

Match Maker/ Sustainable Ingredients/ 3 Feb 2023

# Novel process for the manufacturing of Trehalose, a high-value rare sugar

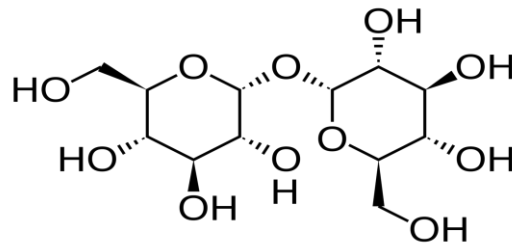
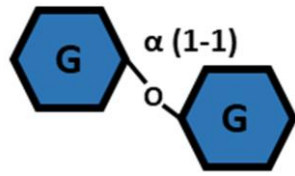
**Lead Inventor: Dr. Sudhir Singh, FNAAS**

Organization: CIAB, Mohali

TechEx.in Case Manager: Pradnya Aradhye (pradnya@venturecenter.co.in)

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# Trehalose



- ◆ Frozen food applications
- ◆ Pharma
- ◆ Personal care products

## Food industry

- Reduction of freezing point of food product, prevention of the formation of ice crystals and freeze-thaw damage
- Stable in a wide range of pH
- Prevents starch aging
- Water retention capability
- Extended shelf-life of food products
- Maintains the polyphenolic content and antioxidant properties
- less cariogenic
- Low sweetness with a clean finish, and can improve food flavor and quality
- Daily intake of 10 g improves glucose tolerance, mitigates insulin resistance

## Pharmaceutical industry

- Stabilizer of the cell membrane and proteins; cryopreservation of sperm and blood cells

## Cosmetic industry

- A moisture-retaining agent
- Helps in reducing the human body odor by degrading unsaturated fatty acids



Limitations in industrial production



- Poor thermal stability of enzyme
- Low product yield

# The Opportunity

The global trehalose market is predicted to grow from USD 240 million in 2021 to about USD 330 million by 2029 at a Compound Annual Growth Rate (CAGR) of 5 percent during the forecast period 2019-2029.

- 1) Healthy option, offers health benefits (Food and beverages)
  - 2) Prevents protein denaturation (frozen meat, fish)
  - 3) Offers skin protection and provide nourishment (creams and shampoos)
- ◆ Price ~ INR 1000/kg (depends on quality and purity)
  - ◆ Major global suppliers: Hayashibara Co., Ltd, Life Sciences Advanced Technologies, Cargill Inc, Shanghai Danfan Network Science & Technology Co., Ltd, Sika(China), Otto Chemie, Profoods, Van Wankhum (Netherlands)
  - ◆ **No manufacturer in India**

# Who should be interested and why?

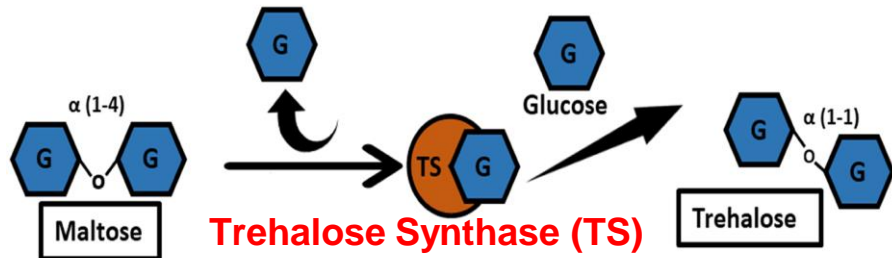
Who?	Why?
Frozen food products industry	<ul style="list-style-type: none"><li>• No imports required</li><li>• Ingredient with good performance</li><li>• Expansion of the product portfolio</li></ul>
Pharma industry	Good quality trehalose as no contaminants are produced in enzymatic conversion
Suppliers to FMCG, beauty care products	New products and forays into new markets

# About the technology

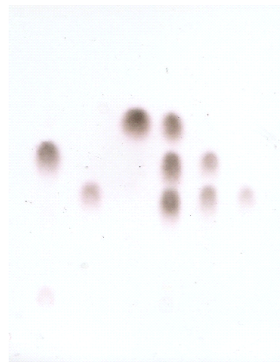
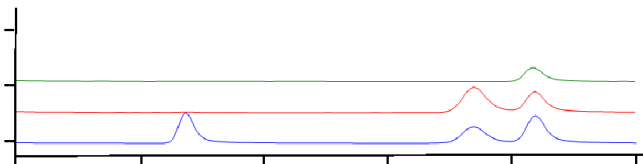
## Process technology features:

