# TARGETING CARBAPENEM-RESISTANT INFECTIONS THROUGH PROBIOTIC-BASED LIVING MATERIALS

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## BACTERIAL MULTIRESISTANCE CRISIS

Superbugs are multidrug-resistant bacteria which represent one of the greatest challenges to global public health, causing more than 35,000 deaths in the U.S. last year. In particular, carbapenem-resistant bacteria (CRB) attract special attention, as carbapenem antibiotics are one of the last-resort treatments for multidrug-resistant bacteria. CRB *Enterobacteriaceae* and CRB *Pseudomonas aeruginosa* have been identified by WHO ( World Health Organization) as critical priority bacteria for which new therapies are urgently needed[1].

## PROBIOTIC CELLULOSE AS A NOVEL ANTIMICROBIAL

We have prepared a bacterial cellulose material (BC) loaded with different bacteria of the *Lactobacillus* genus, classified as GRAS (generally recognized as safe), what we call probiotic cellulose (PC)[2].

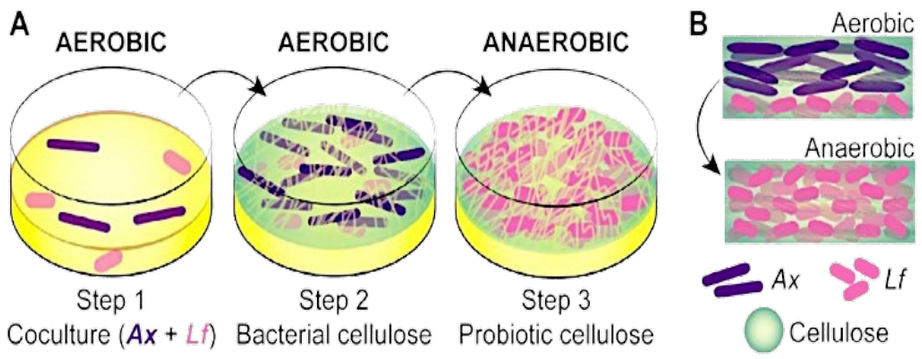


Fig1. (A) Representation of the PC formation protocol. (B) Graphical representation (cross-section) of BC obtained under aerobic conditions and probiotic cellulose produced by switching to anaerobic conditions. Image extracted from reference[2]. Here *Lf* represents probiotic bacteria of the *Lactobacillus* genus (growing under anaerobic conditions) and *Ax* represents the cellulose-producing acetobacter bacteria (growing under aerobic conditions)

## PC CHARACTERIZATION

PC of *Lactobacillus fermentum (Lf)* and *Lactobacillus plantarum (Lp)* were synthesized and characterized.

