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Research Article

# Trehalose Production at Different Mediums in *Bacillus cereus* and *Ralstonia eutropha*

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## Abstract

Trehalose; It is a non-reducing disaccharide consisting of 2 glucose molecules, which is abundant in nature. This disaccharide is a sugar that serves both as an important storage carbohydrate for living things and as a protector against various environmental stresses (drought, freezing, excessive salt environment, etc.). It can produce many living species, from bacteria to insects and invertebrates. The aim of this study was to compare the production of trehalose produced by *Bacillus cereus* Gr (+) and *Ralstonia eutropha* Gr (-) in different carbon sources. The highest Trehalose production in NB (Nutrient Broth) medium was found 172,235(U/ml) in *Bacillus cereus* at 150 rpm. The highest trehalose production in environments containing different carbon sources (1% Glucose, 1% Fructose, 1% Dextrose, 1% Xylose, 1% Maltose, 1% Ramnose, 1% Ribose); It was found 20,794 (U/ml) in *B. cereus* in PBS + maltose broth and 17,485 (U/ml) in PBS + ribose broth in *R. eutropha*. The lowest trehalose production was found to be 7,917 (U/ml) in *B. cereus* in PBS medium. In this study, it was observed that *R. eutropha*, a good PHB (Polyhydroxy butyrate) producer, and *B. cereus*, an endospore-forming soil bacterium, were able to produce trehalose in rich and poor, aeration conditions (0, 150 rpm). This study shows that inexpensive and suitable media can be used to produce trehalose, an important metabolite. Many Gr (t) and Gr (-) bacterial groups seem to be an important microbial source preferred for trehalose production.

**Key words:** Trehalose; *Bacillus cereus; Ralstonia eutropha*

## Introduction

Trehalose; It is a non-reducing disaccharide sugar commonly found in nature, consisting of 2 molecules of glucose, linked by α,α - (1,1) glycosidic bonds [1]. It is white crystalline and its molecular formula is $C_{12}H_{22}O_{11}$. $2H_2O$; its molecular weight is 378. 33. It has a weak sweetness, can be dissolved in water and hot alcohol, but not in ethyl alcohol. It is non-toxic and harmless. It has a stable chemical property [2]. Due to its resistance to heat and acidity, it has a large range of implementation in the nutrient industry. Extends shelf life of starch-containing food products. It suppresses the bitter taste in food and therefore makes trehalose an ideal sugar source for the nutrient industry. In addition to its use in the nutrient industry, it also has an important place in the cosmetics industry thanks to its ability to retain skin moisture and its stabilizing effect on bioactive material. Trehalose's elevated water holding ability ensures the survival of plants and animals during anhydrobiosis. Along with these, hydrophobicity is gained by esterification of sugars such as trehalose, glucose, galactose, sucrose, and mannose and changes its functionality [3]. It is stored as carbohydrate and energy source in some organisms. However, it is a compatible solute in responding to various stresses (drought, cold, heat, osmotic stress, etc.) [4].

Trehalose is an anti-metabolite of many microorganisms and has an important role in biological resistance. Protein has a vital value for organisms as it can preserve the lipid membrane, the integrity of the biofilm and maintain biological activity [2]. Trehalose producing microorganisms; *Pseudomonas aeruginosa*, *Pseudomonas fragi*, *Cryptococcus neoformans*, *C. gattii*, *Saccharomyces cerevisiae*, *E. coli*, *Salmonella*. Apart from these, many living groups including fungi, insects and invertebrates can produce trehalose.

Trehalose gains major significance as the best therapy touch against cancer due to its advantageous properties (solubility in water, being able to metabolize in the cell, natural disaccharides, etc.) It is considered an anti-cancer agent. Trehalose has been reported to have a pleiotropic role in diverse diseases. For example; oxidative stress preservation, prevention of inflammation, interference of protein denaturation, and neurodegeneration are physiological conditions [5].

*Bacillus cereus*; It is a facultative aerobic Gr (+) bacterium that is abundant in soil, air and water, has the ability to form endospores [6]. *B. cereus* strains are both psychrotrophic and mesophilic. Psychrotrophic strains are found in frozen foods and in some cases fresh nutritions. Mesophilic ones can grow at 37°C and alive at temperatures below 10°C. The most important feature of these bacteria is that they can form endospores and biofilms in the most difficult environmental conditions (drought, extreme cold, freezing, various radiations, etc.) [7].

