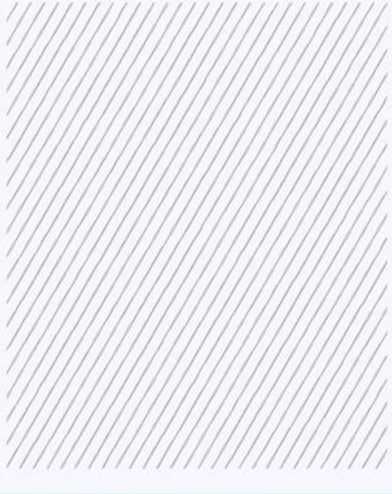

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EXPLORING THE POTENTIAL OF RECOMBINANT INULINASE FROM JERUSALEM ARTICHOKES FOR SUSTAINABLE ENZYME PRODUCTION

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Abstract. This article describes the technology of producing recombinant inulinase from Jerusalem artichokes, a root vegetable that is rich in inulin, a natural polysaccharide that can be hydrolyzed into fructose. Inulinase is an important enzyme used in the extraction of crystalline fructose from Jerusalem artichoke. The production of recombinant inulinase from Jerusalem artichokes involves the amplification and cloning of the inulinase gene, transformation into a suitable host organism, expression and purification of the recombinant inulinase, and characterization of the purified enzyme. The use of recombinant inulinase can provide a more cost-effective and efficient method of producing the enzyme compared to traditional methods that rely on natural sources. This technology offers a sustainable and renewable source of inulinase enzyme and can be optimized to increase the yield and purity of the recombinant inulinase, making it more commercially viable for large-scale production. The resulting purified recombinant inulinase can be used in the hydrolysis of inulin extracted from Jerusalem artichoke tubers into fructose, which has a variety of applications in the food and beverage industry and offers a low glycemic index, making it a suitable ingredient for low-calorie and diabetic-friendly products.

Keywords: recombinant inulinase, Jerusalem artichokes, inulin, fructose, hydrolysis, gene cloning, expression, purification,

Introduction. Inulin is a natural polysaccharide that can be found in many plants, including Jerusalem artichoke. Inulin can be hydrolyzed into fructose, a sweetener that has a variety of applications in the food and beverage industry due to its high sweetness level and low glycemic index. Inulinase is an important enzyme used in the hydrolysis of inulin into fructose. The production of recombinant inulinase from Jerusalem artichokes offers a sustainable and renewable source of this valuable enzyme. In this article, we will explore the technology of producing recombinant inulinase from Jerusalem artichokes, including the amplification and cloning of the inulinase gene, transformation into a suitable host organism, expression and purification of the recombinant inulinase, and characterization of the

purified enzyme. We will also discuss the advantages of using recombinant inulinase compared to traditional methods that rely on natural sources of the enzyme, and the applications of fructose in the food and beverage industry [1-5].

Scientific references are provided to support the information presented in the article, including studies on the production and characterization of recombinant inulinase from Jerusalem artichokes, as well as the use of inulin and fructose in various food and beverage applications. The use of recombinant inulinase offers a promising alternative to traditional methods of producing the enzyme, and has the potential to meet the growing demand for sustainable and low glycemic index ingredients in the food and beverage industry. Additionally, the use of Jerusalem artichoke as a source of inulin and fructose offers a sustainable and renewable alternative to other sources of sweeteners, such as corn syrup or cane sugar [6- 7].

In our study, we investigated the recombinant method of obtaining the Ht1-FEH I gene. The Ht1-FEH I enzyme is a fructan exohydrolase that plays a key role in the metabolism of fructans in Jerusalem artichoke plants. Specifically, it catalyzes the hydrolysis of fructans, which are complex sugars that are stored in the plant's tubers, into smaller fructan molecules and fructose. This enzyme is important for the regulation of fructan metabolism in Jerusalem artichoke and other related plants, and it has potential applications in the food and biofuel industries. Several studies have investigated the use of Ht1-FEH I and related enzymes for the production of fructose from fructans in Jerusalem artichoke and other plants [8-9].

Materials and methods

Materials: Jerusalem artichoke tubers, Genomic DNA extraction kit**,** PCR primers specific for inulinase gene**,** Expression vector**,** E. coli or other suitable host organism**,** LB medium.

