**Bacterial Chalcone Isomerases: Identification of a Thermostable Variant for Use in Flavonoids Biotechnology**

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**Abstract**

Chalcone isomerases (CHI) are key enzymes for the synthesis of flavonoids, colorful metabolites naturally present in plants with rising interest in Thermostable and food industries. Currently, chalcone isomers are either directly extracted from plants or chemically synthesized. However, the chemical synthesis of some flavonoids is difficult and raised the definition of “natural” in food industry, therefore increasing their price. Developing a white biotechnological process for flavonoid synthesis will contribute to its sustainability, but requires finding robust and thermostable enzymes that would ensure the competitiveness.

Chains catalyze the conversion between isomeric forms of chalcones, which are precursors of interesting molecules such as chalcones, flavones, anthocyanins and tannins. CHIs were thought to be unique to plants until 2004 when they were also described in a few bacterial genomes. These previous works defined two types of chalcone isomerases: one with similarity to plant CHIs, identified mainly in Proteobacteria, and another, chordlike and unique, identified in the Firmicutes family (Halobacterium salinarum). However, only the bacterial CHI identified in *Eubacterium brockii* (highlighted in green color) showed catalytic activity against both substrates. The Firmicutes-type chalcone isomerase showed only residual activity towards flavonoids and thermostable representatives. A chalcone isomerase obtained from *Halobacterium salinarum* showed catalytic activity against both substrates, being the only CHI isomerase in this work that did not show a difference in thermostability according to temperature, and was resistant at the highest temperatures. This enzyme is highly conserved and makes it a good candidate for the development of a thermostable biotechnological process for flavonoid synthesis, opening a window of opportunity to design white biotechnological processes based on plant CHIs.

**CHI as a Key Enzyme in Flavonoids Biosynthesis**

**Only described in Plants until 2004...**

**Diversity**

**Secondary Structure Model**

**Enzymatic Activity**

**Colourimetric Assay**

**Protein Purification**

**Substrate: Naringenin Chalcone**

**Substrate: Butein**

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A sequence alignment of some of the identified proteins showed that important residues for catalysis (highlighted in green) and for discrimination (in blue) were conserved in the identified proteins, suggesting a CHI catalytic role for these proteins.

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**CHI Eubacterium-nulla-type**

**Substrate: Naringenin chalcone**

**Substrate: Butein**

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A catalytic activity of proteins was demonstrated by a colourimetric assay, using a naringenin chalcone or butein as substrates. These compounds display a wide range of colors, from blue to yellow, whereas the corresponding flavone and 4-hydroxychalcones isomers are colorless. Activity is measured as the decrease in absorbance at 410 nm. Proteins were purified after chromatographic expression (i.e., from host protein with an affinity binding domain (MBP)). Assays were performed either at 25°C or 40°C. Only residual activity can be detected when buffer is used as substrate, in which case some flavone isomers was used to detect catalytic activity.

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**CHI Shewanella-nulla-type**

**Substrate: Naringenin Chalcone**

**Substrate: Butein**

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