

# Physicochemical and Antimicrobial Properties of Chitosan Extracted from Black Soldier Fly Puparia

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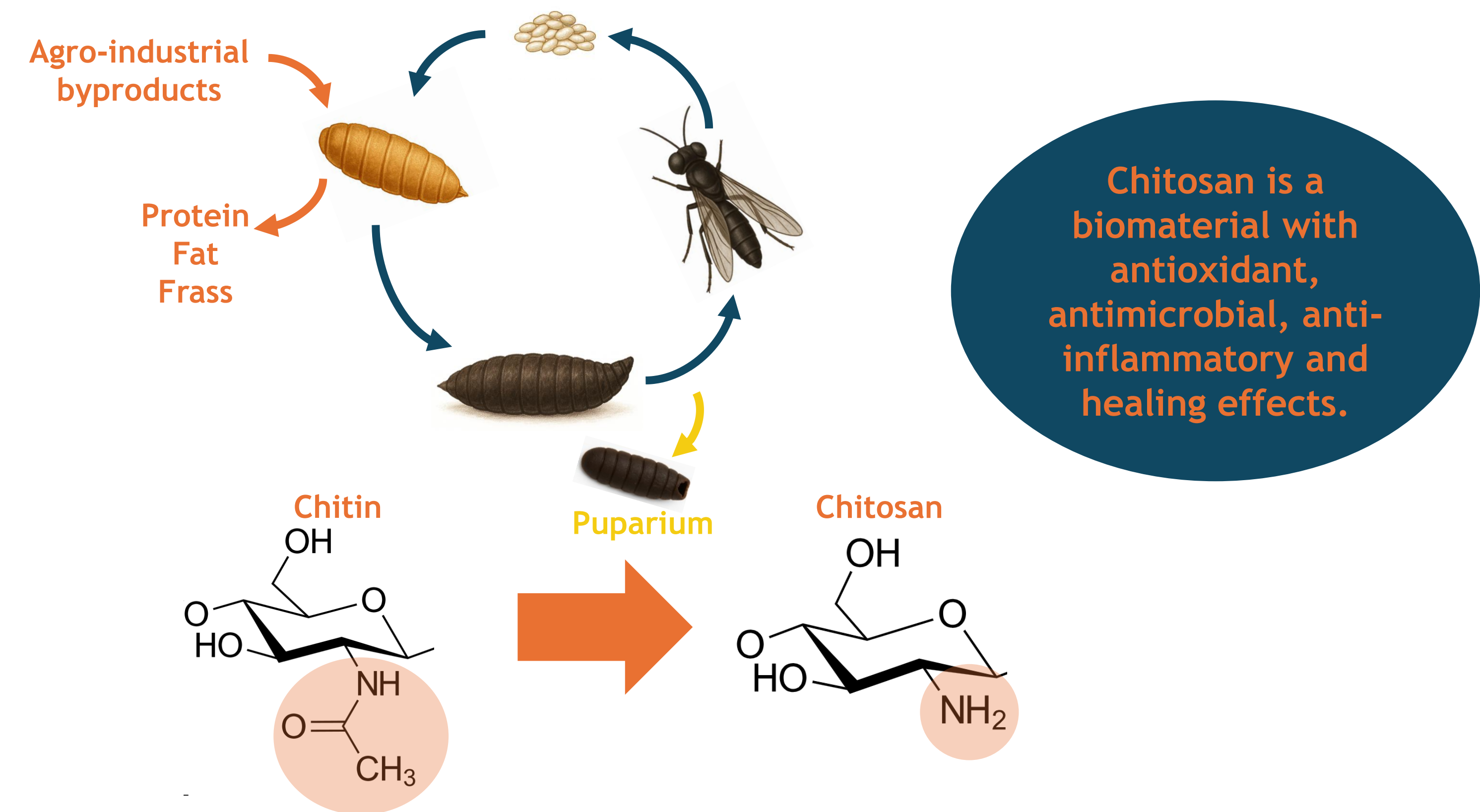


