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plantarum and one of *E. mundtii* produced CPS. Respect to amylolitic activity, 50% of the evaluated strains showed capacity to hydrolyze starch. The majority of *L. plantarum* strains isolated from amaranth sourdough demonstrated capacity to produce ropiness and amilolytic activity.

These results put in evidence the positive effects of autochthonous LAB isolated from amaranth sourdough for the development of healthy novel foods with higher quality and nutritional value.

Código de Resumen: BF-013

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

CreC, A NEW TOOL FOR METABOLIC MANIPULATIONS IN *Escherichia coli*

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Succinic acid is used as a specialty chemical in the agricultural, food, and pharmaceutical industries. Although succinic acid is currently produced from petroleum derived maleic anhydride, considerable interest in the fermentative production of succinate from sugars has emerged. Many genetic strategies have been performed to enhance succinate production in *Escherichia coli*, a natural but poor producer of this compound. The pleiotropic regulation over celullar functions carried out by two-component systems (TCSs) makes them interesting targets for genetic manipulations to achieve this purpose. CreC is a TCS responsive to the carbon source present in the media, whose absence was seen to enhance succinate production. In this work, additional strategies were tested to increase its production in *creC* mutants. Two plasmids carrying carboxylating enzymes were introduced in the parental and *creC* strains: pEcPpc, that overexpresses the carboxylating enzyme phosphoenolpiruvate caboxilase (Ppc) from *E. coli,* and pSBF2, that overexpresses the formate dehydrogenase from *Candida boidini* (Fdh). In both plasmids the genes are under the control of the *lacZ* promoter, and can be induced by IPTG. Two different concentrations of IPTG (0,1mM and 1M) were used to get a better estimation of the relative weight of the conversion catalyzed by Ppc and Fdh on succinate production. With the lowest concentration of IPTG, the *creC* mutant produced 3 times more succinate than the parental strain. These concentrations were only slightly higher than those observed in the absence of plasmids for both strains. However, when IPTG was supplied in a higher dose (1 mM), succinate production was triggered, with marked increases in all cases. The mutant strain overexpressing both plasmids produced more than four times what it had produced with 0,1 mM IPTG, and 40% more than the wild type in the same condition. In order to eliminate side products and increase NADH availability, the ethanol pathway was deleted and *ackA* was also eliminated to conserve carbon atoms in the form of acetyl-CoA, a substrate for succinate formation via the glyoxylate pathway. The double mutant *creC*, *adhE* and the triple mutant *creC*, *adhE*, *ackA* were cotransformed with pEcPpc and pSBF2, and succinate was measured in cultures of these strains grown in the same conditions previously described ($NaHCO_3$ 100mM, IPTG 0.1mM and 1mM). In contrast to what was expected, these strains did not present significantly higher amounts of succinate when compared to the simple mutant *creC* harboring both plasmids. In all cases, a very marked increase was observed with higher amounts of IPTG, indicating that in the *creC* background, overexpression of Ppc and Fdh had an important effect on succinate production, while the mutations in *adhE* and *ackA* did not. These results indicate that CreC appears as a good candidate for genetic manipulation in order to improve a reduced compound of commercial interest, such as succinate.

Código de Resumen: BF-014

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

LACTIC ACID BACTERIA FROM ARTISANAL TANNERIES: ISOLATION AND EVALUATION OF ACIDIFYING ABILITY

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The tanning process allows to transform animal skin in stable and non-putrescible products namely leather. This process consists in various steps such as washing skins, liming, depilation, lime removal by washing, purging and tanning. During purging, the skins are immersed in a cereal mix which is let to ferment for 12-24 h at room temperature (18ºC-37°C). After this period, the pH decreased to 4.5-5.0, a condition that is required for the final steps. Under these homemade working conditions, the cereal fermentation is quite variable and affects the quality of the leather. The objective of this study was to isolate lactic acid