*Ralstonia eutropha* are rod-shaped microorganisms with an expanse of 0.5-1.0 µm and a long of 1.8-2.6 µm. It can use different organic compounds as carbon sources. Optimum breeding temperatures are about 30 $^0$C. It can reduce nitrates to nitrogen gas (8). In addition, it is one of the non-pathogenic proteobacteria belonging to the Gr (-) and facultative chemoliotrophic β- class [9]. *R. eutropha* increases to very high cell densities (281 g/L) under nutrient-rich conditions and stores big amounts of PHA (232 g/L) when the nitrogen or phosphate resource is scarce [10].

## Material and Methods

### Chemicals

Anthron, KCl, $KH_2PO_4$, NaCl, $Na_2HPO_4$, Nutrient Agar, Salicylic acid, Trehalose, Tris.

### Microorganisms and nutrient medias

In this study; *Bacillus cereus* (ATCC 10876) and *Ralstonia eutropha* (ATCC17699) were used. These microorganisms were produced in an oven at 37°C for 24 hours, overnight (O/N), by passing them into NA plates with a loop at intervals of 20 days. The next day, the plates were removed from the oven and stored at + 4°C [11].

### Carbon sources

1% Glucose, 1% Fructose, 1% Maltose, 1% Nutrient Broth, 1% Rhamnose, 1% Ribose, 1% Sucrose, 1% Xylose.

### Method

Bacteria were inoculated with loops and incubated at 37 °C for 24 hours overnight. They were placed in nutrient media containing different carbon sources (1% NB, 1% Dextrose, 1% Fructose, 1% Glucose, 1% Maltose, 1% Ramnose, 1% Ribose, 1% Sucrose, 1% Xylose) from 100 µl of culture samples. 100 µl of supernatant, 300 µl of salicylic acid, 100 µl (140 mM) Trehalose were placed in eppendorf tubes and kept statically in the oven for 15 minutes after vortexing. 500 µl (500 mM) Tris was placed on the samples. In the antron process; 990 µl $dH_20$ + 10 µl sample + 1.5 ml Antron was added. It was kept in boiling water for 10 minutes. It was cooled in tap water. Readings were taken against the blind (Antron) on the $OD_{620}$. The study was conducted in a static and 150 rpm environment and with 3 replications [12].

### Findings

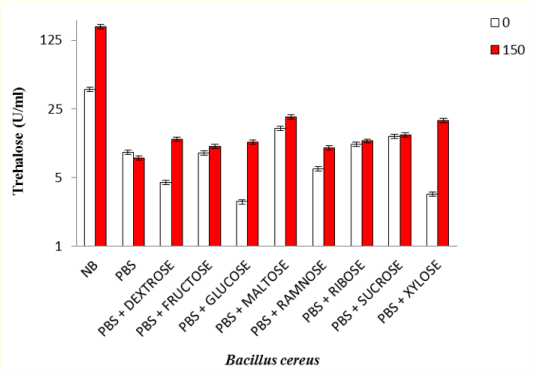


**Figure 1:** Trehalose production of Bacillus cereus at 0 and 150 rpm at 37 oC in different environments.

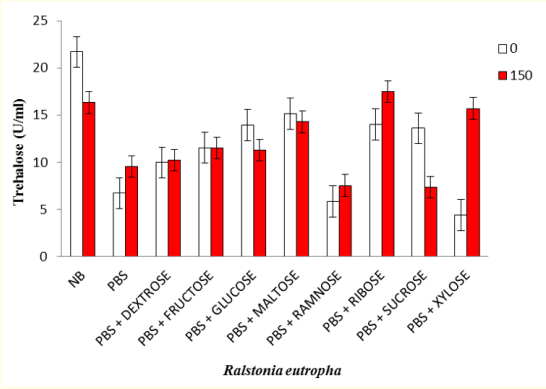


**Figure 2:** Trehalose production of Ralstonia eutropha at 0 and 150 rpm at 37 oC in different environments.

## Discussion and Conclusion

The highest trehalose production is at 150 rpm in media containing different carbon sources (1% Glucose, 1% Fructose, 1% Dextrose, 1% Xylose, 1% Maltose, 1% Ramnose, 1% Ribose); It was found 20,794 (U/ml) in *B. cereus* in PBS + Maltose broth and 17,485 (U/ml) in PBS + Ribose broth in *R. eutropha*. The lowest trehalose production was found to be 7,917 (U/ml) in *B. cereus* in PBS medium. It is seen that microorganisms try to survive by producing trehalose in poor environments such as PBS. In addition, this study shows that inexpensive and suitable media can be used to produce trehalose, an important metabolite. Many Gr(t) and Gr(-) bacterial groups seem to be an important microbial source preferred for trehalose production.

## Competing İnterests

The authors declare that they have no competing interests.

## Acknowledgements

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