EVALUATION OF 
*Kluyveromyces marxianus* FOR THE PRODUCTION OF 
LACTASE SIMULTANEOUSLY TO PECTINASE OR INULINASE

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SUMMARY

Five strains of *K. marxianus* were evaluated for the production of intracellular lactase, intra and extracellular pectinase and intra and extracellular inulinase. The strain NRRL-Y-1109 showed the highest lactase activity, but the strain CDBB-L-278 produced notably higher activities of inulinase and pectinase than the rest of the strains tested. The strain CDBB-L-278 was selected for the simultaneous production of two enzymes. Two enzymes fermentations were achieved with productions of 44% lactase and 53% pectinase, or 26% lactase and 47% inulinase compared to the single enzyme levels.

INTRODUCTION

*Kluyveromyces marxianus* has been widely used for the production of lactase (Castillo 1990). In addition, other enzymes from this yeast have economic potential. For instance, endo-polygalacturonase has proved to be useful for apple juice clarification (Gómez-Ruiz et al 1988), vegetable tissues maceration (Kobayashi and Matsuo 1979; Lim et al 1980; Voragen et al 1980) and cloud stabilization of orange juice (Krop and Pilnik 1974). Similarly, inulinas have been widely studied for the production of fructose from inulin (Bucke 1981;
The simultaneous production of two valuable products in a single fermentation step should be of interest from the economic point of view. García-Garibay et al. (1987a,b) have proposed the simultaneous production of single-cell protein and pectinase from whey; clearly, if two enzymes instead of one are obtained in parallel to biomass a more attractive process could be envisioned. Hewitt and GrootWassink (1984) have proposed the simultaneous production of lactase and inulinase from K. marxianus. Lactase is an intracellular enzyme, pectinase is extracellular and inulinase can be found also extracellularly. The separation of biomass from the culture medium can provide a basis for lactase recovery after cell disruption, while the other two enzymes could be selectively precipitated from the supernatant.

MATERIALS AND METHODS

Strains.
The following strains of Kluyveromyces marxianus were evaluated: CDBB-L-278 (CINVESTAV IPN, Mexico City, Mexico), UCD-C-351 (University of California, Davis, U.S.A.), NRRL-Y-1109 (Peoria U.S.A.), NRRL-Y-1195 (Peoria U.S.A.) and CDBB-L-337 (CINVESTAV IPN, Mexico City, Mexico).

Culture media.
For pectinase production the following medium composition was used: 2% glucose (Baker), 0.2% yeast extract (Bioxon), 0.1% (NH₄)₂SO₄ (Baker), 0.05% KH₂PO₄ (Baker), 0.05% MgSO₄ (Baker); 0.5% of pectin (Sigma) was added for pectinase induction. For lactase production lactose (Baker) was used instead of glucose. For inulinase production a medium with 1% inulin from dahlia tuber (Sigma) and 0.5% yeast extract (Bioxon) was used. For the simultaneous production of lactase and pectinase the same medium for lactase production was used supplemented with 0.5% of pectin. For the simultaneous production of lactase and inulinase 0.5% of inulin was added instead of pectin. The pH was adjusted to 5.0 with H₂SO₄ (Baker) in every case.

Culture conditions.
Yeast growth was conducted in 250 ml baffled erlenmeyer flasks with 50 ml of medium incubated in a New Brunswick G-24 rotatory shaker (250 rpm, 30°C) with 5 ml of inoculum of the same composition.

Analyses.
Biomass and endo-polygalacturonase were determined according to García-Garibay et al. (1987a). Lactase was determined by the method reported by García-Garibay et al. (1987c). Inulinase activity was determined on sucrose (Baker) as reported by GrootWassink and Fleming (1980), using the Nelson-Somogyi's technique (Nelson 1944) to monitor the increase in reducing groups. Specific standard curves were prepared with o-nitrophenol (Sigma), D-galacturonic acid (Sigma) and an equimolecular mixture of glucose and fructose (Baker) for the activities of lactase, pectinase and inulinase respectively. A Pye-Unicam (model SP-30 UV) spectrophotometer was used for all the assays. Extracellular activities were determined in the culture supernatant after centrifugation (3000 rpm, 15 min). Intracellular activities were determined using permeabilized cells as reported by García-Garibay et al. (1987c). One unit of lactase (uL) was defined as the amount of enzyme needed to hydrolyze one μmole of ONPG (Sigma) per minute at 40°C, pH 6.6; one unit of endo-polygalacturonase (uPG) was defined as the amount of enzyme which liberates one μmole of reducing groups from polygalacturonic acid (Sigma) per minute at 30°C, pH 5; one unit of inulinase (uI) was defined as the amount of enzymes which liberates one μmol of reducing sugars from sucrose (Baker) per minute at 50°C, pH 5.0.

All fermentations and analyses were performed in duplicate. Values given are those obtained at the end of exponential phase.