A citrus by-product bioformulation (Bioact-LM) to control blue mold and brown rot in postharvest value chain

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# Why this topic in FoodWaStop: rationale and objectives

- Postharvest rots drive losses in the citrus chain (blue mold, brown rot).

- Control options are limited and still rely on a few treatments.

- Citrus processing generates large peel side streams.

- Bioact-LM (lemon peel fermentation) as a case study aligned with FoodWaStop themes.

Source: PeelPioneers

Aim and approach

Aim: Develop Bioact-LM to reduce citrus postharvest losses and fit a circular approach.
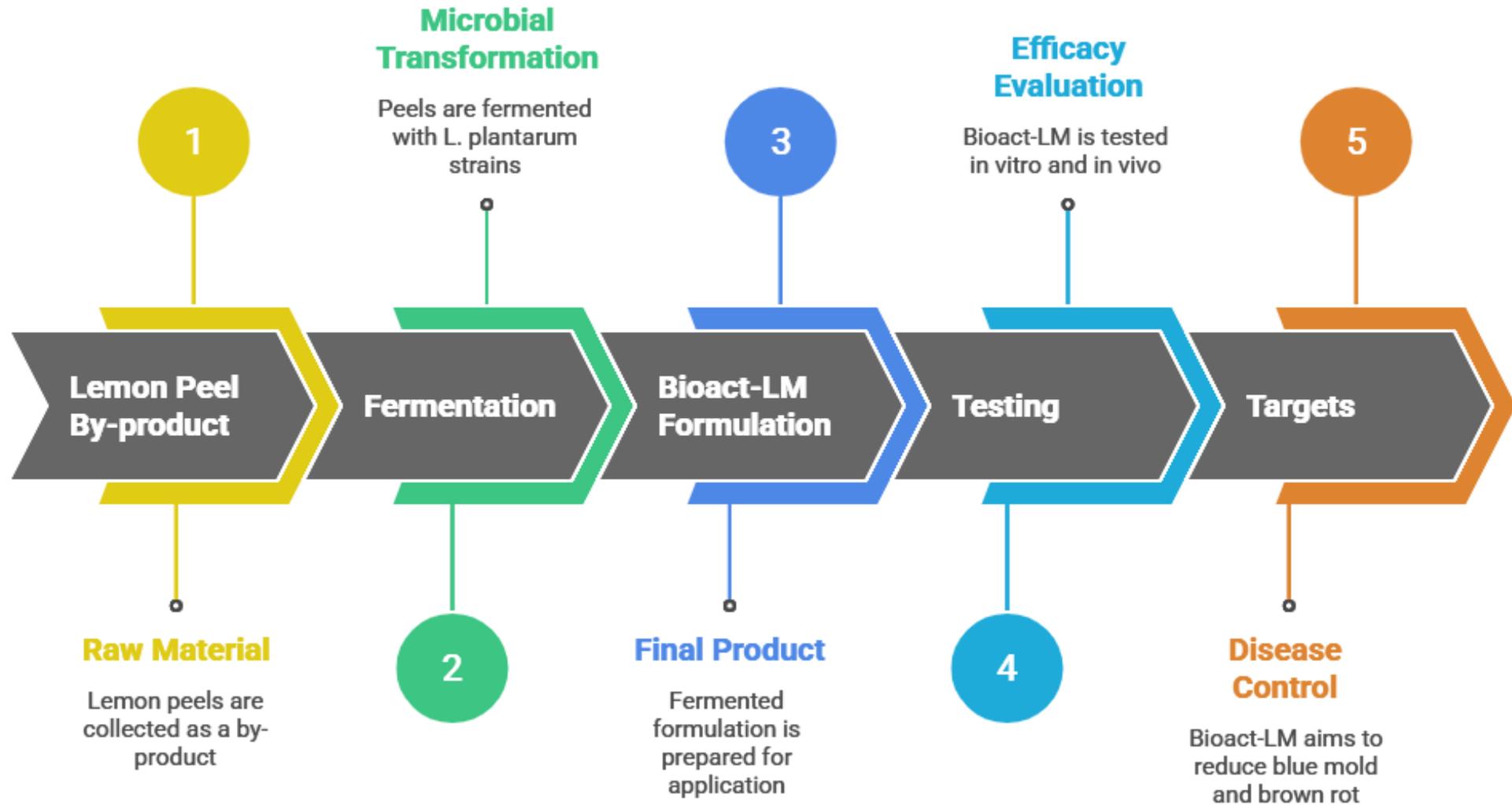
Bioact-LM: production in brief

