BIOTRANS
LA ROCHELLE 2023

N°131 / PC
TOPIC(s) : Biocatalytic cascade reactions

A cell-free platform for the synthesis of the active form of Vitamin B6 from xylose

AUTHORS
Renan Yuji MIYAMOTO / BRAZILIAN BIORENEWABLES NATIONAL LABORATORY, RUA GIUSEPPE MAXIMO SCOLFARO, 10000, CAMPINAS
Vivian Pascal WILLERS / TECHNICAL UNIVERSITY OF MUNICH, SCHULGASSE, 16, STRAUBING
Ricardo Rodrigues DE MELO / BRAZILIAN BIORENEWABLES NATIONAL LABORATORY, RUA GIUSEPPE MAXIMO SCOLFARO, 10000, CAMPINAS
Gabriela Felix PERSINOTI / BRAZILIAN BIORENEWABLES NATIONAL LABORATORY, RUA GIUSEPPE MAXIMO SCOLFARO, 10000, CAMPINAS
Volker SIEBER / TECHNICAL UNIVERSITY OF MUNICH, SCHULGASSE 16, STRAUBING
Corresponding author : Leticia ZANPHORLIN / leticia.zanphorlin@lnbr.cnpem.br

PURPOSE OF THE ABSTRACT
Vitamin B6 is a fundamental nutrient involved in more bodily processes than any other vitamin. Although vitamin B6 is essential for all forms of life, humans cannot synthesize it, so this micronutrient must be obtained from foods or isolated supplements. Currently, technological routes through chemical or fermentative processes can produce vitamin B6. Chemical synthesis employs expensive and/or hazardous resources while biological pathways still suffer to produce high titers due to the toxic features of its intermediates, and because vitamin B6 itself can interact with a wide range of metabolic reactions. Cutting-edge biotechnology relies on a genetically modified bacteria from the Rhizobium genus to produce vitamin B6. This process takes seven days and uses glucose as the building block in a complex media. Alternatively, the synthetic biochemistry approach can handle metabolic engineering cases that face highly complex challenges, but usually requires a formidable amount of enzymes. Here we propose a bio-based strategy via a synthetic biochemistry approach to produce the active form of vitamin B6 that uses xylose and ammonium sulfate as substrates and requires only six enzymes. Thermodynamical analysis demonstrated the cascade's feasibility, giving us the initial conditions to test in vitro. Because the obtained yield was only 15.6 % (0.4 mM), all the enzymes of the cascade were characterized. Two possible limiting steps were identified regarding the low thermal stability of the phosphoketolase and the low activity of the PLP synthase. The rational prospection of novel targets was performed using the sequence similarity network (SSN) tool and genome mining, crossing those data with thermophilic organisms available in our laboratory strain collection. This way, the final phosphoketolase and synthase presented melting temperatures 35 and 13 °C higher, and their activities improved by 21 and 2-fold, respectively. The optimized system was able to produce 74 % higher vitamin B6 titers. Several approaches were made to optimize even more the cascade, including three ATP recycling systems, relieving the side-reaction of the phosphoketolase, and providing an amidotransferase partner for the synthase. However, none of those strategies was effective in the further improvement of the cascade. Although there is still room for improvement to reach higher titers, our system to produce vitamin B6 from xylose presented higher productivity than the state-of-art bio-based technology that uses glucose as a building block (15.9 vs 7.7 mg L-1 h-1), and higher yields (27.2 vs 2.2 %).

FIGURES

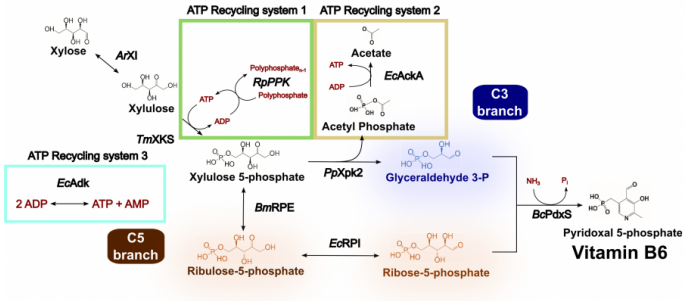

FIGURE 1

Enzymatic cascade

Description of the cascade starting from xylose

FIGURE 2

KEYWORDS

Cascade | Synthetic biochemistry | Xylose | Vitamin

BIBLIOGRAPHY

Rosenberg J, Ischebeck T, Commichau FM. Biotechnol. Adv., 2017

Acevedo-Rocha CG et al, Curr. Opin. Biotechnol., 2019

Guédez G et al, Structure, 2012.