# SYMPOSIUM PROGRAM & ABSTRACT BOOK

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This paper presents a method for the immobilization of *Candida molischiana* 35M5N β-glucosidase (E.C.3.2.1.21) to Duolite A-568 resin, evaluates its efficiency and demonstrates the potential use in industry of the immobilized enzyme for flavor enrichment of wine and fruit juice. The possibility of rapidly developing the aroma of terpenes in a continuous process may therefore be of great interest in oenology and in fruit juice industry. The immobilized β-glucosidase system using Duolite A-568 resin was found to be quite stable for the hydrolysis of bound aroma precursors under fruit juice or wine conditions. This enzyme can be used repeatedly for different treatment and give the evidence that is possible to enhance greatly the fruit juices and the wines flavor by enzymatic hydrolysis of glycoside flavors precursors.

## OPTIMIZATION OF GROWTH CONDITIONS ON BIOSYNTHESIS OF EXTRACELLULAR Β-GLUCOSIDASE *BY DEBARYOMYCES HANSENII*

### P5-6

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Yeasts of oenological origin are under investigation for the presence of some hydrolytic enzymes (extracellular proteases, esterases, glycosidases) that could be correlated with the formation of volatile aromatic compounds responsible for the quality of a wine. The study of β-glucosidase activity in yeasts was investigated in order to use these yeasts themselves or the enzyme produced by them to increase the varietal character of the wine. A strain of *Debaryomyces hansenii* has previously been shown to be capable of producing a constitutive extracellular β-glucosidase, whose, biosynthesis was dependent on the aerobic conditions of growth, medium pH and composition, and the metabolic state of the cell, i.e. growth phase. In this study we report the results of the optimization of some growth conditions for the maximum production of β-glucosidase. Response surface design was applied to determine the optimum ammonium sulphate and potassium phosphate concentration and aeration/agitation rate for β-glucosidase biosynthesis. Each factor was used at five levels and ranged between 0.1-1% for ammonium sulphate, 0.1-2% for potassium phosphate and 1/5-1/40 for aeration/agitation rate, respectively. The results indicated that the maximum yield of β-glucosidase biosynthesis corresponded to potassium phosphate concentration of 1.5%, ammonium sulphate concentration of 0.5% and a rate of aeration/agitation of 1/40. The validation of these predictions has been confirmed by three experiments carried out in that area.

## EXCRETION IMPROVEMENT OF ENDO-POLYGALACTURONASE OF *KLUYVEROMYCES MARXIANUS BY* 2-DEOXYGLUCOSE

### P5-7

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*Kluyveromyces marxianus* produces endo-polygalacturonase, an extracellular pectinase. The strain *K. marxianus* CDBB-L-278, a very good producer of this enzyme, was compared with other three strains. It was found that concentrations of 0.4% of 2-deoxyglucose resulted highly inhibitory to the growth of all strains, except for *K. marxianus* CDBB-L-278 which did not show any significant reduction in its growth rate even at concentrations of 0.6% of this compound. Additionally, 2-deoxyglucose led to a higher production of endo-polygalacturonase by all the strains, but particularly CDBB-L-278 which pectinase production was increased twofold when 0.6% of the glucose analog was added. A change of cell morphology was observed when 2-deoxyglucose was added to the culture medium, suggesting that the noticed increase of pectinase was due to a facilitated excretion of the enzyme on account of structural changes in the cell wall. Consequently, experiments using Tween 80 were performed to facilitate the excretion of the enzyme. Similar increments were observed comparing enzyme excretion when either 2-deoxyglucose or Tween 80 were added to the culture media. Considering that intracellular endo-polygalacturonase activity was not found, but the addition of compounds able to modify the permeability of the cellular wall led to a very high extracellular pectinase activity, it is suggested that the endo-polygalacturonase could be an intracellular inactive zymogen which is processed during its excretion resulting in the active enzyme.

## KLUYVEROMYCES MARXIANUS CDBB-L-278: A WILD INULINASE HYPERPRODUCING STRAIN

### P5-8

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The yeast *Kluyveromyces marxianus* has been widely studied for the production of inulinase (EC 3.2.1.7) focused on the production of fructose syrups from the inulin of Jerusalem artichoke. *K. marxianus* CDBB-L-278 is an inulinase hyperproducing strain. This strain has been in the CDBB culture collection of the Center of Research and Advanced Studies (CINVESTAV), National Polytechnic Institute, Mexico City, for more than 20 years, but unfortunately there are no records documenting its origin. It was able to grow in a medium containing inulin as the unique carbon source in the presence of 2-deoxyglucose. It produced up to 3.3 times the activity of the control strain *K. marxianus* NCYC-1429 (which has been widely studied as an inulinase producer by other authors) in an inulin medium, and 3.6 times in a medium with glycerol as the sole carbon source. Although the strain CDBB-L-278 was able to produce inulinase in the presence of 2-deoxyglucose, it was demonstrated that it is not a de-repressed strain since enzyme production was reduced when the concentration of glucose or fructose was increased in the medium. Since inulinase was produced in a glycerol medium without an inducer, it can be considered that the enzyme production was partially constitutive in *Kluyveromyces marxianus* CDBB-L-278 as well as strain NCYC-1429. The inulinase from *K. marxianus* CDBB-L-278 was characterized. It had a higher affinity for inulin than for sucrose. Temperature and pH profiles were different for both of these two substrates. The enzyme was stable to high temperatures, with a half-life of 180 min at 50°C.

## β-MANNANOLYTIC SYSTEM OF *AUREOBASIDIUM PULLULANS*

### P5-9

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The strain *Aureobasidium pullulans* Y-2311-1, known as a hyperproducer of endo-β-1,4xylanase [1], was found to be also one of