- Starting material: lemon peel powder (by-product).
- Fermentation with *L. plantarum* N3B2/M2B2 → bioactive formulation (Bioact-LM).
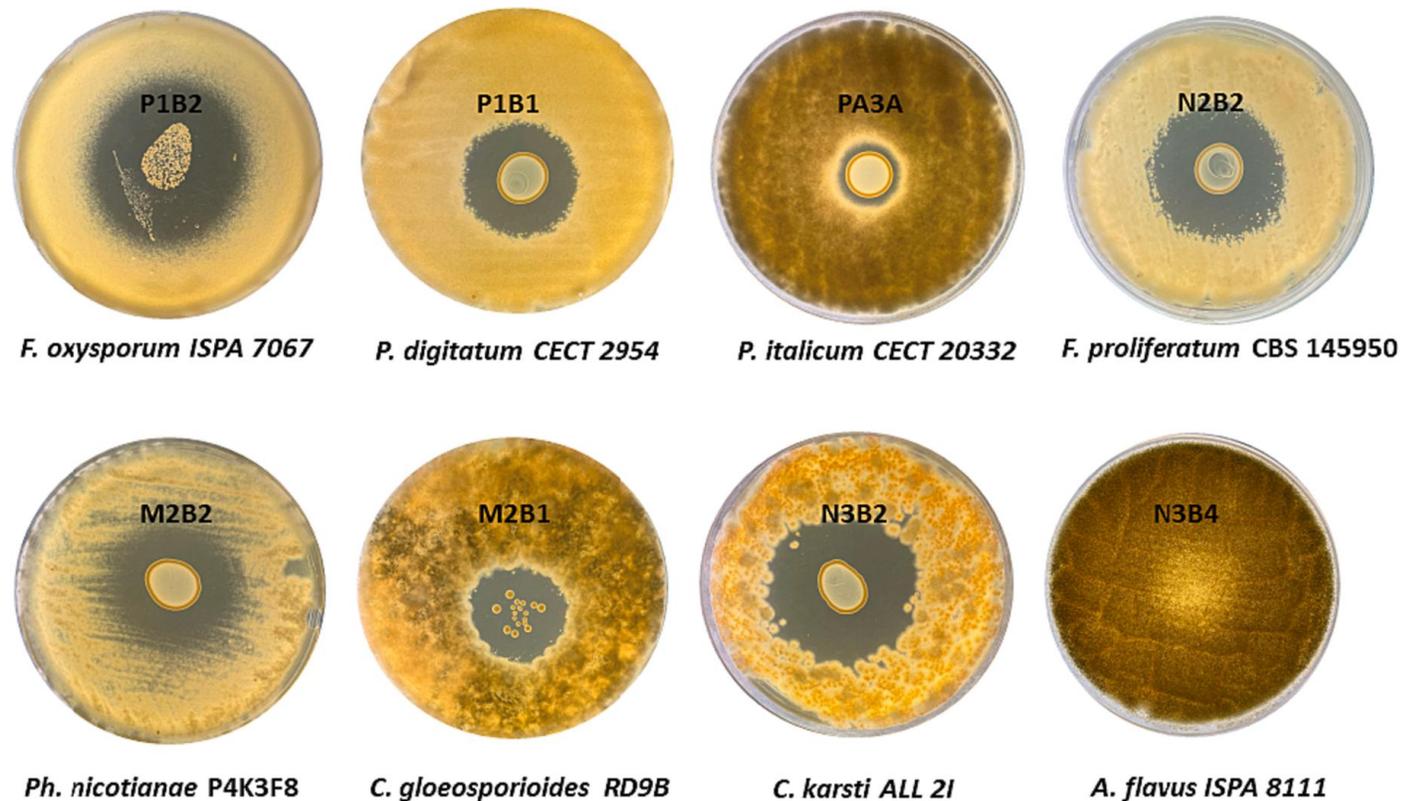- Tested by contact + volatile-mediated activity (VOCs).

Key idea

Use fermentation to enrich lemon peel-derived matrices with antimicrobial metabolites (organic acids, phenolics, VOCs).

References: Riolo et al., Biological Control (2024); La Spada et al., Journal of Agriculture and Food Research (2024).

Screening pipeline and broad-spectrum activity

- 13 LAB isolates recovered from citrus peel and identified (MALDI-TOF).
- *In vitro* screening with dual-culture overlay and agar diffusion tests.
- Pathogen panel: fungi and oomycetes (including *Penicillium* and *Phytophthora* spp).
- Two *L. plantarum* isolates (N3B2, M2B2) selected for highest and consistent inhibition.

