TITLE: BETA-GALACTOSIDASE ACTIVITY OF ANTARCTIC BACTERIA: AN IMPORTANT BIORESOURCE FOR THE DAIRY INDUSTRY

AUTHORS: SANTOS, A[1,2]; FARAH, S[1]; BARRIENTOS, L[1,2]; LAMILLA, C[1,2]; PAVEZ, M[1,2], SCOTT, F. [3]

INSTITUTION: [1]LABORATORIO DE BIOLOGÍA MOLECULAR APLICADA; CENTRO DE EXCELENCIA EN MEDICINA TRASLACIONAL, AVENIDA ALEMANIA 0458; [2]NÚCLEO CIENTÍFICO Y TECNOLÓGICO EN BIORECURSOS (BIOREN), UNIVERSIDAD DE LA FRONTERA, AVENIDA FRANCISCO SALAZAR, 01145 TEMUCO, CHILE; [3]FACULTAD DE INGENIERÍA Y CIENCIAS APLICADAS UNIVERSIDAD DE LOS ANDES, AV. MONS. ÁLVARO DEL PORTILLO 12.455 LAS CONDES, SANTIAGO, CHILE.

ABSTRACT:

The extreme climatic conditions of Antarctica have exerted strong selective pressures in favor o evolution of bacteria, capable of producing metabolites of biotechnological interest. Finding new sources of active enzymes at low temperatures could cover relevant needs to the industrial sector. The aim of this work was to determine the production of low-temperature active β-galactosidases from 304 bacterial strains isolated from Antarctica. The determination of β-galactosidase activity was performed at 15 °C on lactose agar (Meat Extract (0.3%), Peptone (0.5%), Lactose (0.5%), Agar agar and IPTG (1mM)).β-Bluo-Gal (300ug/ml) was used as an indicator of β-galactosidase activity. Molecular identification of the bacterial isolates was carried out using the 27F and 1492R universal primers whilst PCR products were sequenced in Macrogen (Korea). The most promising strains were cultured at 15 °C in lactose broth. For obtention of β-galactosidases, cells were lysed by sonication and the cell lysate centrifuged at 4000 RPM for 30 min at 4 ° C. The supernatant was used as an enzymatic extract. For quantification of β-galactosidase activity, 20uL of the enzyme extract were added to a 22mM oNPG solution (o-nitrophenyl-BD-galactopyranoside). The reaction was carried out at 15 °C for 15 minutes at pH 6.5 and the amount of released ONP measured at 420 nm. A unit of β-galactosidase (U) activity was defined as the amount of enzyme releasing 1umol ONP from the ONPG per minute under experimental conditions. A total of 81 strains (27%) showed β-galactosidase activity from which, the five most promising strains were selected: Se 8.10.12, 104, So9b, Se5 and So 13.3. Molecular identification indicates that the selected strains correspond to Non-cultivable Bacteria, *Arthobacter psychrolactophilus*, *Arthrobacter scleromae*, *Arthrobacter cryoconiti* and *Streptomyces sp,* respectively. The strains with the greatest activity were Se 8.10.12 (1741 U), followed by So9b (246 U), 13.3 (190U), Se5 (182U) and 104 (119U). Our results indicate that the studied Antarctica bacterial strains show high enzymatic activity of β-galactosidases at 15ºC. These data serve as baseline for the exploration of improvements in the culture of microorganisms helping increase the production of enzymes towards possible biotechnological applications.

KEYWORDS: Antarctic bacteria, β-galactosidase, cold active enzymes