- ◆ Cost effectiveness
- ◆ Low cost starting material
- ◆ Efficient enzymatic conversion
- ◆ High thermal stability of enzyme
- ◆ Ample raw material available
- ◆ Scalable process
- ◆ Dual catalysis

## Enzymatic conversion of Maltose to Trehalose



Maltose to Trehalose conversion (> 70%)



## Expression of Trehalose Synthase (TS) in *E. coli* cells

Culturing of cells in LB medium at 37°C, 100 rpm for 24 h



## Enzyme extraction and purification

Cells harvesting by centrifugation. The cell pellet is suspended in 50 mM Tris buffer of pH 7, containing 300 mM NaCl, followed by disruption of the cells by sonication for 3 min. Chromatographic separation of enzyme (TS) from cell lysate.



## Enzymatic reaction

Maltose solution (100 g/L) + Enzyme (50-100 mg / L)

pH 6-7; Temp 30°C  
Incubation time Approx. 3 h



Maltose to Trehalose conversion ~70%



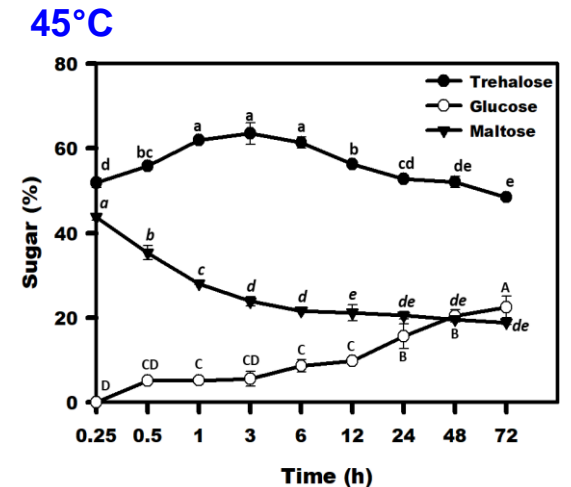
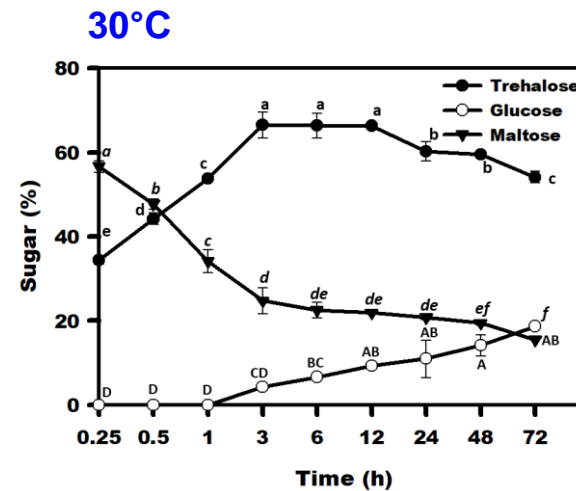
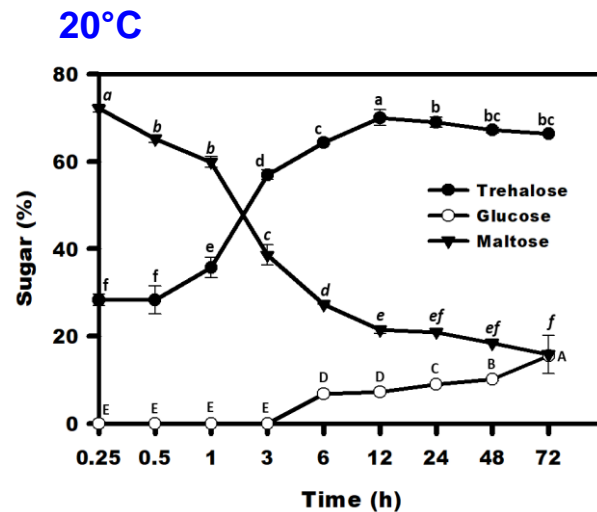
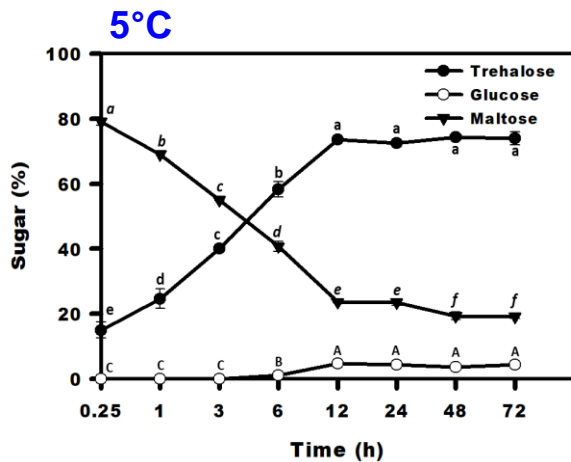
Simulated moving bed chromatography for separation of Trehalose

