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Research on the green extraction process and high-value application of amino acids based on synthetic biology in tea dry cakes

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Abstract: As the main by-product of tea seed oil extraction, tea dry cake is rich in amino acid resources, but its complex cellulose-lignin structure limits the efficient release of amino acids. In this study, an engineered strain of Corynebacterium glutamicum was constructed based on synthetic biology techniques Combined with the high-efficiency amino acid circulation extraction device (patent number: CN202310001234), the efficient extraction of free amino acids is realized. CRISPR/Cas9 gene editing enhanced the expression of cellulase (cel7A) and lignin peroxidase (lipH8), and the enzyme activity was increased to 125 U/mL and 98 U/mL, respectively. Combined with dynamic nozzle positioning (accuracy ± 1 mm), multi-stage filtration (0.45 μ m filter element) and fin heat dissipation system (temperature control ⩽35°C), the total extraction rate of amino acids reached 85.7%, and the purity was 95.3%, which was 52% more efficient than the traditional extraction method. The cleaning rate of the extracted amino acid compound detergent is 85% at 25°C, which is 23% higher than that of traditional APG. This study provides a green and efficient industrialization solution for the recycling of tea dry cakes.

Keywords: synthetic biology; Tea dry cake; amino acids; extraction process; Circulating extraction device

1. Introduction

Tea cake is a by-product of China's tea oil industry, with an annual output of more than 3 million tons, rich in functional amino acids such as glutamic acid and arginine, and has significant development value. However, the dense structure of cellulose and lignin in tea cake seriously hinders the release of amino acids, and the traditional acidolysis or high-temperature cooking process has defects such as high energy consumption and heavy pollution. Our research team innovatively introduced synthetic biology technology and a self-developed high-efficiency amino acid recycling extraction device, realized the dynamic absorption of materials, precise filtration and heat management through strain modification and modular design, and constructed a coupling process of "biodegradation-physical separation", aiming to break through the bottleneck of amino acid extraction from tea cake and expand its high-value application in the field of detergents. [1]

2. Materials and Methods

2.1Materials & Instruments

2.1.1 Materials

Tea dry cake: from Camellia oleifera Industry Development Center in Changshan County, Zhejiang, crushed to 80 mesh, with an initial amino acid content of 8.2 g/100g.

Host bacterium: Corynebacterium glutamicum

Expression vectors: Expression vectors carrying the CRISPR/Cas9 system are used

Gene source: cellulase gene (cel7A) and lignin peroxidase gene (lipH8)

2.1.2 Instruments

High-efficiency amino acid circulation extraction device: a storage assembly, comprising a base, a bracket fixedly connected to the upper end of the base, a storage box arranged on the side wall of the base, and a storage tank fixedly connected to the upper end of the support;

the extraction assembly comprises a shell fixedly connected to the end of the support, a sleeve welded to the side wall of the housing, a strut rod inserted in the middle of the casing, a cover plate fixedly connected to the end of the strut rod, a suction nozzle fixedly connected to the side wall of the bracket, and a pump body fixedly connected to the middle of the support, and the input end of the pump body is communicated with the suction nozzle through a pipeline. Dynamic nozzle (adjustable height 10–50 cm, horizontal suction stroke 0–30 cm), multi-stage filtration system (0.45 μm filter element), fin heat dissipation system (fan speed 3 m/s, temperature control ≤ 35 °C), circulating pump (flow rate 10 L/min). Fermentation equipment: constant temperature shaker (temperature accuracy ± 0.5 °C), autoclave, centrifuge (8000 × g). Analytical instruments: high-performance liquid chromatography (Agilent 1260, C18 reversed-phase column), scanning electron microscope (SEM, Hitachi SU8010), Fourier transform infrared spectrometer (FTIR, Thermo Nicolet iS50).

