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BRIEF REPORT

Native yeasts for alternative utilization of overripe mango pulp for ethanol production

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KEYWORDS
Mango fruit;
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Abstract Mango fruits (*Mangifera indica* L.) are highly perishable, causing postharvest losses and producing agroindustrial waste. In the present work, native yeasts were used to evaluate ethanol production in overripe mango pulp. The two isolated strains showed similar sequences in the 18S rDNA region corresponding to *Kluyveromyces marxianus*, being different to the data reported in the NCBI database. Values of up to 5% ethanol (w/v) were obtained at the end of fermentation, showing a productivity of 4 g/l/day, a yield of up to 49% of ethanol and a process efficiency of 80%. These results represent a viable option for using the surplus production and all the fruits that have suffered mechanical injury that are not marketable and are considered as agroindustrial waste, thus achieving greater income and less postharvest losses.

PALABRAS CLAVE
Mango;
Desechos agroindustriales;
Kluyveromyces marxianus;
Producción de etanol

Levaduras nativas para la utilización alternativa de pulpa de mango senescente para la producción de etanol

Resumen Las frutas de mango *(Mangifera indica* L.) son altamente perecederas, lo cual causa pérdidas poscosecha y produce desechos agroindustriales. En el presente trabajo, se utilizaron 2 levaduras nativas para evaluar la producción de etanol en pulpa de mango senescente. Las 2 cepas aisladas mostraron similitud en la región 18S ADNr, correspondiente a *Kluyveromyces marxianus*, la cual es diferente a lo reportado en la base de datos del NCBI. Se obtuvieron valores de hasta el 6% de etanol (v/v) al final de la fermentación, con una productividad de hasta 4 g/l/día, un rendimiento de hasta 49% de etanol y una eficiencia en el proceso fermentativo

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del 80%. Esto representa una opción viable para utilizar excedentes de producción o frutos que han sufrido daño mecánico y no son comercializables, al lograr más ingresos y menos pérdida poscosecha.

Mango fruits (*Mangifera indica* L.) are highly perishable and, under tropical conditions, ripen within 6–7 days and become overripe and spoiled within 15 days after harvest[13]. The overripe mangoes, characterized by over softening, desiccation and microbial infection are not marketable and cause postharvest losses[12].

The edible pulp makes up 33–85% of the fresh fruit, while the peel and the kernel corresponds with 7–24% and 9–40%, respectively[15]. Thus it can take advantage of comprehensive manner of the fruit pulp due to the lack of treatment of overripe mangoes which are considered as agricultural waste. Due to the 18–20%[8] of sugar content, alcoholic fermentation is a viable alternative to use surplus mangoes. There is no information about the microbial flora associated with the mango fermentation process. The aim of this work was to evaluate ethanol production from overripe mangoes by fermentation using native yeasts, previously isolated from the fruit itself.

Overripe mangoes (*M. indica* L.) cv. 'Haden' and 'Tommy Atkins' were obtained from a local market in Mexico City, Mexico. Mangoes were peeled, ground and the juice was extracted using a Turmix® extractor. Mango juice (20 ml) was placed in reactors and incubated for 24 h at 27 ± 2 °C. Six samples (0.1 ml) were taken from each reactor after 24 h of culture time. All samples were diluted (10^{-3}), inoculated in Potato Dextrose Agar (PDA, Bioxon®) and incubated at 27 °C for 72 h. Two different colonies were isolated, purified and conserved in a cryoprotective medium (glycerol and skimmed milk) at −50 °C.

The strains designated KM1 and KM3 (isolated from Haden and Tommy-Atkins mangoes, respectively) were propagated in yeast extract peptone dextrose (YPD-Bioxon®) at 30 °C for 72 h under stirring (200 rpm). Biomass was recovered by filtration (Whatman #44) and the recovered cells were used for DNA extraction using the protocol described by Ausubel et al.[1] DNA integrity was evaluated by agarose gel electrophoresis (1.5%). PCR primers PN3 (5′-CCGTTGGTGAACCAGCGGAGGGATC-3′) and PN10 (5′-TTCGCTTATTGATATGCTTAAG-3′) were used to amplify a 600 bp fragment of the 18S rDNA gene. PCR experiments were performed using a PCR Thermal Cycler Px2 (Thermo Electron) system. A hundred (100) ng of template DNA were used for 25 μl PCR reaction, prepared by using *Taq* DNA polymerase (Invitrogen) according to the manufacturer's instructions in biological triplicates. The DNA fragment was sequenced using the *Taq* FS Dye Terminator Cycle Sequencing Fluorescence-Based method in an automated model 3730 capillary sequencer (Applied Biosystems, IBT-UNAM, Mexico). The obtained sequences were aligned (MAFFT V6, http://mafft.cbrc.jp/alignment) along with others previously reported. Phylogenetic and molecular evolutionary analyses were done using the MEGA 5 software by neighbor-joining analysis of Tamura-3 parameter distance estimates. The tree robustness was determined by bootstrap analysis (1000 replicates). Homology searching was performed using the BLAST algorithm in the NCBI database.

For ethanol production, KM1, KM3 (isolated in this study) and *Saccharomyces cereviseae* (Viticulture and Enology Center of Galicia, Spain, EVEGA) were used. The strains were propagated in 25 ml of YPD medium, incubated at 27 °C (48 h), collected and counted in a Neubauer chamber. The juice obtained from overripe Haden (H) and Tommy Atkins (T) mango pulp was used as a substrate for ethanol production. In order to release the sugars present in the mango juice, 0.1% (v/v) of the Novoferm® 61 (Novozymes A/S, Denmark) enzyme complex was added. Sugar content was adjusted at 20 °Brix and pH at 4.6. To inhibit bacterial growth, 70 mg/l of SO_2 were added; 400 ml of juice were placed in a 500 ml-glass reactor inoculated (10^6 cell/ml) and incubated at 16 °C. °Brix, pH and temperature were monitored. Fermentation was stopped when the °Brix value was stable for three days. Samples were centrifuged (4000 rpm/15 min) and filtered (0.45 μm, Millipore®) for analytical determinations. Sugars (glucose and dextrose) and ethanol quantification were carried out by High Performance Liquid Chromatography (HPLC Varian ProStar) with a refractive index detector (RI ProStar 350) under the following conditions: MetaCarb 67H Organic Acids column (300 mm × 6.5 mm), flow rate of 0.6 ml/min, 10 μl of sample, 40 °C in column and 35 °C in the detector for 30 min. The mobile phase was 0.04 N H_2SO_4. Glucose, dextrose, ethanol and cocktail stock solution (0–2000 ppm) were prepared for the construction of calibration curves. Productivity (g/l/day) was defined as the ratio between the maximum ethanol concentration (g/l) and culture time (day). Ethanol yield (Yp/s) was defined as the ratio between ethanol concentration (g/l) and sugar consumption (g/l). Efficiency percentage was defined as the ratio between ethanol concentration (g/l) and maximum theoretical yield (g/l) multiplied by 100.

To evaluate the effect of the strains used on productivity and efficiency in ethanol production, a completely randomized design with factorial arrangement 2 × 3 × 9 was established. Levels were: mango variety with two levels [Haden (H) and Tommy Atkins (T)], strains with three levels [*S. cerevisiae* (S), *Kluyveromyces marxianus* KM1 and KM3] and time with nine levels (0, 2, 4, 6, 8, 10, 12, 14 and 16 days). ANOVA was carried out for data analyses using SAS 9.0.

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