bacteria (LAB) from the fermented cereal mixture in artisanal tanneries and to evaluate *in vitro* the acidifying activity of the strains. This is the first step for the formulation of a starter culture for tanneries to normalize the process. Samples of fermented cereals were taken from a tannery located in San Pedro de Colalao, Tucumán, Argentina, and colonies were isolated in MRS agar medium. The primary identification of the isolates included Gram stain, microscopic observations and catalase reaction. Gram positive, catalase negative strains (23) were cultured in a CERELA medium formulated for the production of lactic ferment and incubated at 18°C and 37°C for 24 h. At intervals, pH and titratable acidity (TTA) were determined. In most samples, a prevalence of cocci (95%) respect to bacilli was observed. The total isolated strains (56), 23 strains were Gram (+) and catalase (negative) which were selected. At 37 °C, most strains (21) acidified the culture medium within the first 8-h reaching a final pH $\leq$ 5.0 and a TTA $\geq$100ºD. At 18 °C, all strains showed a lower growth; however, the decrease in pH was 4.5-5.0 and the acidity developed (100-140ºD) after 24 hours of fermentation were similar to values obtained at 37°C. The best acid-producer strains were identified as *Enterococcus faecium* CRL 1943 and *Leuconostoc citreum* CRL 1945by phenotypic and genotypic techniques. Currently, studies are being conducted to formulate a lactic inoculant on the basis of these strains and their metabolites for artisanal tanning.

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Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EFFECT OF STIRRING ON GROWTH AND TOLERANCE TO HEAT STRESS OF THE PROBIOTIC *Lactobacillus rhamnosus* CRL1505

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The market for functional foods, particularly those that incorporate probiotic bacteria, is constantly expanding, a fact that involves new technological challenges. The development of probiotic dietary supplements in powder requires feasible techniques at industrial scale such as spray drying. However, the process involves thermal stress to the cell with loss of viability and or metabolic activity. Previous studies showed that the fermentation conditions strongly affect robustness of lactic starter cultures. The aim of this study was to evaluate the effect to stirring on growth, acidification and tolerance to heat stress of the probiotic *Lactobacillus rhamnosus* CRL1505. Bach cultures were performed in CERELA medium (Under patenting process, pH 6.3) without pH control, under (150 or 400 rpm) or without stirring (control) at 37ºC. An active culture (16-h old) was inoculated (1%, w/v) and fermentation proceeded for 24h. Samples were aseptically withdrawn at 0, 4, 6, 8, 10, 12 and 24h. Growth (OD_{620nm} and plate-dilution method), pH, lactose consumption (HPLC) and organic acid produced (HPLC) were evaluated. The heat stress tolerance (60ºC/ 5 min.) of cells harvested at a late stationary phase was also determined. Slight differences in growth between stirred ($1.8\ 10^9$ uf/ml) and static ($8.4\ 10^8$ uf/ml) cultures were observed, after 24 h. Lactic acid production increased more rapidly under aerated conditions than in static cultures. Acetic acid and ethanol formation was detected only in agitated cultures (9-16 mM), The cells grown with agitation were significantly more resistant than cells grown in static condition to heat stress. After 5 minutes at 60ºC, viable counts for stirred cultures were 3.2-fold higher than for static cultures regardless the stirring rate. These results are encouraged for drying probiotic cultures by spray.

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Modalidad: Poster

MACROLIDE MEGOSAMINYLATION IN BACTERIAL SYSTEMS

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Glycosyltransferases from polyketide gene cluster determinate the glycosylation patterns of macrolides which define the bioactivities of these molecules. Previously we have demonstrated substrate flexibility of the UTP-dependent glycosyltransferase pair MegDI-MegDVI from megalomycin gene cluster toward both the TDP-zugar and macrolide substrates. Thus, a new megosaminyl-azitromycin derivative with improved antimalaria and antibiotic activity were produced by bioconversion experiments in *E. coli*. In order to study structural contribution for antibacterial and antimalarial activity of this compound, new derivatives were produced. Modifications into desosamine residue were introduced by synthetic chemistry and megosamine residue was introduced by bioconversion experiments generating two new megosaminil-azitromycin derivatives. The structures of the compounds were confirmed by mass spectrometry. Scaling up of this process will allow validating its structure and the biological activity will be analyzed in order to test the effect of structural modifications on activities. In addition, due to low