# Hayashibara

# vs CIAB Technology

Organism	Optimum pH	Optimum temp.(°C)	Trehalose Yield(%)	Trehalulose Yield (%)	Thermostability
<i>Pimelobacter</i> sp. R48	7.5	20	81.8% at 5°C in 48h	-	40% (40°C, 60 min)
<i>Pseudomonas putida</i> H262	7.5	60	75% at 20°C in 24 h	-	NR
<i>Thermus aquaticus</i> (ATCC 33923)	6.5	65	80.7% (40 °C) in 48h	81% (40 °C) in 96 h	90% (80°C, 60 min)
<b>(CIAB Technology)</b>					
<b>Hot spring metagenome</b>	<b>6-7</b>	<b>5-45°C</b>	<b>52% (45 °C) in 15 min;</b> <b>60% (45°C) in 1 h;</b> <b>66% (30 °C) in 1 h;</b> <b>70% (20 °C) in 12 h;</b> <b>74% (5°C) in 12h</b>	<b>~85% (50 °C) in 12 h</b>	<b>80% (45°C, 120 h);</b> <b>60% (50°C, 120 h);</b> <b>50% (60°C, 96 h)</b>
Source: unknown 88% identity to uncharacterized protein sequence from <i>Thermanaerotherix daxensis</i>					

CIAB technology offers enzymatic biosynthesis of Trehalose at wide range of temperature (5 to 45 °C)



# Current status

## Technology status:

- ❖ Demonstrated at lab scale (1 L)
- ❖ Sample available
- ❖ Patent filed

## Patents:

- ❖ Priority document: : A process for the production of trehalose and trehalulose employing a novel trehalose synthase from thermal spring metagenome. Indian Patent Appl. No. 202111022212 (May, 2021)
- ❖ Coverage: IN
- ❖ Approved: Pending

## Publications:

- ❖ Agarwal N, Singh SP (2021) A novel trehalose synthase for the production of trehalose and trehalulose. *Microbiology Spectrum* 9: e01333-21 (IF 9.043)

# Next Steps

- ◆ The CIAB team has invented a new process for the production of trehalose. The process has been demonstrated at lab scale.
- ◆ The next steps are to identify an industrial partner for co-development of the technology involving process optimization, develop purification methods, scale up to pre-commercial levels, carryout detailed testing and produce trial quantities for customer inputs.

## Seeking:

- ❖ Industrial partners interested in technology licensing
- ❖ Industrial partners interested in sponsoring further technology advancement and scale-up
- ❖ Industrial partners interested in raising 3<sup>rd</sup> party funds for a collaborative project.
- ❖ Industry interested in tapping scientist capabilities as an expert/consultant.



# Team and Organization



**Lead Scientist:**  
Dr Sudhir Singh, FNAAS  
Scientist-D, CIAB

Fellow, National Academy of Agricultural Sciences, India

Member, National Academy of Sciences, India

HIRALAL Chakravarty Award (ISCA, DST)

Gandhian Young Technological Innovation Award to the team, SRISTI

Young Scientist Award, IBA (France)

SBS-Madurai Kamraj Univ Genomics Award, BRSI

Track record of tech transfer to industry

**Expertise:** Gene mining, biocatalyst engineering, bioprocessing, bioproduct generation and enzyme characterization



## Center of Innovative and Applied Bioprocessing, Mohali

- ◆ CIAB is an autonomous institute of DBT, India
- ◆ Key assets and strengths of the team:
  - ◆ **10** Indian patents filed , **8** granted. **More than 45** publications in bioprocess, enzyme characterization and functional biomolecules.
  - ◆ Well equipped labs and analytical facilities
    - ◆ 5 L fermenter
    - ◆ HPLC and Gas chromatography
    - ◆ Enzyme characterization assays
    - ◆ Protein Purification system
    - ◆ Membrane separation unit
- ◆ Industry project/ tech transfer
  - ◆ Track record of technology transfer and working with industry; attractive models of engagement and flexible terms for IP

For more information, contact:

Pradnya Aradhya  
pradnya@venturecenter.co.in  
+91-88050-09010

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