



The Make or Break of Precision Fermentation

The choice of the Host Organism

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The presenter



Ravinder Sharma

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Ravinder Sharma is a Food Technologist at Tetra Pak, responsible for the Biotech for Food platform.

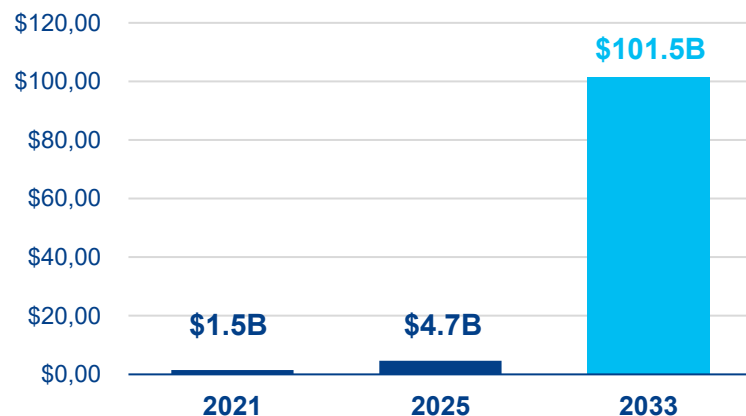
With over three years at the company, **Ravinder brings deep expertise in biotechnology and fermentation technology.** Holding a PhD from Guru Nanak Dev University, India, and fluent in English, Hindi, and Punjabi, **Ravinder is actively engaged in advancing research and building knowledge in the New Food area.**



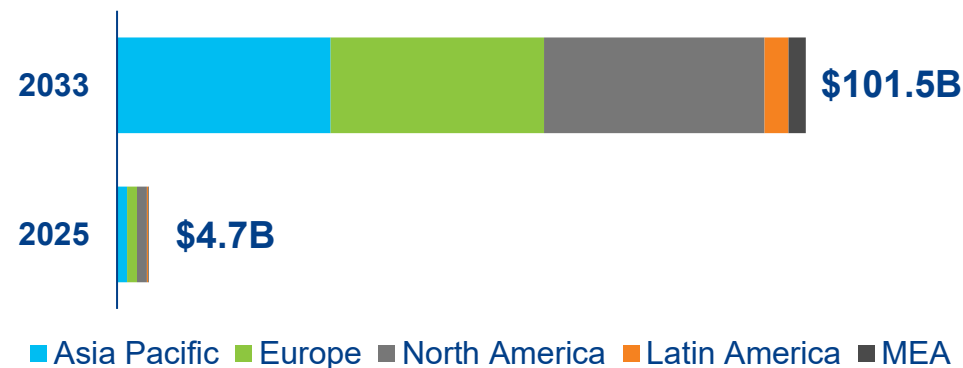
Global Precision Fermentation Market (2021- 2033)

Market size

CAGR 2026-2033: 48.3%



By Region



- **Rapid projected growth \$101.5B by 2033**
- **A very strong CAGR of 48.3%**
- **North America, Europe, and Asia Pacific appear to dominate the 2033 market**



