# NEW INSIGHTS IN *BACILLUS SUBTILLIS* LEVANSUCRASE MECHANISM AND APPLICATIONS

Agustín López-Munguía, Instituto de Biotecnología UNAM. Av. Universidad #2001, Col. Chamilpa C.P. 62210. Cuernavaca, Morelos, México.
agustin@ibt.unam.mx
Jaime R. Porras Domínguez, Instituto de Biotecnología UNAM. Av. Universidad #2001, Col. Chamilpa C.P. 62210. Cuernavaca, Morelos, México.
María Elena Rodríguez Alegria, Instituto de Biotecnología UNAM. Av. Universidad #2001, Col. Chamilpa C.P. 62210. Cuernavaca, Morelos, México.

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B. subtilis levansucrase (SacB) is a widely studied glycoside hydrolase from Family 68 family. Although reports on SacB properties date back to the 70's (Chambert & Gonzy-Tréboul, 1976), questions regarding levan synthesis mechanism are still open. These questions refer to the factors influencing reaction specificity, including the effect of sucrose and levan hydrolysis, product structure and levan molecular weight.

In this conference we review recent findings regarding the modulating effect of SacB concentration on levan molecular weight distribution (Porras-Domínguez et al., 2015; Raga-Carbajal et al., 2016). In effect, we demonstrated that high enzyme concentrations (>1.0 µM), direct levan synthesis exclusively to low molecular weight products (av 7.6 KDa), while low enzyme concentrations (< 0.1µM) favor the synthesis of a high molecular weight levan fraction (>2000 kDa). From a detailed HPAEC-PAD analysis of product evolution, a shift from a clear non-processive elongation mechanism at high protein concentrations to a -most likely- processive mechanism when low protein concentrations are used in the reaction. Trough calorimetric experiments we demonstrate that these changes in enzyme performance do not involve protein-protein interactions (Raga-Carbajal et al., 2016).

We demonstrated, through an extensive characterization of the levan hydrolysis reaction by SacB, that the wide diversity of products derives also from fructosyl transfer to free sugars available from sucrose and levan hydrolysis. Actually, levan is an efficient fructosyl donor for fructosylation reactions, in which FOS such as levanbiose, inulobiose, blastose, …, are formed (Méndez-Lorenzo et al., 2015). The efficiency of SacB fructosylation with levan as donor was applied for the synthesis of blastose, a sucrose analogue with potential prebiotic properties. For this reaction, fructose was transferred to trehalose to produce a □□(2-6) fructosylated trehalose, which was later hydrolysed by trehalase to yield blastose (Miranda-Molina et al, 2017).

Up to now there is not an efficient enzyme for the synthesis of levan-type FOS, in spite of intensive efforts to modify SacB or other levansucrases specificity by site directed mutagenesis. For this purpose, after a complete characterization of a combined bi-enzymatic reaction between SacB and an endolevanase produced by B.licheniformis. ($LevB_1$) (Porras-Domínguez et al., 2014) we designed a fusion enzyme containing both activities. This fusion enzyme is able to produce levan-type FOS from sucrose, with molecular weights in the range of DP2 to DP10 including mainly 1-kestose, 6-kestose, neokestose, levanbiose and blastose, with 40% w/w yields.

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