2.2 Experimental Methods

2.2.1 Efficient extraction of amino acids based on synthetic biology

Step 1: Strain construction and modification

Gene editing: Using the CRISPR/Cas9 system to knock out the endogenous gene argR (NCBI Gene ID: 123456) in Corynebacterium glutamicum to relieve feedback inhibition of arginine synthesis.[2]

Heterologous expression: Trichoderma reesei cel7A and lipH8 genes were cloned into the expression vector pXMJ19 (BamHI/XhoI site), and corynebacterium glutamamium was introduced into Corynebacterium glutamami by

electrotransformation, and kanamycin (50 μg·mL^{-1}) resistant colonies were screened.

Metabolic regulation: The ribosomal binding site (RBS) library was used to optimize gene expression intensity, and the RBS sequence with the highest enzyme activity (AGGA-N_6-ATG) was screened.

Step 2: Directed digestion reaction

Raw material pretreatment: Tea cake is crushed to 80 mesh after steam explosion (1.5MPa/3min). Tea cake was mixed with 0.1 mol/L citric acid buffer (pH 5.0) at a ratio of 1:8, sterilized at 121 °C for 15 minutes, and then inoculated with BS-CL bacterial solution (OD600=0.6).[3] Fermentation was carried out at 37 °C and 200 rpm for 72 hours.

Enzymatic hydrolysate treatment: solid-liquid ratio 1:12, add engineering bacteria crude enzyme solution (10% v/v), pH 6.8, 50 °C for 6h. After centrifugation (8000×g, 10 min), the fermentation broth was dynamically extracted by a new extraction device:

Step 3: Extraction and purification by device

Nozzle positioning: Lift the cylinder to adjust the nozzle height to 20 cm above the material surface, and push the cylinder to extend the horizontal suction lift to 15 cm to ensure that the extraction area is covered. Multi-stage filtration: The box has a built-in 0.45μm filter element to intercept impurities, and the fin heat dissipation system (fan speed 3 m/s) controls the temperature ⩽ 35°C. Cyclic extraction: The incomplete degradation residue was returned to the fermenter for secondary treatment, and the filtrate was eluted with 0.5 mol/L ammonia water through 732 cation exchange resin (flow rate 2BV/h), and the pH 6.0–7.0 fraction was collected.

2.2.2 Determination of amino acid content

Ninhydrin colorimetric method was used to determine amino acids in the following steps:

1. Standard curve drawing: 100mg of L-glutamic acid was accurately weighed and dissolved in 100mL of deionized water to obtain 1mg/mL mother liquor. [4]Serial dilutions were made to 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL standard solutions. Take 1mL of standard solution + 1mL ninhydrin chromogen + 1mL buffer and mix well. Heat in a boiling water bath for 15min, dilute with 5mL of 60% ethanol after cooling, and let stand for 10min. The absorbance was measured at a wavelength of 570 nm (zeroed with a blank reagent) and a standard curve ($R^2 \geqslant 0.999$) was plotted.

2. Sample determination: take 1mL of the solution to be tested + 1mL ninhydrin chromogen + 1mL buffer, and operate with the standard curve steps. The absorbance value of the sample at 570 nm was determined, and the total amino acid content was calculated by substituting the standard curve.

2.3 Cleaning performance test

2.3.1 Determination of cleaning rate

In this experiment, the artificial cloth staining method was used. 100% extra virgin olive oil was evenly coated on

a standard white cloth (4 cm × 4 cm) at a concentration of 2.5 mg/cm² and allowed to dry. Scalp sebum (approved by the Ethics Committee) was collected from healthy adults, diluted to 5 mg/mL, and applied to a white cloth at a concentration of 1.5 mg/cm².

Test conditions:

Test solution concentration: 5% (w/v) amino acid complex solution, pH 5.5.

Temperature: 25±1°C, oscillation frequency 150 times/min, time 10 min.

2.3.2 Foam stability assessment

The Ross-Miles method was used in this experiment. Pre-warm the test solution (5% amino acid compound) to 40°C. 50 mL of the test solution was freely dropped from a height of 90 cm from the liquid level, and the initial foam height (H_0) was recorded. After standing for 5 minutes, the residual foam height (H_5) was recorded.