Qualities for Appropriate Host Organisms for Recombinant Protein Production

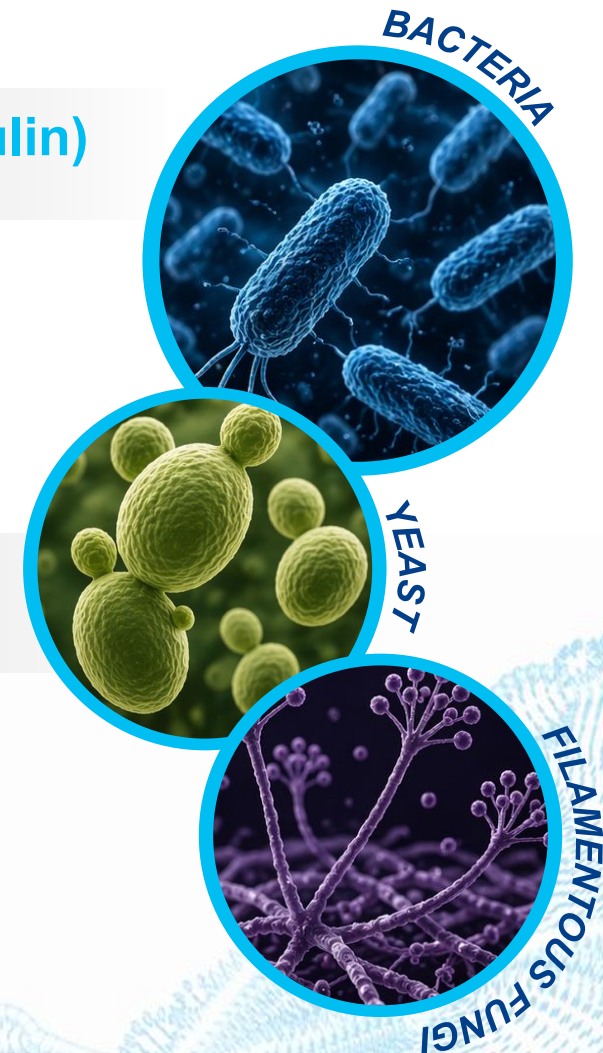
Physico-Chemical Properties of Target Protein (β -Lactoglobulin)

(Farrell, 2004)

- **Globular Protein** (~18.27kDa monomer, 162 amino acids)
- **PI value** (~5.13), Negative Charge
- **Required Protein folding and Post translational modifications**

Bacterial, Yeast and Filamentous fungal based expression systems

- **Ease of genetical modifications**
- **Highest expression level**
- **Stable & Scalable Process for high Process robustness**
- **Enable Cost-effective Product recovery in DSP**





Microbial Host Platforms for Precision Fermentation

Bacterial Strains

OPTION 1: **Gram Positive: *Lactic Acid Bacteria (LAB)* (*L. Lactis*) & Non-Toxigenic *Bacillus* strains (i.e. *B. Subtilis*)**

OPTION 2: **Gram Negative: *E.coli* (K-12 and BL21)**

Filamentous Fungal Strain

Trichoderma reesei

Yeast

Komagataella Phaffii

Pros. & Cons. of using Gram Positive Bacteria as Host

Pros.



Accepted as Safe
with Food grade vector ✓

Lack of Endotoxins ✓

Secretes Protein
(Bacillus) ✓

Simple DSP ✓



OPTION 1: QPS

*Lactobacillus Spp. &
Bacillus subtilis*

(Gram Positive)

Rozanov et al, 2025
Tagliavia and Nicosia 2019
Phan et al, 2005

Cons.



✗ Complex/Specialised Genetic
modifications

✗ Longer development Time

✗ Increased strain development cost

✗ Proteases degrade the product

✗ Less cell density and Lower expression

✗ Sporulate under O₂ limitations (Bacillus)

Pros. & Cons. of using Gram Negative Bacteria as Host

Pros.



Ease of Genetic Modifications



Shorter Development Time



Easy to reproduce the strains



Higher Protein Production
than Gram Positive



OPTION 2: Non-QPS

E.Coli
(K12 & BL21)

(Gram Negative)

Rozanov et al, 2025
Tagliavia and Nicosia 2019
Phan et al, 2005

Rosano and Ceccarelli, 2014

Cons.



Antibiotic-resistance gene require
for selective pressure (Genetic stability)



Cell Lysis is required.
High DNA Content



Inclusion Bodies. **Challenging scale-up**



Complex DSP. **Require additional
folding step and protein loss**



Endotoxins released.
Additional purification step require

Pros. & Cons. of using Filamentous Fungus as Host

Pros.



Higher protein secretion capacity



Post-Translational modification capability



No cell lysis require



Ability to grow on cheap substrates



Overall low production cost



**Trichoderma
reesei**

Wang et al, 2020
Lubeck & Lubeck, 2022
Aro et al, 2023

Cons.



