## Accepted Manuscript

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PII: DOI: Reference:	S0141-0229(17)30214-4 https://doi.org/10.1016/j.enzmictec.2017.11.008 EMT 9158
To appear in:	Enzyme and Microbial Technology
Received date:	14-10-2017

 Revised date:
 19-11-2017

 Accepted date:
 24-11-2017

Please cite this article as: Liu HongLing, Yang ShaoJie, Liu Qiang, Wang Ruiming, Wang Tengfei. A Process for Production of Trehalose by Recombinant Trehalose Synthase and Its Purification. *Enzyme and Microbial Technology* https://doi.org/10.1016/j.enzmictec.2017.11.008

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# ACCEPTED MANUSCRIPT

#### A Process for Production of Trehalose by Recombinant Trehalose Synthase and Its Purification

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### Highlights

- Industrial-scale production of trehalose by means of a recombinant TreS in E. coli is proposed.
- A set of batch fermentation strategies to improve the enzymatic activity is built. And the cell lysate containing TreS was directly used to convert maltose into trehalose.
- The isolation and purification of trehalose by continuous chromatographic separation represent a new technological breakthrough

#### Abstract

The process for production of trehalose using trehalose synthase (TreS) to convert maltose into trehalose in one step is highly desirable in the industry. Nonetheless, the studies on industrial-scale production of trehalose by recombinant TreS in *Escherichia coli* are still scarce. In this study, a TreS from *Pseudomonas putida* ATCC47054 was expressed in *E. coli* BL21(DE3) via plasmids pET15b and pET22b. pET15b-*treS* showed better plasmid stability and TreS expression, which revealed that the highest activity, 39866  $\pm$  1420 U/(g dry cell weight) at the final lactose concentration of 4 g/L for 7 h at 27 °C in a 5-L fermentor at pH 8.0. The use of 30% (w/v) high-maltose syrup as a substrate can extend the temperature tolerance of TreS to 60 °C. More than 64% of maltose can be converted into trehalose by adding 200 U of TreS per gram of maltose at 50 °C for 24 h. The total

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