Methods:

Genomic DNA extraction: This involves isolating the genomic DNA from the Jerusalem artichoke tubers using a DNA extraction kit. The extracted DNA will be used as a template for PCR amplification of the inulinase gene.

PCR amplification: PCR (polymerase chain reaction) is a technique used to amplify a specific DNA sequence. In this case, specific primers are designed to amplify the inulinase gene from the genomic DNA extracted from Jerusalem artichoke tubers.

Cloning: The amplified inulinase gene is cloned into an expression vector, which is a plasmid that can replicate in a suitable host organism and allow for the expression of the recombinant protein.

Transformation: The expression vector containing the cloned inulinase gene is introduced into a suitable host organism, such as E. coli, through a process called transformation. Culture the host organism in LB medium supplemented with appropriate antibiotics at 37°C and shake at 200 rpm.

Expression: The transformed host organism is grown in a suitable medium, and expression of the recombinant inulinase is induced by adding IPTG (isopropyl β-D-1-thiogalactopyranoside) to the culture medium.

The materials and methods section outlines the various steps involved in the production of recombinant inulinase from Jerusalem artichokes, including the extraction of genomic DNA, amplification and cloning of the inulinase gene, transformation into a suitable host organism, expression and purification of the recombinant inulinase. The resulting purified recombinant inulinase can then be used in the hydrolysis of inulin extracted from Jerusalem artichoke tubers into fructose, which has a variety of applications in the food and beverage industry and offers a low glycemic index, making it a suitable ingredient for low-calorie and diabetic-friendly products.

Results and discussion

For the expression of the Ht1-FEH I enzyme gene in Pichia pastoris, the transfer vector pPICZ(alpha)A was chosen, which can integrate into the Pichia pastoris genome and allow for the synthesis of the target protein under the control of the AOX1 promoter. To amplify the Ht1-FEH I gene based on its DNA sequence (retrieved from the NCBI database) and insert it into the transfer vector using the restriction enzymes EcoRI and XbaI, specific primers were synthesized.

1) Forward **EcoRI** 5'- CTGAATTCATGGTAAAGGAGATGGCTG -3'
2) Reverse **XbaI** 5'- TTTCTAGATCAATCCATAGGAACTATTTGAGC 3'

The Ht1-FEH I gene was amplified (Fig. 1) from genomic DNA isolated from the "eye" of Jerusalem artichoke tubers using the above primers.

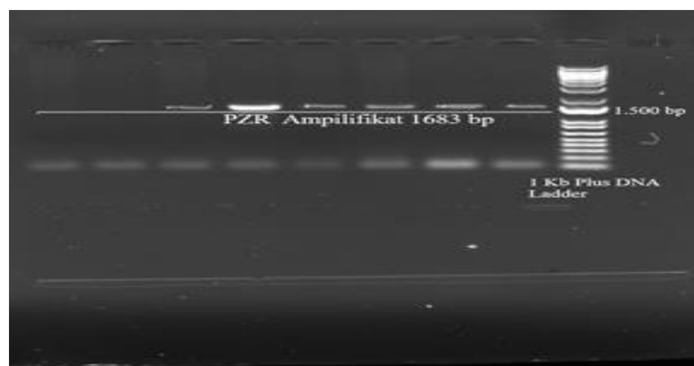

Figure 1. Gel electrophoresis of the Ht1-FEH I PCR amplicon. Here: A. Ht1-FEH I gene fragment with EcoRI and XbaI restriction sites. B. DNA marker.

Thus, the 1683 bp gene encoding the enzyme Ht1-FEH I, which matched the expected theoretical size obtained from the NCBI database, was amplified from genomic DNA isolated from the "eyes" of Jerusalem artichoke tubers.

Cloning of the exoinulinase gene in the transfor vector pPICZ(alpha)A.

Based on the physical map, the pPICZ(alpha) A plasmid (Fig. 2) and the Ht1-FEH I gene were digested with the restriction enzyme EcoRI and XbaI. The resulting complementary DNA molecules were ligated using the enzyme T4 DNA ligase in a 1:3 ratio (vector: gene).