Complex/ specialized Genetic Manipulation



Longer strain development Time



Proteases degrade the product



Secondary metabolite production



High viscous broth results in O₂ limitations may also cause Sporulation

Pros. & Cons. of using Yeast Cell as Hosts

Pros.



- Higher protein secretion capacity than E.coli. ✓
- Well established molecular tools (Mut⁺, mut^s); Stable construct ✓
- Comparatively Safer than Bacteria ✓
- Post-Translational Modification capability ✓
- No cell lysis require ✓
- Comparatively low production cost ✓
- Availability Methanol free Constitutive (pGAP) Promoters ✓



Komagataella Phaffii

Wang et al, 2020
Lubeck & Lubeck, 2022
Gao et al, 2021

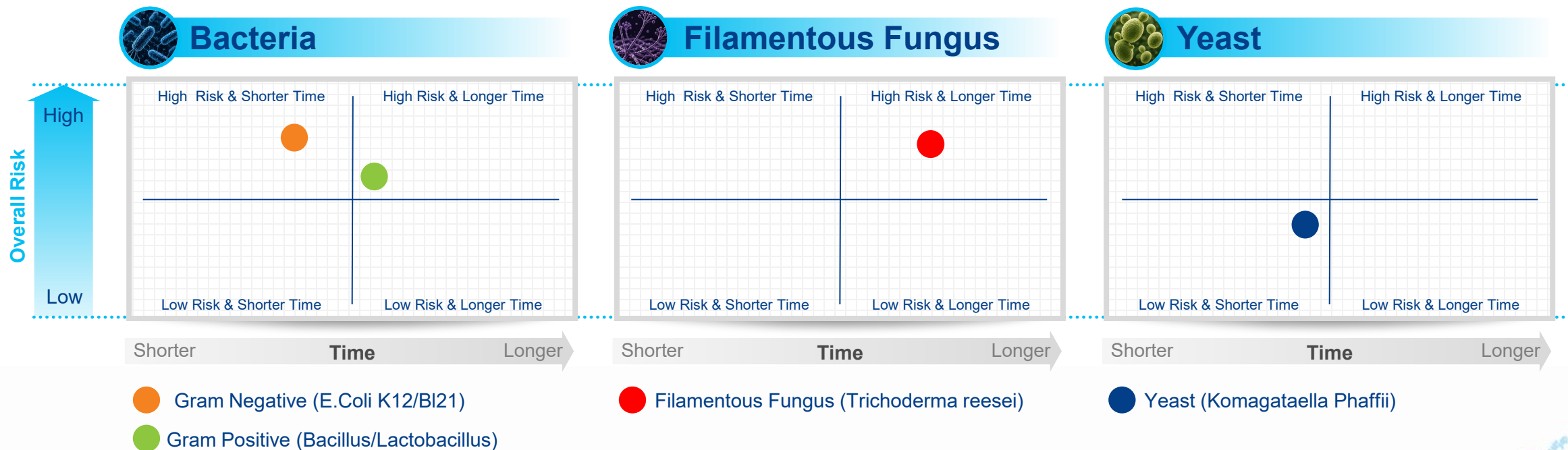
Cons.



- Specialized Genetic Manipulation include high throughput screening ✗
- Differential glycosylation ✗
- High mannan (non specific PTMs) ✗
- Strain with Methanol induced promoter may have scale-up issue ✗



