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***Yersinia enterocolitica* RECOVERY BY IMMUNOMAGNETIC SEPARATION**

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Yersinia enterocolitica, a human enteropathogen, is transmitted through contaminated water and food. Its recovery by culture is difficult when a low number of this bacterium is found. The immunomagnetic separation (IMS) is a separation and concentration method which uses paramagnetic polystyrene particles (PMP) covered with specific antibodies against surface antigens of microorganisms under study. In the present work, IMS was performed for evaluating the *Y. enterocolitica* recovery from an enrichment broth. The local virulence plasmid bearing strains, *Y. enterocolitica* 2/O:9 and *Y. enterocolitica* 3/O:3 were grown in trypticase soy broth (TSB) at 22° C overnight. Their initial concentrations were standardized at OD_{600} 0.2. Serial dilutions of each strain were performed and spread on trypticase soy agar (TSA), and countings of 2 x 10^8 CFU/ml for 2/O:9 and 1x 10^9 CFU/ml for 3/O:3 were obtained. One milliliter of each dilution was transferred to 9 ml of TSB and these bacterial suspensions were utilized on the same day (day 0) and 24 h after being incubated at 22° C (day 1). Dilutiones 10^{-2} to 10^{-5} were assayed on day 0 and dilutions 10^{-6} to 10^{-9} were assayed on day 1. *Y. enterocolitica* counting obtained without IMS treatment (wIMS) were compared to those obtained after IMS treatment (aIMS). The 2,8 µm diameter PMP covered with sheep anti rabbit-IgG (Dynalbeads) reacted with rabbit anti *Y.enterocolitica* 2/O:9 IgG and rabbit anti *Y.enterocolitica* 3/O:3 IgG and were ready for the challenge against suspensions of *Y.enterocolitica* O:9 and *Y. enterocolitica* O:3. The antibody-antigen reaction was performed at 35° C for 30 min with gentle agitation. The procedure was concluded by performing three washes with PBS-0.02% Tween 20 and final suspensions in 100 µl PBS. A measured volume of each Eppendorf tube was spread on Mac Conkey agar for estimating the *Y. enterocolitica* recovery after IMS. This counting was compared to that obtained before performing IMS. The best performance of IMS in the *Y. enterocolitica* recovery was observed for the highest bacterial dilutions (dilutions 10^{-6} on day 0 and 10^{-9} on day 1). Thus, counts of 80 CFU/ml (wIMS) and 1322 CFU/ml (aIMS) for the strain 2/O:9 and 70 CFU/ml (wIMS) and 1535 CFU/ml (aIMS) for the strain 3/O:3 were obtained on day 0. Also, counts of 4 x 10^4 CFU/ml (wIMS) and 6,3 x 10^4 CFU/ml (aIMS) for the strain 2/O:9 and < 200 CFU/ml (wIMS) and 6090 CFU/ml (aIMS) for the strain 3/O:3 were obtained on day 1. The *Y. enterocolitica* recovery was up to 30 times more effective by culture after IMS than by culture without IMS.

BF-P12

PECTINOLYTIC ACTIVITY EXPRESSED BY YEASTS ISOLATED FROM OENOLOGICAL ENVIRONMENTS.

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Introduction: Pectolytic enzymes play an important role in the winemaking process due to the fact that they improve the extraction of colour and aroma compounds. They also improve clarification and filtration processes of musts and wines. These enzymes break up pectin and weaken the cell wall, reducing the viscosity of musts and improving the extraction of the different compounds. Pectinases used in the food industry are commercially produced by *Aspergillus niger*. *Saccharomyces* and non- *Saccharomyces* yeasts present an alternative source for the large-scale production of commercial enzymes. Yeasts have advantages compared to filamentous fungi with regard to the production of pectinases, because they are unicellular, their growth is relatively simple, and in some species the growth medium does not require an inducer. The aim of this work was to study the pectinase activity of 162 isolated yeasts (47 non-*Saccharomyces* and 115 *Saccharomyces* sp.). Qualitative assay was carried out on plates with pectin as substrate, at 25ºC, pH 4.0 and 6.5 during 72h, in order to select yeasts having pectinolytic activity. Results were considered positive when colonies were surrounded by a degradation halo. After that, those isolates were grown anaerobically, for 72 h at 30ºC using two media: an inducing medium (0,67% YNB; 0,5% pectin and 10% glucose), and a non-inducing medium (0,67% YNB; and 10% glucose). For determination of pectinolytic activity, a reaction mixture of 0.1 ml of supernatant, 0.9 ml of 0.5% (w/v) pectin in 0.05 M sodium acetate buffer (pH 5) was used. It was prepared and incubated in a water bath at 37°C for 1 h (yeasts inoculated in inducing medium) and 24 h (yeasts inoculated in non-inducing medium). Pectinolytic activity was determined by estimation of reducing sugars by DNS technique. Results: of the 162 yeasts, 24 isolations were able to hydrolyze pectin under both pH conditions (14 *Saccharomyces* sp. and 10 non- *Saccharomyces*). Highest amounts of yeast isolations were detected at pH 6.5. All yeasts, developed in inducing and non- inducing media, expressed pectinolytic activity. Fifty percent of species from *Saccharomyces* genus registered the greatest values of pectinolytic activity growing in inducing medium. All species belonging to the non-*Saccharomyces* genera which were cultured in non-inducing medium expressed highest activity. This suggests that pectinase synthesis may be

partially constitutive for these non- *Saccharomyces* yeasts. Conclusion: this study clearly revealed the potential of indigenous yeasts to produce useful enzymes to catalyze desired biotransformations during wine fermentation and they offer an alternative source of these enzymes as well.