Isolate code	Species	Host (Species and Cultivar)	Geographical Origin
M1B1	*Lactiplantibacillus plantarum*	*Citrus × clementina*	Tavernes blanques, Valencia, Spain
M1B2	*La. plantarum*	*Citrus × clementina*	Tavernes blanques, Valencia, Spain
M2B1	*La. plantarum*	*Citrus × clementina*	Tavernes Blanques, Valencia, Spain
N3B2	*La. plantarum*	*Citrus sinensis* 'Washington Navel'	Torrent, Valencia, Spain
N1B2	*La. plantarum*	*C sinensis* 'Washington Navel'	Torrent, Valencia, Spain
N2B2	*La. plantarum*	*C. sinensis* 'Washington Navel'	Torrent, Valencia, Spain
M2B2	*La. plantarum*	*C. sinensis* 'Washington Navel'	Torrent, Valencia, Spain
N3B1	*Leuconostoc mesenteroides*	*C. sinensis* 'Washington Navel'	Torrent, Valencia, Spain
N1B1	*L. mesenteroides*	*C. sinensis* 'Valencia'	Alicante, Valencia, Spain
N3B4	*L. mesenteroides*	*C. sinensis* 'Valencia'	Alicante, Valencia, Spain
P2A3	*La. plantarum*	*Citrus limon* 'Verna'	Alicante, Valencia, Spain
P1B2	*La. plantarum*	*C. limon* 'Verna'	Alicante, Valencia, Spain
P1B1	*La. plantarum*	*C. limon* 'Verna'	Alicante, Valencia, Spain

F. oxysporum ISPA 7067   P. digitatum CECT 2954   P. italicum CECT 20332   F. proliferatum CBS 145950

Ph. nicotianae P4K3F8   C. gloeosporioides RD9B   C. karsti ALL 2I   A. flavus ISPA 8111

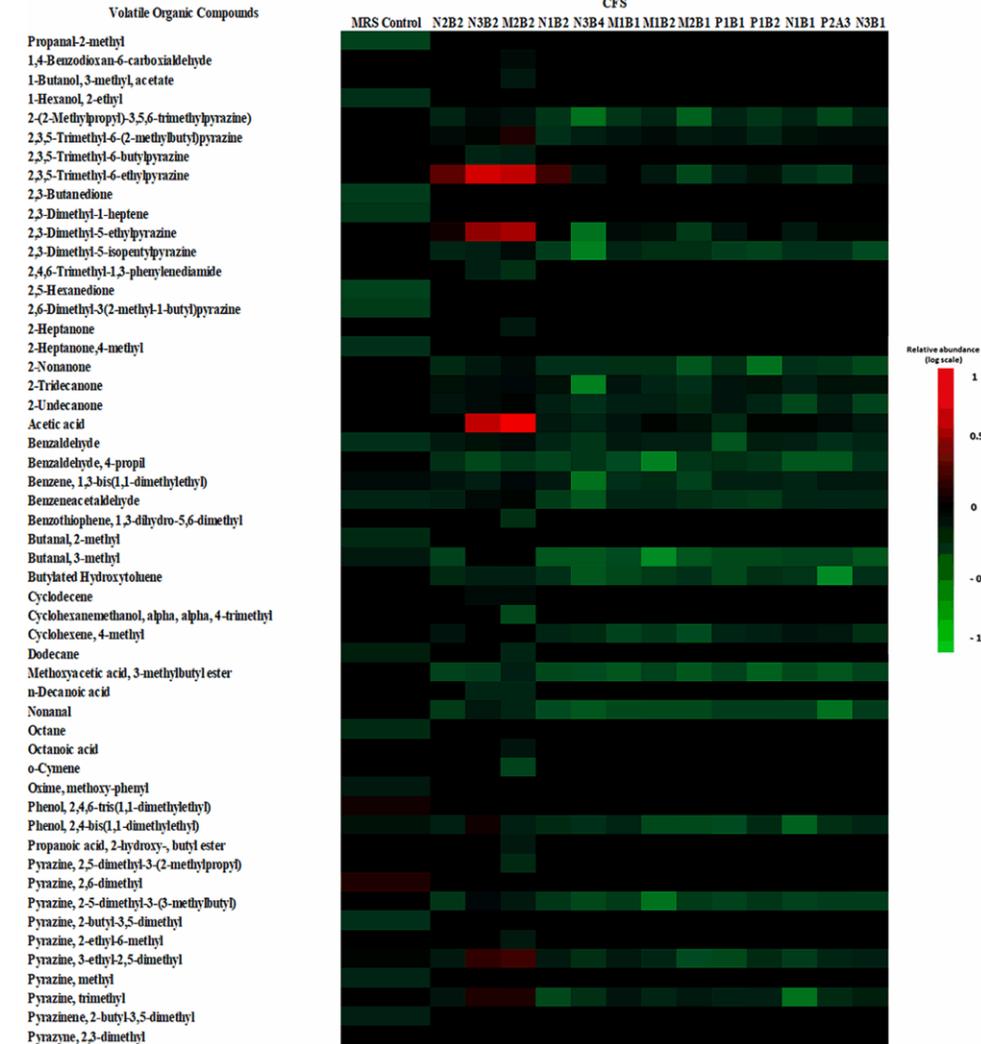# Chemical profile of Bioact-LM

Main metabolite classes detected in fermented lemon medium

- **Major**: lactic acid, acetic acid.