Risk Assessment for using different Microbial Hosts

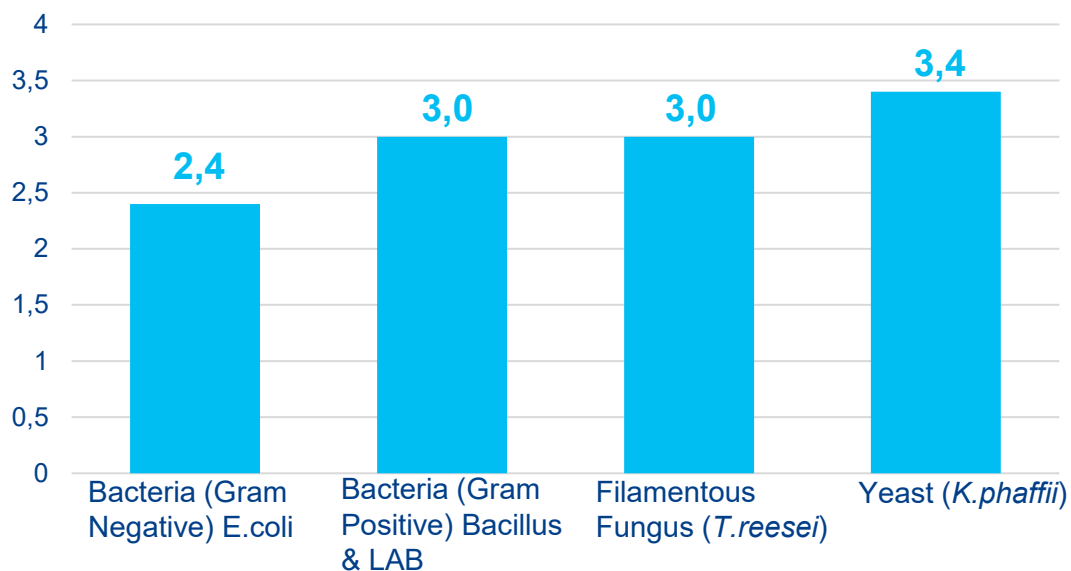


Yeast strains (Komagataella Phaffii): Low to Moderate Risk and Moderate Development time



Evaluation of Overall Process Robustness with different Microbial Hosts

Overall Process Robustness



Parameters to evaluate Overall Robustness

Grading

Strain development duration	Long (1) to Short (5)
Process Control	Difficult (1) to Easy (5)
Protein Secretion	Low (1) to High (5)
Protein folding (PTMs)	No (1) to Complete folding (5)
Cost Effectiveness	Very Low (1) to Very high (5)
Regulatory Acceptance	Full (1) to No critical evaluation (5)
Ease of Overall Process development	Difficult (1) to Easy (5)