BF-P13
SELECTION OF INDIGENOUS YEASTS ACCORDING TO THEIR OENOLOGICAL AND VINIFICATION CHARACTERISTICS

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In Oenology exists a controversy about differences between pure culture wine fermentations in comparison to those performed with indigenous yeasts. Despite the advantages of using pure cultures of *Saccharomyces cerevisiae* with regard to the easy control and homogeneity of fermentations, wine produced with pure yeasts monocultures lacks the complexity of flavour, stylistic distinction and vintage variability caused by indigenous yeasts. Mixed cultures could assure the maintenance of the typical sensory properties and characteristic profile of the wine of each area. The aims of this work were a) to evaluate oenological characteristics, b) to detect enzymatic activities and c) to compare vinification results of pure and mixed cultures of *Saccharomyces* / non-*Saccharomyces* indigenous yeasts. One hundred and sixty two yeast isolations were used. Qualitative enzymatic assays were carried out on plates with specific substrate, at 25ºC, pH 4.0 and 6.5 during 72h, in order to select yeasts expressing enzymatic activities. Based on these results, 15 strains were selected and oenologically characterized according to protocols of Vazquez et al. (2001). To be tested as starters in wine fermentations, three of these strains were selected by their valuable oenological characteristics, BSc562-04 (*S.cerevisiae*), BHv438-92 (*Hanseniaspora vinae*) and BTd259-04 (*Torulaspora delbrueckii*) in pure and mixed cultures of *Saccharomyces* / non-*Saccharomyces* (1-99% and 10-90%) were seeded in 3 l Pedro Ximenez grape must, without press. Final values (g/l): residual reducing sugars, residual total sugars, volatile acidity, total acidity, glycerol, density, and ethanol (%v 20ºC), viscosity (Pa s), conductivity (mS) were determined in all fermentations. Yeasts employed in all fermentations were selected by their relevant enzymatic activities (xylanase, celulase, amylase, β-glucosidase, protease, pectinase, esterase and lipase) and desirable oenological characters (tolerance and high ethanol production, efficient sugar consumption, growth at high sugar concentration, resistance to killer toxins). Significant differences were found in analyzed parameters of pure and mixed conditions. Pure *T.delbrueckii* fermentations showed the greatest volatile acidity, viscosity and density. It also presented the highest concentration of residual reducing sugar and residual total sugars. The highest total acidity and glycerol production was produced by *H.vinae*. However, mixed assays of all strains do not negatively influence the evolution of fermentation and analyzed parameters. In all mixed cultures, dry wines were obtained with similar or better analytical profiles to those produced by pure *S.cerevisiae*. To enhance the complexity of wine flavours in winemaking, multistarter fermentations could be an interesting alternative to a guided fermentation using a starter culture of *S.cerevisiae*.

BF-P14
EFFECTS OF THE GLOBAL REGULATOR ROB IN THE PRODUCTION OF PHB IN RECOMBINANT *E.coli* STRAINS.

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Polyhydroxyalkanoates (PHAs) are thermoplastic biopolyesters accumulated by numerous microorganisms under unfavorable growth conditions. These polymers, that act as energy reserve and electron sink in the cells, have drawn much attention as environmentally-friendly plastics because they are completely biodegradable. Poly(3-hydroxybutyrate) (PHB) is the best characterized PHA. Accumulation of PHB in recombinant *E. coli* has many advantages, including the capability to synthesize it from several carbon sources. In the last years, there has been an important increase in the production of glycerol, a by-product in the synthesis of biodiesel. For this reason, glycerol has become an attractive substrate for bacterial fermentations. The manipulation of metabolic fluxes is a strategy used to redirect the flow of carbon and reducing power to increase the use of substrates for the synthesis of desired products. Traditionally this is done through genetic manipulations that inactivate competing pathways, or enhance the efficiency of reactions involved in the synthesis of intermediaries for the biosynthesis of the products. In recent years an alternative that involves introducing modifications in global metabolic control has been established. This affects several pathways at the same time, altering carbon and reducing power availability. There are several regulators that affect carbon and energy flow in *E.coli*, among them are redox regulators, such as *arcA*, and regulators that affect catabolism, such as *cre*. Mutations in both of these regulators, which are in the same chromosomal region, have been seen to affect PHB production in recombinant *E.coli*. The poorly known *rob* regulator is located in the same region, close to *arcA* and