- **Minor**: phenolic acids (incl. salicylic acid), phenyllactic acid.

- **VOCs**: volatile mixture (second mode of action).

- M2B2 showed higher lactic acid; N3B2 showed higher DL-3-phenyllactic acid.

Table 7

Quantification of phenolic compounds in mg/L (DL-3-Phenyllactic acid, 3-4-Dihydroxyhydrocinnamic acid, salycilic acid, vanillic acid) and organic compounds in g/L (lactic and acetic acid) produced in LM formulations. Data are means ± SD. Letters indicate statistically significant differences as determined using one-way ANOVA Tukey HSD post hoc test (p < 0.01). ND, not detected.

Phenolic and organic compounds	Lemon peel medium (LM)		
	Control	N3B2	M2B2
DL-3-phenyllactic acid	ND [c]	24.51 ± 0.93 [a]	19.44 ± 0.74 [b]
3-4-dihydroxyhydrocinnamic	ND [b]	12.01 ± 0.46 [a]	11.76 ± 0.65 [a]
Salycilic acid	ND [b]	0.84 ± 0.24 [a]	0.73 ± 0.21 [a]
Vanillic acid	ND [b]	0.07 ± 0.02 [a]	0.05 ± 0.02 [a]
Lactic acid	ND [c]	13.52 ± 0.38 [b]	15.64 ± 0.41 [a]
Acetic acid	ND [b]	1.45 ± 0.21 [a]	1.61 ± 0.16 [a]

Interpretation: multi-compound, multi-target activity rather than a single active ingredient.

References: Riolo et al., Biological Control (2024); La Spada et al., Journal of Agriculture and Food Research (2024).

Blue mold control on oranges (*Penicillium italicum*)

In vivo test (orange fruit)

- Treatments: control vs fermented LM with N3B2 or M2B2.
- Outcome: reduced disease severity over time and reduced conidia production.

Interpretation for the value chain

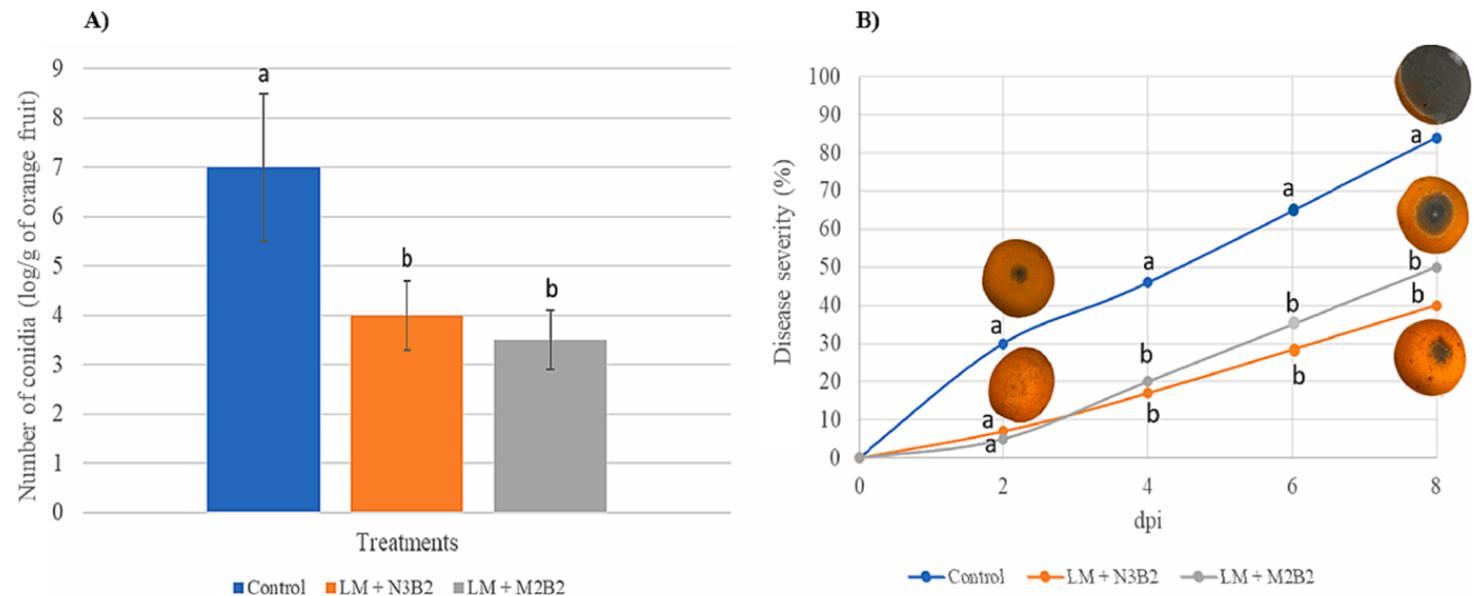- Lower sporulation may reduce cross-contamination in packing and storage.