Schutz et al, 2023

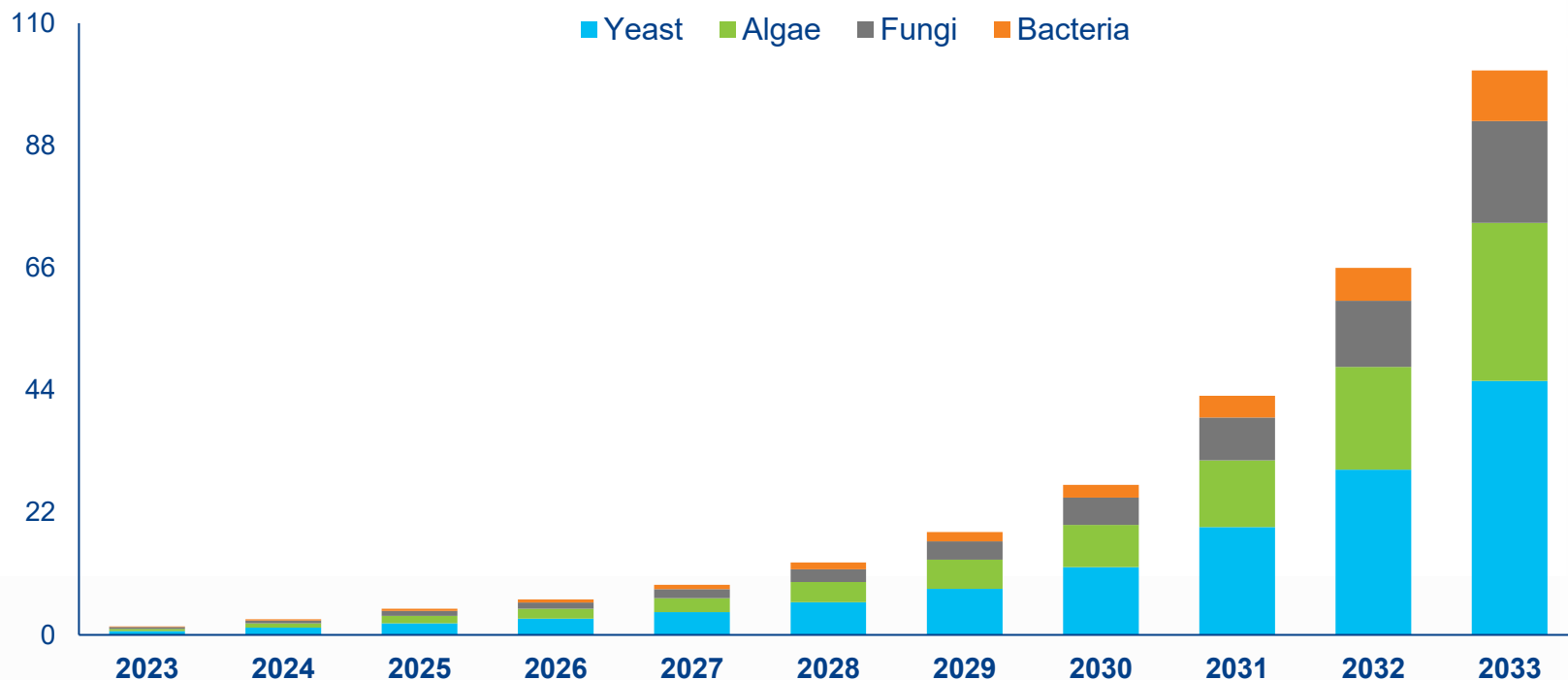


Yeast strains (*Komagataella Phaffii*) is found to be highly suitable for development of β -Lactoglobulin



Precision Fermentation Market (2021- 2033)

Size, by Microbe, 2023-2033 (USD Billion)



Yeast segment has the largest revenue share of 43.1% in 2025





Tetra Pak Team at AFT 2026



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Authors of Abstract at AFT 2026



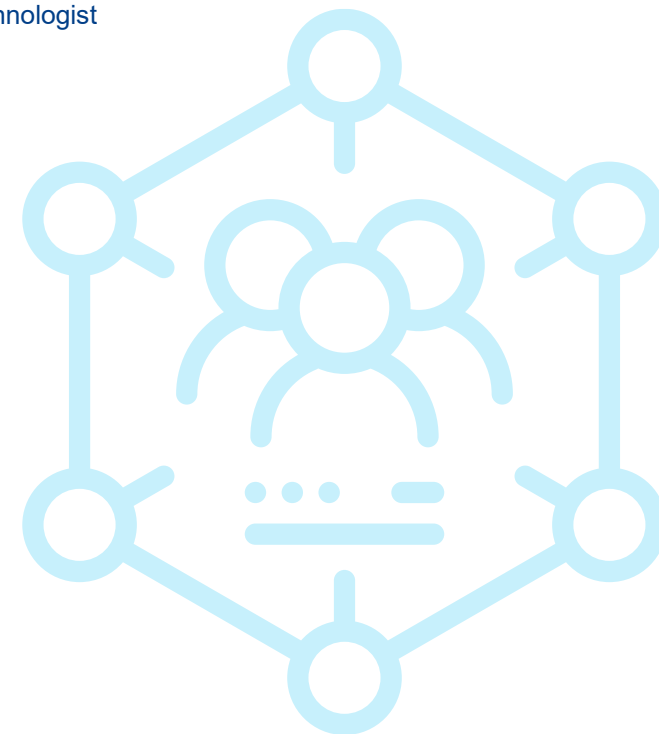
Joana Campos

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We're here to make food safe and available. It's why we provide advanced food production systems. In collaboration with our customers and suppliers, driven by more than 24,000 dedicated employees worldwide, we protect food sustainably every day for hundreds of millions of people in more than 160 countries.

Because we're here to fulfil a purpose:

**We commit to making food safe and available, everywhere, and we promise to protect what's good:
food, people and the planet.**

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