *Saccharomyces cerevisiae* isolates (I1, N4 & V4) from fermented maize dough

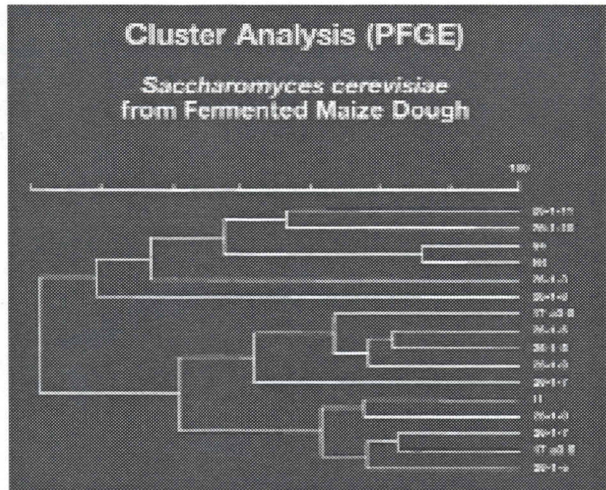

Fig. 3:
Dendrogram showing the clustering of *Saccharomyces cerevisiae* based on their chromosomal profile and evaluated using the Pearson product moment correlation coeffiecient (r) and the unweighted pair group algorithm with arithmetic averages (UPGMA)

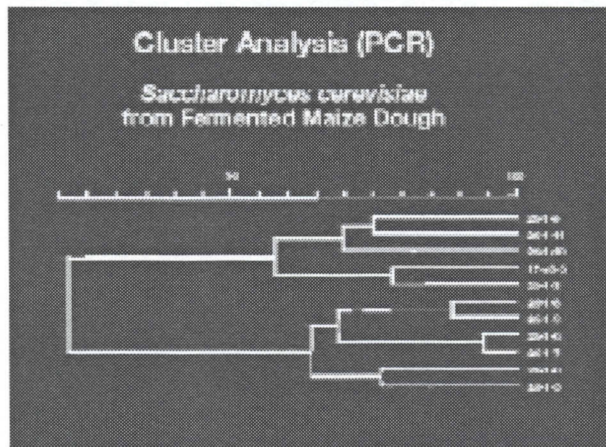

Fig.4:
Dendrogram showing the clustering of *Saccharomyces cerevisiae* strains based on their PCR profiles evaluated as in Fig. 3

Discussion

In order to discriminate between the different strains of *Sacch. cerevisiae* two DNA based techniques were investigated. Both the PCR and the PFGE analyses gave a diversity of profiles. Each strain had different profiles and for the PCR profiles the specificity of the patterns revealed significant polymorphism between the strains which made both techniques highly discriminative. For *Sacch. cerevisiae* a high number of bands was observed with evidence of chromosome polymorphism.The PCR method is rapid and less laborious and is equally reproducible and discriminative as the PFGE method. We conclude that *Sacch. cerevisiae* strains isolated from fermented maize dough show variation in their genetic profile based on the methods employed. Both the PFGE and PCR techniques can be used for identification of *Sacch. cerevisiae* at both species and subspecies level.

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