Fig. 5. Efficacy of treatments with LM-based formulations fermented with two diverse *La. plantarum* isolates in reducing the severity of blue mold incited by *P. italicum* and pathogen sporulation on artificially inoculated sweet orange fruit. A. Sporulation of *P. italicum* after 7 days of incubation at $16 \pm 2\,°C$, expressed as number of conidia produced in infected wounds per gram of plant matrix ($\log g^{-1}$) (means $\pm$ SE) (left). Different letters indicate statistical significance according to Fisher's protected LSD test ($p < 0.05$). B. Disease severity (mean % of fruit surface affected by rot) on orange fruit at various time intervals after inoculation (dpi). Different letters indicate statistical significance according to Fisher's protected LSD test ($p < 0.05$) (right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

References: Riolo et al., Biological Control (2024)

Brown rot (*Phytophthora* spp.): contact activity

In vitro MIC/MFC of Bioact-LM

- Test range: from 0.6 to 300 mg/mL (microplate assay).

- Range: MIC 18.75–150 mg/mL; MFC 37.5–300 mg/mL.

- Species-dependent response (e.g., MIC 75–150 mg/mL;

  MFC up to 150–300 mg/mL depending on isolate).

Table 2
Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the bioactive Lemon Medium (LM) -based formulation against diverse *Phytophthora* species.

Phytophthora isolates	Bioactive LM-based formulation (mg/mL ± ME[a])	
	MIC	MFC
Phytophthora. × *cambivora* MSQ2C	18.75 ± 0.1	37.50 ± 0.1
P. cinnamomi CECT 2965	150.00 ± 0.1	150.00 ± 0.1
P. citricola CECT 20567	150.00 ± 0.1	150.00 ± 0.1
P. citrophthora IMI 396200	150.00 ± 0.1	300.00 ± 0.1
P. multivora CycC2	150.00 ± 0.1	300.00 ± 0.1
P. nicotianae T3-B-K1A	75.00 ± 0.1	150.00 ± 0.1
P. oleae V2–K10A	75.00 ± 0.1	150.00 ± 0.1
P. palmivora CP2B	75.00 ± 0.1	150.00 ± 0.1
P. parvispora I-1Q1A	150.00 ± 0.1	300.00 ± 0.1
P. pseudosyringae CBS 111775	18.75 ± 0.1	37.50 ± 0.1
P. ramorum CECT 20519	37.50 ± 0.1	37.50 ± 0.1

[a] Measurement Error (ME).

Why relevant for postharvest
- Contact activity supports applications such as dips, sprays, or coatings.
- Target pathogens include citrus brown rot agents (*P. nicotianae*, *P. citrophthora*, *P. palmivora*, etc.).