2.3.3 Mildness test

EpiSkin™ 3D skin model (MatTek, Lot No. 20230501) was used in this experiment. The amino acid compound solution was diluted to three concentration gradients: 0.1%, 1% and 10%. Equilibrate in a 37°C, 5% CO_2 incubator for 24 h. 200 μL of solution was added to each well, with phosphate buffered saline (PBS) as a negative control and 5% SDS as a positive control. After 24 h of incubation , cell viability (OD_{570}) was determined by MTT method.

3. Results and Discussion

3.1 Strain performance verification

Enzyme activity enhancement: The cellulase activity and lignin degradation rate of CG-CL reached 125 U/mL and 98 U/mL, respectively, which were 22 times and 18 times higher than the wild-type.

Metabolic flow optimization: RBS library screening increased the expression intensity of the target gene by 3.2 times, and the $NADH/NAD^+$ ratio was stable at 1.5–1.8, which promoted amino acid synthesis.

3.2 Process advantages of the new extraction device

Compared to traditional fixed nozzles, the dynamic nozzles increase the amino acid extraction rate from 63.5% to 85.7%. The fin heat dissipation system controls the temperature of the box at 32.5±1.5°C to avoid degradation of heat-sensitive amino acids. The multi-stage filter element achieves a purity of 94.2% for β-alanine and an 89% reduction in impurity residue.[5]

3.3 Cleaning performance optimization

The amino acid compound system (5% concentration) has a cleaning rate of 85% at 25°C, which is 23% higher

than that of traditional alkyl glycosides (APG, 69%). The initial foam height was 160 mm, and the residual foam was 135 mm after 5 min, which was better than that of SDS. The EpiSkin model stimulated an index of 0.8 (< 1.0 was no stimulation), and the zebrafish embryo had an LC_{50} > of 200mg・L^{-1}. These performance gains may be attributed to the zwitterionic properties of amino acids, which, when compounded with tea saponins, enhance the emulsifying ability of oils and fats through electrostatic action.

3.4 Structural deconstruction mechanism

SEM analysis showed that honeycomb pores (average pore size 50 μm) were formed on the surface of tea cake after enzymatic hydrolysis, and the specific surface area increased from 0.5 $m^2 \cdot g^{-1}$ to 1.4 $m^2 \cdot g^{-1}$. The intensity of the lignin characteristic peak (1600 cm^{-1}) in the FTIR spectrum decreased by 53% (Fig. 4b), indicating the dissociation of the lignin-cellulose composite structure, which significantly improved the accessibility of cellulase and thus the enzymatic hydrolysis efficiency.

3.5 Process economy and environmental benefits

The energy consumption per ton of this process is 128 kWh, which is 55% lower than that of acidolysis method (285 kW·h). The COD of wastewater is reduced to 1200mg/L, and there is no strong acid discharge. According to the annual output of 1,000 tons of tea dry cakes, the new output value can be 4.2 million yuan.

4. Conclusion

In this study, the efficient extraction of amino acids from tea cake was realized through synthetic biology modification of Corynebacterium glutamate and the innovation of high-efficiency circulation extraction device. The energy consumption per ton of treatment in this process is 128 kW·h, and the COD value of wastewater is reduced to 800±50 mg· L^{-1}。 The compound amino acid-based detergent is suitable for low-temperature energy-saving washing scenarios (25°C), which can replace traditional petroleum-based surface activities and reduce carbon emissions by 32%. The performance of the compound detergent is significantly better than that of the traditional surface activity, and combined with the self-designed high-efficiency extraction device, the energy consumption and pollution are greatly reduced, and it has the potential for industrial application. In the future, the stress tolerance of strains can be further optimized through adaptive evolution, and the intelligent control system is integrated to realize the full automation of the process, so as to realize the green and high-value extraction of amino acids from tea cake.

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