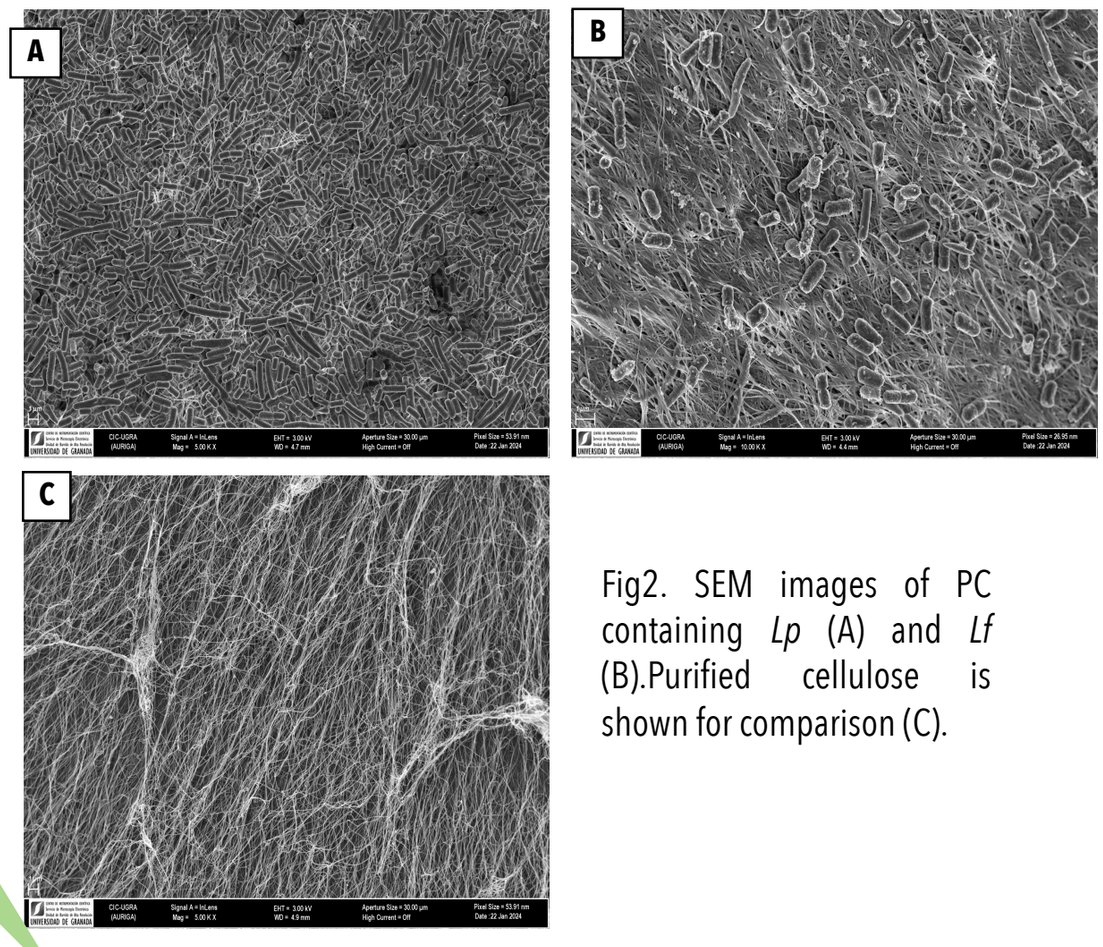


Fig2. SEM images of PC containing *Lp* (A) and *Lf* (B).Purified cellulose is shown for comparison (C).

## PC ACTIVITY TEST

PC maintains the lactic acid activity of the encapsulated probiotics. The pH decay over time was monitored for PC-*Lp* and PC-*Lf* stored for one month at 4°C, both freeze-dried and non-freeze-dried, with and without a cryoprotectant.

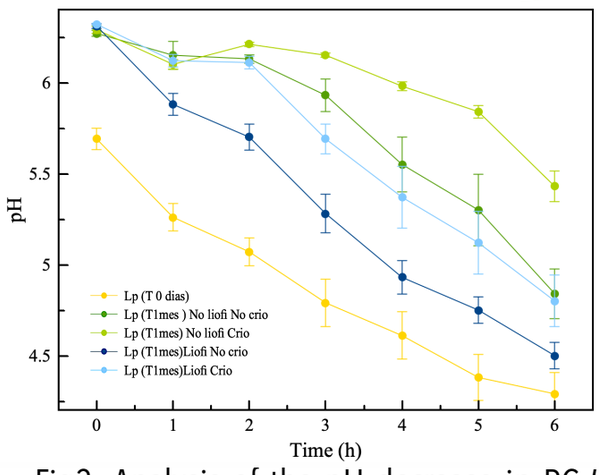


Fig3. Analysis of the pH decrease in PC-*Lp* (stored for one month at 4°C) over 6 hours compared to freshly synthesized PC-*Lp*.

## ACTIVITY OF PC AGAINST CRB

By itself, BC has no antibacterial activity. We tested the efficacy of PC to act as an antibacterial in particular against CRB, isolated from clinical samples from the Hospital Universitario Virgen de las Nieves (Granada). We demonstrated the enhanced stability and activity of probiotics once encapsulated, by comparing PC with free probiotics against CRB.

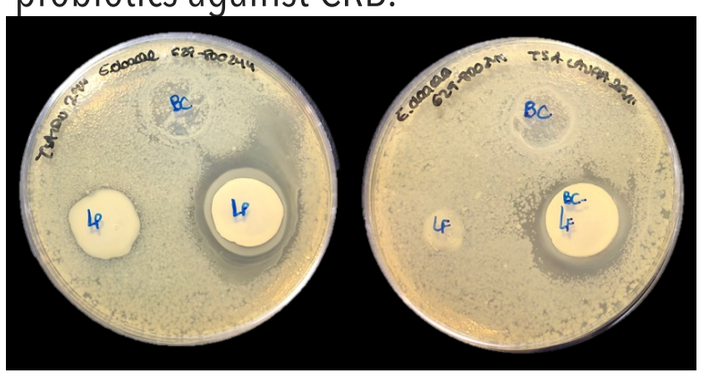


Fig4.Differences of inhibitory activity between encapsulated and free *Lactobacillus* against *Enterobacter cloacae*.

## CONCLUSIONS

The stability and activity of the probiotics incorporated in the cellulose network is enhanced. Thus, probiotic-containing living materials can represent an interesting alternative to treat CRB.

## REFERENCES

[1]Mancuso, G., De Gaetano, S., Midiri, A., Zummo, S., & Biondo, C. (2023). The Challenge of overcoming antibiotic resistance in carbapenem-resistant Gram-negative bacteria:"Attack on Titan". *Microorganisms*, *11*(8), 1912.

[2] Sabio, L., González, A., Ramírez-Rodríguez, G. B., Gutiérrez-Fernández, J., Bañuelo, O., Olivares, M., ... & Dominguez-Vera, J. M. (2021). Probiotic cellulose: Antibiotic-free biomaterials with enhanced antibacterial activity. *Acta Biomaterialia, 124*, 244-253.

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