Brown rot (*Phytophthora* spp.): VOC-mediated inhibition

- Bioact-LM releases volatile organic compounds (VOCs) that inhibit mycelial growth in a sealed-plate headspace assay (no direct contact).
- Species-dependent inhibition (e.g. *P. pseudosyringae* (100%) and a marked effect for *P. ramorum)*
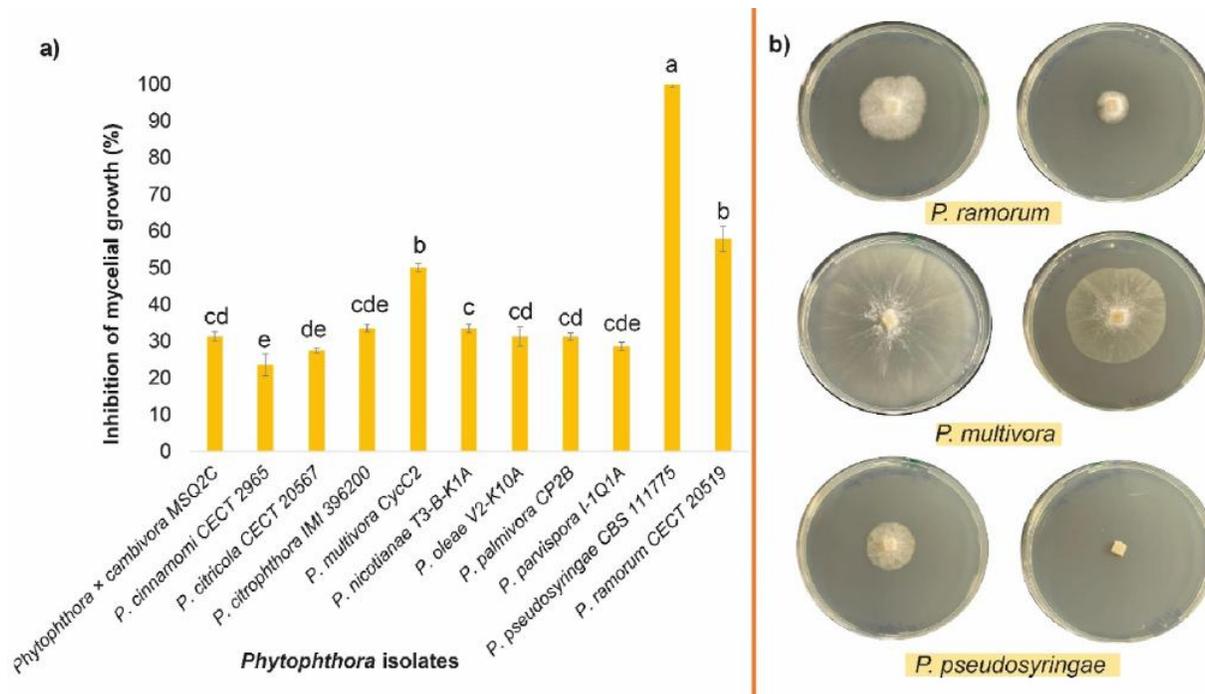- Practical meaning: may contribute under storage conditions.