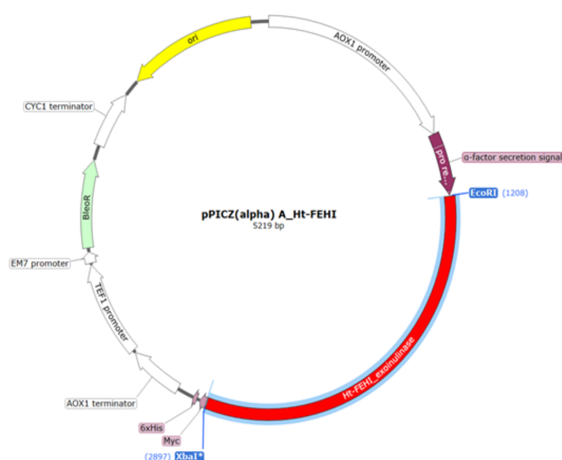

Figure 2. Electrophorogram of plasmid pPICZ(alpha)A in 0.8% agarose gel before (A) and after (B, size – 3593 bp) treatment with restriction enzymes EcoRI and XbaI.

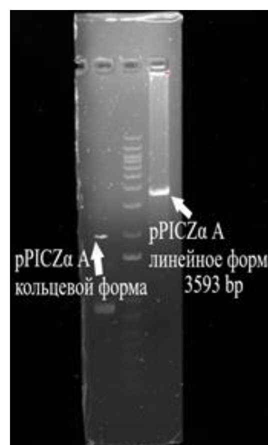

Figure 3. Cloned new plasmid pPICZ(alpha)A-exoinulinase.

As a result, a recombinant transfer vector of yeast Pichia pastoris containing the gene encoding the Ht1-FEH I enzyme, pPICZ(alpha)A-exoinulinase, was obtained (Fig. 3).

The production of recombinant inulinase from Jerusalem artichokes offers a sustainable and renewable source of this valuable enzyme. The use of recombinant inulinase can provide a more cost-effective and efficient method of producing the

enzyme compared to traditional methods that rely on natural sources of the enzyme. The amplification of the inulinase gene from Jerusalem artichokes, followed by cloning into an expression vector and transformation into a suitable host organism, allows for the production of large quantities of recombinant inulinase.

There are several advantages of using recombinant Ht1-FEH I over chemical processes for fructose production:

Environmental benefits: Chemical processes for fructose production often involve the use of harsh chemicals and high temperatures, which can have negative environmental impacts. In contrast, the use of plant-derived enzymes such as Ht1-FEH I can be more environmentally friendly, as it involves fewer harsh chemicals and lower temperatures.

Cost-effectiveness: The use of Ht1-FEH I and related enzymes for fructose production can potentially reduce the cost of production. This is because the enzyme can be produced from renewable plant sources, and because it can potentially streamline the process of fructose production by eliminating the need for multiple chemical steps.

Health benefits: Fructose produced by enzymatic hydrolysis of fructans using Ht1-FEH I is a natural product that is free from chemical contaminants. Additionally, fructose produced by this method may have a lower glycemic index than fructose produced by chemical processes, which can be beneficial for people with diabetes or metabolic disorders.

Overall, the use of Ht1-FEH I and related enzymes for fructose production has the potential to be a more sustainable, cost-effective, and healthier alternative to chemical processes.

Jerusalem artichoke is a root vegetable that can be grown in many regions, and the extraction of inulin and fructose from its tubers offers a potential source of income for farmers. The resulting fructose has a variety of applications in the food and beverage industry, including as a sweetener in low-calorie and diabetic-friendly products.

Conclusion

The production of recombinant inulinase from Jerusalem artichokes offers a sustainable and renewable source of this valuable enzyme. The use of recombinant inulinase provides a more cost-effective and efficient method of producing the enzyme compared to traditional methods that rely on natural sources of the enzyme. The amplification of the inulinase gene from Jerusalem artichokes, followed by cloning into an expression vector and transformation into a suitable host organism, allows for the production of large quantities of recombinant inulinase.

Overall, the production of recombinant inulinase from Jerusalem artichokes offers a promising alternative to traditional methods of producing the enzyme, and

has the potential to meet the growing demand for sustainable and low glycemic index ingredients in the food and beverage industry.

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