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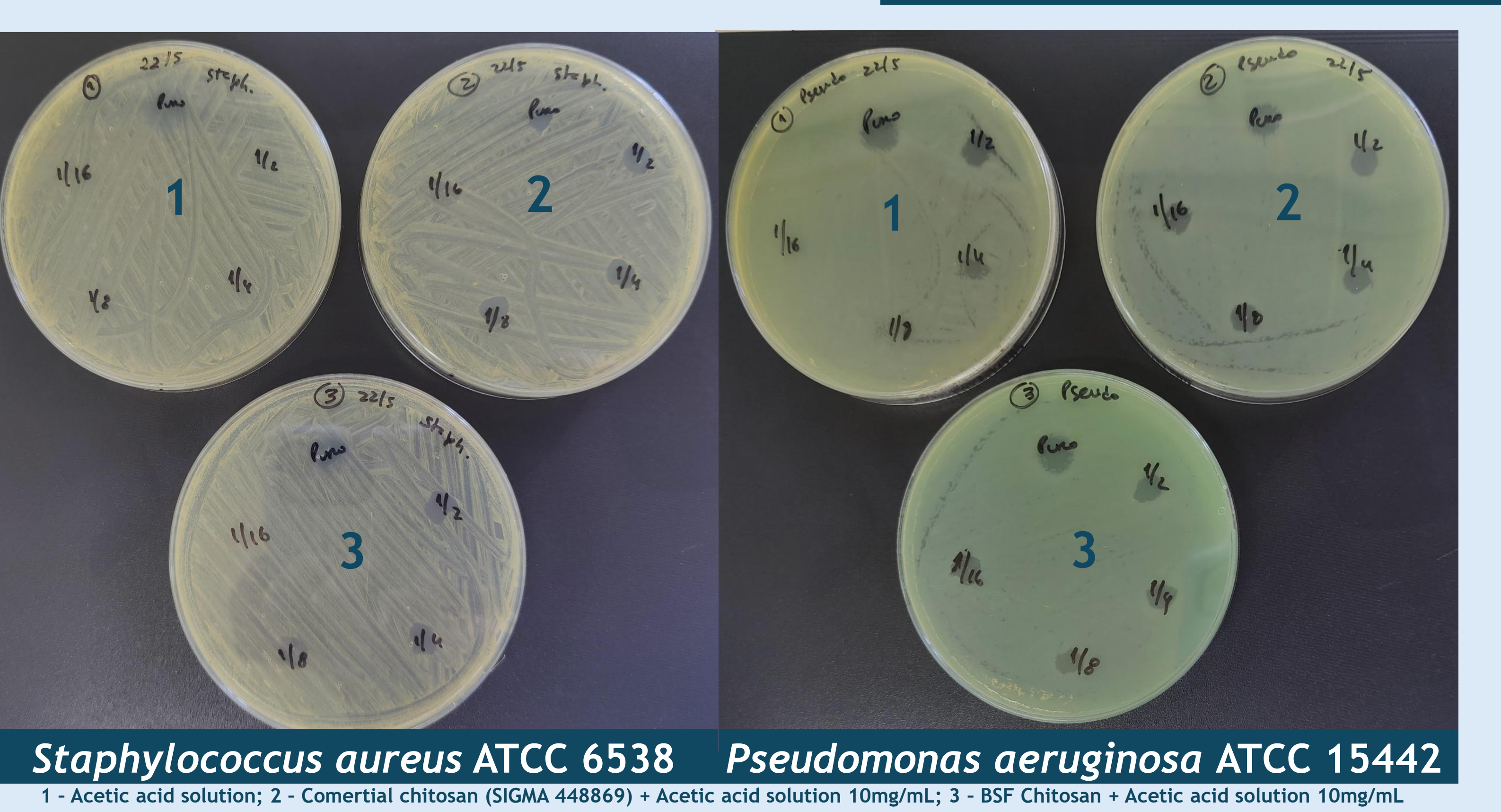
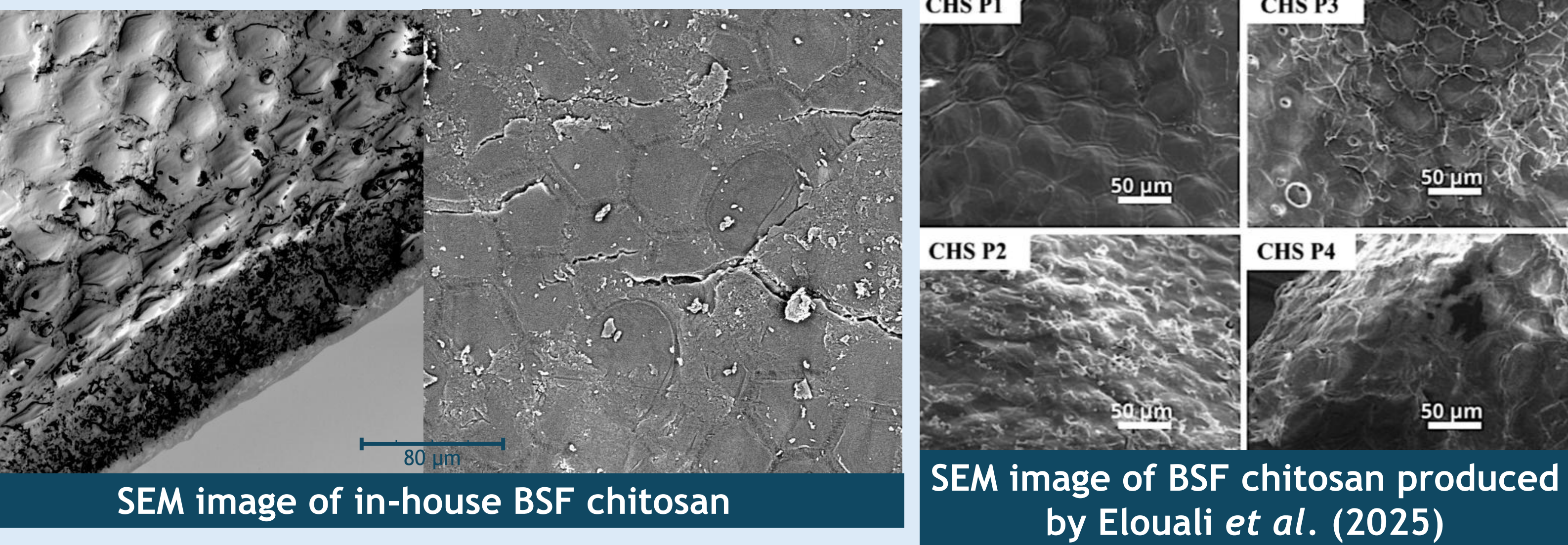
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## Introduction