Fig. 2. *In vitro* inhibitory activity on *Phytophthora* species of volatile organic compounds (VOCs) released by bioactive LM-based formulation. a) Inhibition of mycelial growth (%) of *Phytophthora* strains; values sharing the same letters are statistically similar according to one-way ANOVA Tukey HSD post hoc test ($p < 0.05$); bars represent standard deviation. b) In plate growth inhibition determined by VOCs (on the left) or sterile distilled water (on the right) on *P. ramorum* CECT 20519 and *P. multivora* CycC2, *P. pseudosyringae* CBS 111775.
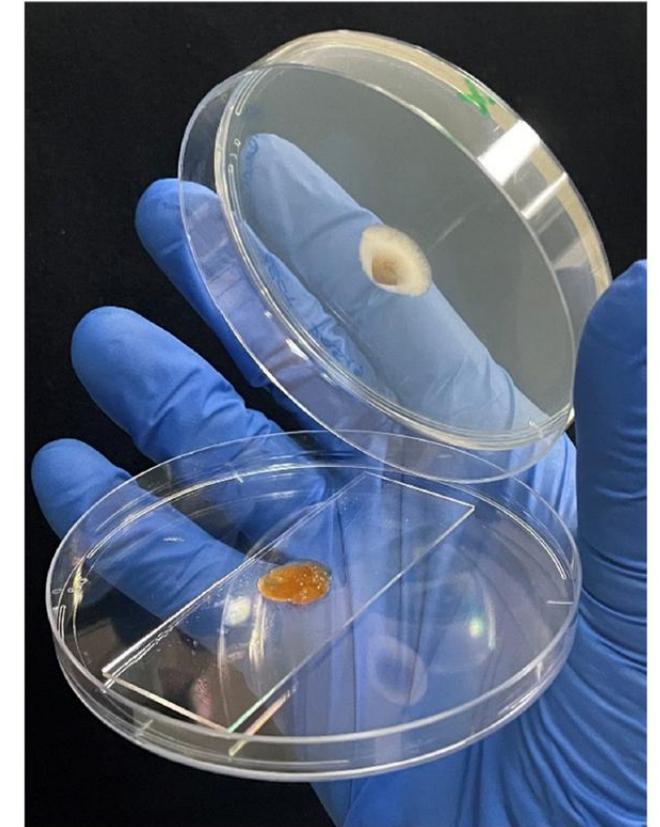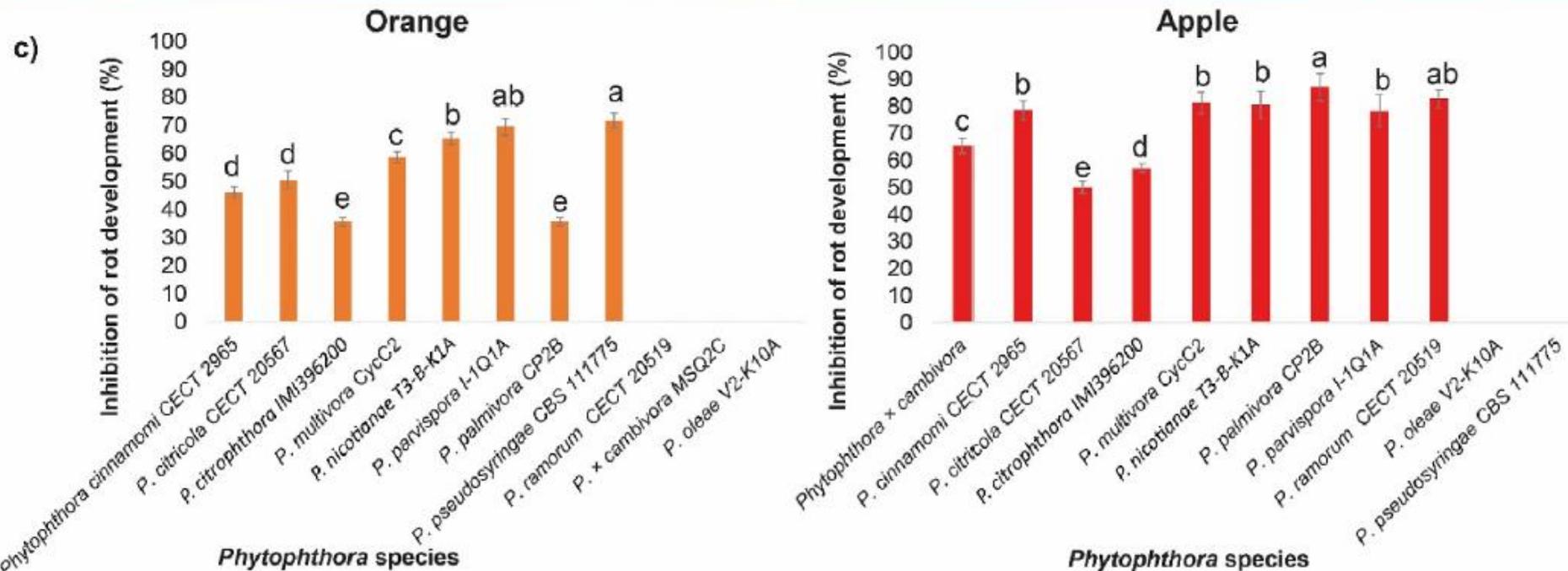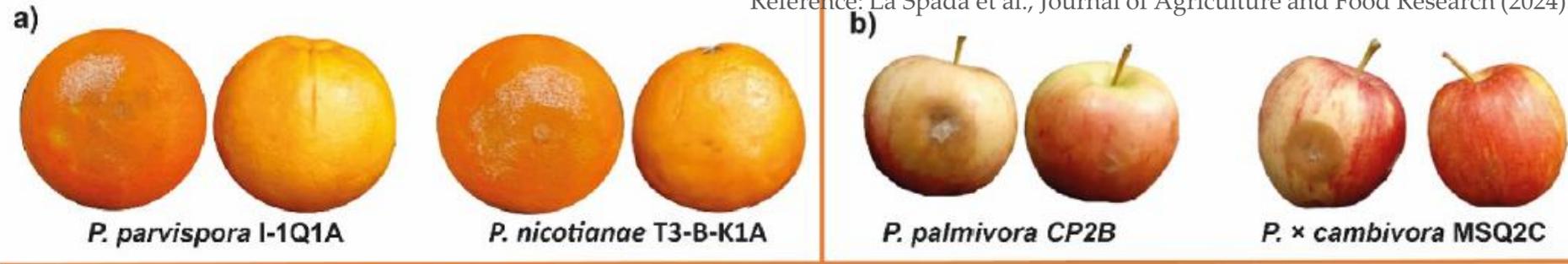

Fig. 1. Assay of the *in vitro* test for evaluating the efficacy of volatile organic compounds (VOCs) released by the bioactive Lemon Medium-based formulation in limiting the mycelial growth of *Phytophthora* species.

Reference: La Spada et al., Journal of Agriculture and Food Research (2024).

# *In vivo* brown rot control (orange and apple)

- High: 65–70%;
- Moderate: 50–60%;
- Lower: ≤35%.
- Species-dependent efficacy.

Fig. 3. Effectiveness of the bioactive LM-based formulation in preventing Phytophthora rot on orange and apple fruit, 7 days post inoculation with different *Phytophthora* species. a) orange fruit inoculated with *P. parvispora* I-1Q1A and *P. nicotianae* T3-B-K1A treated with the bioactive LM-based formulation (fruit on the left) or sterile distilled water (fruit on the right); b) apple fruit inoculated with *P. palmivora* CP2B and *Phytophtora × cambivora* MSQ2C treated with the bioactive LM-based formulation (fruit on the left) or sterile distilled water (fruit on the right); c) *In vivo* Inhibition of rot development (%) on orange (on the right) and apple (on the left) fruits; values sharing the same letters are statistically similar according to one-way ANOVA Tukey HSD post hoc test ($p < 0.05$); bars represent standard deviation.

