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Malolactic fermentation (MLF) occurs during the winemaking of red wines to improve their quality and organoleptic attributes. Nowadays, MLF in the Argentine wineries is mainly produced by commercial lactic acid bacteria (LAB). In the present study, we propose using native lactic acid bacteria isolated from wine waste and red grape must, which were selected for their relevant malolactic activity in *in vitro* assay, high ethanol tolerance, and inability for producing biogenic amines. First, their esterase activity were evaluated to select the strains with the higher aromatic potential for vinification assays. High esterase activity was found in CE of *Oenococcus oeni* strains such as MS46 and B18 strains (from must and Malbec wine lees, respectively), but not in CS or SN. SDS-PAGE of these CE fractions revealed bands with a 38 KDa estimated size. Esterase activity assays were performed in triplicate in 3 cell fractions: culture supernatant (SN), cell suspension (CS), and cell-free extract (CE) using p-nitrophenyl acetate as substrate. The final reaction mixture contained Citrate-Phosphate buffer (pH 5.0), substrate solution (1 mM), and the sample (reaching a final OD_{600} of 0.5 for CS). After incubation at 37°C for 2 h, 0.5 M sodium hydroxide were added to stop the reaction. Absorbance at 410 nm was compared with a p-nitrophenol standard curve. The winemaking process was carried out on Malbec type must (density, 1.115 g/cm3; initial pH, 3.68) and Cabernet Sauvignon must (density, 1.115 g/cm3; initial pH, 3.68) from a winery located in Colalao del Valle (Tucumán, Argentina). For the alcoholic fermentation (AF), both must types were inoculated in duplicate with the *Saccharomyces cerevisiae* strain mc2. Musts incubation lasted 10 days and the volumetric mass was monitored at 20 °C. At the end of AF process, wines with the following values were obtained: ethanol 14.5% v/v, pH 3.72, residual sugars <2.00 g/L, and L-malic acid 2.87 g/L (Cabernet Sauvignon), and 2.50 g/L (Malbec). For the MLF, *O. oeni* MS46 and B18 strains were grown until end of growth exponential phase in adaptation medium (In g/L: MRS 50, Fructose 40, Glucose 20, L-malic acid 4, Tween 80 1, Pyridoxine 0.1 mg/L, Ethanol 7%). After centrifugation, they were inoculated in duplicate at 10^7 UFC/mL. A control assay without inoculation was included. MLF was controlled by L-malic acid consumption (R-Biopharm enzymatic kit). In addition, viable cells counts in MRS medium supplemented with Fructose (5 g/L) and L-malic acid (4 g/L) and pH variation were determined. A major goal here was that the *O. oeni* strains B18 and MS46 demonstrated high esterase activity in CE. In addition, SDS-PAGE of these CE fractions revealed bands with a 38 KDa estimated size. So, these strains were selected to inoculate in fermented musts. In Malbec wine, the malic acid concentration reached levels < to 0.02 g/L after 21 days with both strains tested. Similar values were obtained for the Cabernet Sauvignon after 28 days while in the control assay, L-malic acid slightly changed. Both MS46 and B18 strains showed a great capacity to complete MLF, not presenting marked differences in their behaviors and showing a count of 10^3 UFC/mL at the MLF process end.

PLANTS

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CBM20CP, A NOVEL FUNCTIONAL PROTEIN OF STARCH METABOLISM IN GREEN ALGAE.

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Ostreococcus tauri is a marine picoalga, the smallest free-living eukaryotic and the simplest photosynthetic organism described to date, which has a single chloroplast and mitochondrion. The *O. tauri* genome codes for less than 8000 genes with low genetic redundancy, however, the pathway of starch metabolism would be conserved. This alga has all the enzymes that participate in the synthesis of starch in higher plants encoded in its genome, at least one ADPGlucose pyrophosphorylase (ADPGlc PPase), one GBSS, SSs I-III (SSI, II, and III), SBEI-II and ISA1-3, however, a sequence coding for a SSIV was not found. It is well known that SSIV regulates the number of starch granules in *Arabidopsis* and would also participate in the initiation of starch synthesis. The fact that *O. tauri* contains a single starch granule could be related to the lack of this enzyme. Moreover, we previously described the presence of three different isoforms of SSIII with a variable number of Starch binding domains (SBDs), suggesting that the synthesis and regulation of starch metabolism in this organism is highly complex. SBDs are a special subfamily of CBMs that bind to starch and have acquired the evolutionary advantage of being able to disrupt the surface of their substrate due to the presence of two binding sites. These domains have been classified into thirteen families, in special SBDs included in CBM20 family were first found in starch hydrolases, however, they are present in several amylolytic and non-amylolytic enzymes from plants, mammals, archaea, bacteria, and fungi. In general, CBM20 are attached also to a CD and many of them have regulatory functions and a moderate affinity to starch. Only few proteins from algae containing a CBM20 have been characterized, such a laforin homolog from the red algae *Chondrus crispus* and a the SAGA1 protein from *C. reinhardtii*, which is involved in shaping starch plates. Although the *O.tauri* genome is fully sequenced, there are still many genes and proteins to which no function was assigned. Here, we identify the OT_ostta06g01880 gene that encodes CBM20CP, a plastid protein which contains a central carbohydrate binding domain of the CBM20 family, a coiled coil domain at the C-terminus and lacks catalytic activity. We demonstrate that CBM20CP has the ability to bind starch, amylose and amylopectin with different affinities. Furthermore, this protein interacts with OsttaSSIII-B, increasing its binding to starch granules, its catalytic efficiency and promoting granule growth. The results allow us to postulate a regulatory role for CBM20CP in starch metabolism in green algae.

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