Black soldier fly (BSF, *Hermetia illucens*) larvae can convert agro-industrial by-products into sustainable feed and high-quality organic fertilizer. The *puparium* (cocoon left behind when the black soldier fly emerges) is a by-product of this industry and is rich in chitin.



## Results and Discussion



1 - Acetic acid solution; 2 - Commercial chitosan (SIGMA 448869) + Acetic acid solution 10mg/mL; 3 - BSF Chitosan + Acetic acid solution 10mg/mL

## Conclusion

Our results emphasize the need for small adjustments to our current protocol in order to produce chitosan with a lower molecular weight. These modifications will improve water solubility, broadening its potential applications. Importantly, the chitosan produced in this study has already shown antimicrobial activity against both microorganisms tested. By fine-tuning the production process, we aim to further enhance its bioactivity, bringing us closer to our ultimate goal of developing a clinically viable antimicrobial adhesive gel for dentures.

References: Elouali, S., Ait Hamdan, Y., Benali, S., Lhomme, P., Gosselin, M., Raquez, J. M., & Rhazi, M. (2025). Extraction of chitin and chitosan from *Hermetia illucens* breeding waste: A greener approach for industrial application.

## Methods

### Chitosan Production

#### 1. Demineralization

3 hours at 30°C with HCl (1M)  
Dry overnight at 60°C

#### 2. Deproteinization

2 hours at 80°C with NaOH 2M  
Dry overnight at 60°C

#### 3. Depigmentation

2 hours at 80°C with H<sub>2</sub>O<sub>2</sub> (30%)  
Dry overnight at 60°C

#### 4. Deacetylation

3 hours at 90°C with NaOH 50%  
Dry overnight at 60°C

### Physicochemical Characterization

#### FTIR

Fourier-Transform Infrared Spectroscopy  
(Spectrum 65 Perkin Elmer & ATRmiracle™)

#### WDXRF

Wavelength Dispersive X-Ray  
Fluorescence spectrometry  
(Bruker S4 Pioneer)

#### TEM & SEM

Transmission Electron Microscopy and  
Scanning Electron Microscopy  
(JEOL 1200EX & PHEnom Prox)

#### MALDI-TOF

Matrix-Assisted Laser  
Desorption/Ionization Time-Of-Flight  
mass spectrometry  
(Bruker Autoflex maX Matrix-Assisted Laser  
Desorption and Ionization)

#### Microbiology

Antimicrobial activity  
*Pseudomonas aeruginosa* ATCC 15442  
*Staphylococcus aureus* ATCC 6538

Characteristics	Chitosan
Chitosan Yield	10%
Average molecular Weight	200 KDa
Degree of deacetylation	70%
Heavy metals	Not detected
Other Elements	Na, Si and Fe (detected at ppm levels)

**Morphology and Elemental composition**  
SEM & TEM microscopy revealed a sample composed of flakes and particles with different dimensions and irregular morphologies, compatible with the data published by other authors. Standardless Semiquantitative WDXRF methods revealed the success of the demineralisation step, with a reduction in calcium concentration to residual levels (<30 ppm). In addition, the presence of Na, Si and Fe at the ppm level was also observed, which may have been originated from the reagents used in the chitosan production protocol.

**Antimicrobial activity assessment:**  
Preliminary antimicrobial testing was preformed in acetic acid solution. Solutions were prepared using both BSF-derived and commercial (Sigma) chitosan. Acetic acid (10mg/mL) solution was used as a negative control. Both commercial and in-house produced BSF chitosan demonstrated comparable antimicrobial activity, with effective inhibition observed at all tested dilutions in *Pseudomonas aeruginosa* ATCC15442 and in tree of the four dilutions tested in *Staphylococcus aureus* ATCC 6538.

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