Limitations and next steps

Limitations

- Packinghouse protocol not optimised.

- Efficacy depends on conditions (cultivar, wounds, storage).

- Batch-to-batch variability (fermentation and peel batches).
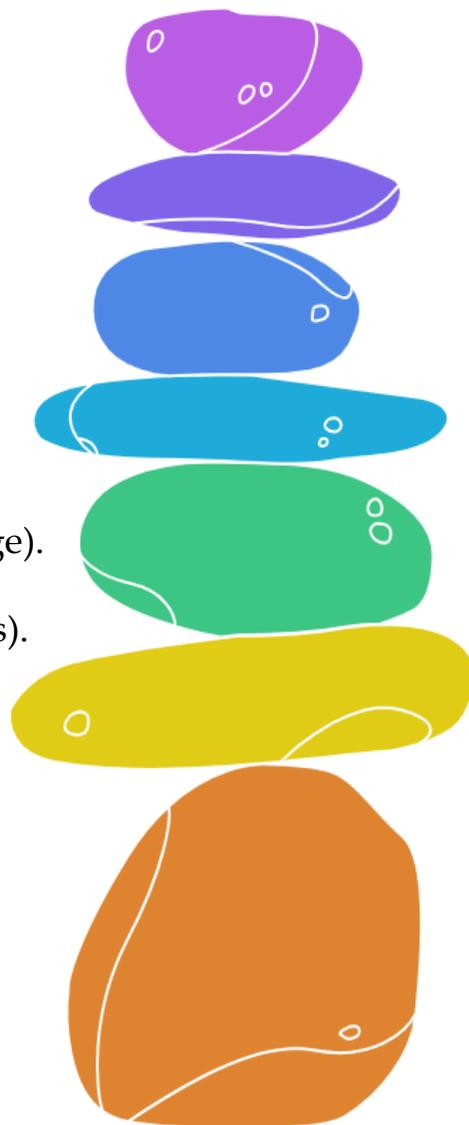
Next steps

- Define dose and contact time (dip/spray).

- Stability + compatibility (coatings/waxes, sensory).

- Semi-commercial validation + regulatory pathway.

Where Bioact-LM can fit in the citrus postharvest chain

- **Packinghouse**: dip or spray after washing/sanitising.

- **Cold storage:** support disease suppression; reduce cross-contamination risk.

- **Formulation route**: liquid treatment or ingredient for biodegradable coatings.

Peer-reviewed publications

Biological Control 189 (2024) 105443

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

A circular economy approach: A new formulation based on a lemon peel medium activated with lactobacilli for sustainable control of post-harvest fungal rots in fresh citrus fruit

Mario Riolo[a,b,*], Ana Moreno Villena[b], Jorge Calpe[b], Carlos Luz[b], Giuseppe Meca[b,*], Nunzio Tuccitto[c], Santa Olga Cacciola[a]

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HIGHLIGHTS

- LABs isolated from citrus fruit peel showed broad spectrum antifungal activity.
- Development of a lemon peel powder fermented by LAB from citrus.
- Effective activity of bioproduct to prevent blue mould of citrus fruits.
- Organic and phenolic acids were the prevalent active metabolites in the bioproduct.

Riolo et al., 2024 — Biological Control

Journal of Agriculture and Food Research 17 (2024) 101227

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Exploring eco-friendly solutions for Phytophthora disease management: Harnessing the anti-oomycete potential of a fermented lemon waste formulation

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La Spada et al., 2024 — Journal of Agriculture and Food Research

Take-home messages

- Bioact-LM is obtained by fermenting lemon peel by-products with *L. plantarum* strains from citrus peel.

- Bioact-LM reduced blue mold (*P. italicum*) and brown rot (*Phytophthora* spp.) in fruit assays.

- Two complementary effects were observed: contact inhibition and VOC-mediated inhibition.

- This is aligned with FoodWaStop themes: loss reduction and citrus by-product valorisation.

Related publication linked to FoodWaStop

Antifungal activity and sensory analyses of chitosan aromatized with essential oils for the protection of fresh oranges and apples

Mario Riolo [a], Federico La Spada [a,*] , Priscilla Farina [b], Soumia El Boumlasy [a,c,d],
Roberta Ascrizzi [e], Francesca Venturi [b,f], Isabella Taglieri [b,f], Nunzio Tuccitto [g,h],
Antonella Pane [a], Samuel Álvarez-García [i,j], Barbara Conti [b,k,**], Gianfranco Romanazzi [i,*],
Santa Olga Cacciola [a]

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Thank you