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## Role of competition exclusion for trace elements in the antifungal activity of *L. plantarum* L244 and *L. rhamnosus* CIRM-BIA 1759

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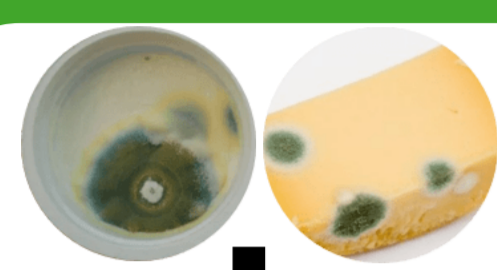
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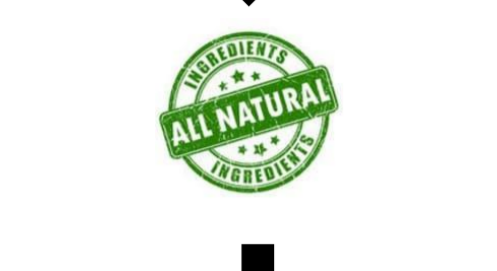
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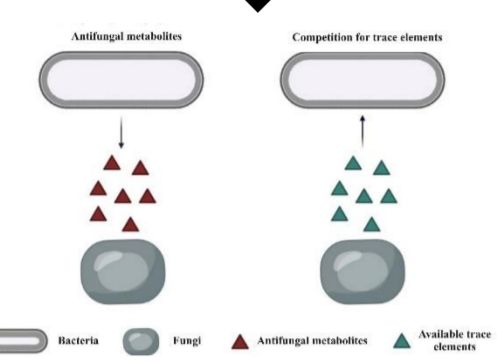
## Context and objectives



Dairy products are susceptible to acid-tolerant spoilage fungi, causing food waste and economic losses.



Antifungal lactic acid bacteria (LAB) cultures are of growing interest as a biopreservation method.



Until recently, production of antifungal compounds and pH decrease were considered as the main factors contributing to LAB antifungal activity in dairy foods but competition-exclusion for manganese (Siedler *et al.*, 2020) was shown to play an important role in their antifungal activity.

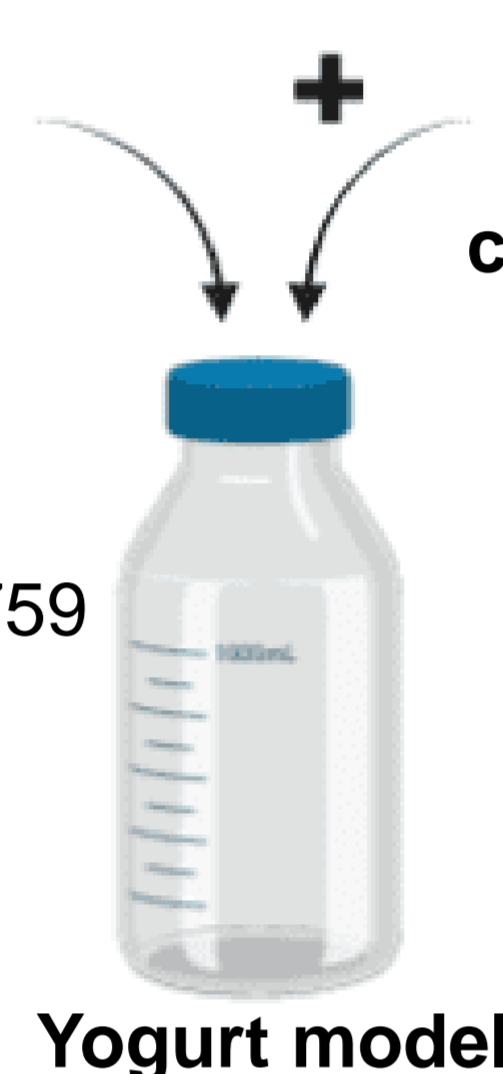


To investigate the competition-exclusion mechanism in two previously characterized antifungal LAB strains, namely *Lactiplantibacillus plantarum* L244 and *Lactiseibacillus rhamnosus* CIRM-BIA 1759, using a yogurt model (Leyva Salas *et al.*, 2018).

## Experimental strategy

### 1 – Effect of trace element addition before fermentation on L244 and CIRM-BIA 1759 antifungal activity

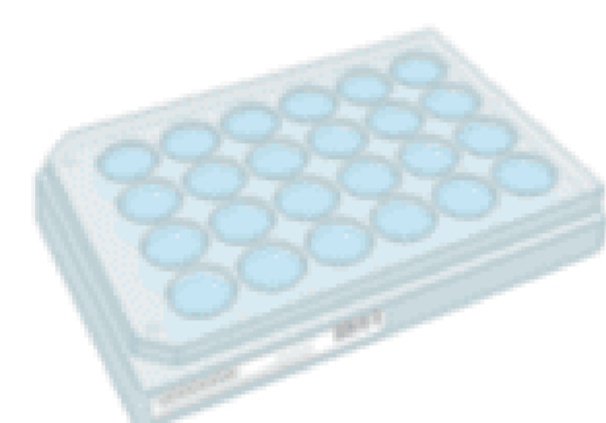
- Skimmed milk
- Skimmed milk powder
- Yogurt starters
- LAB strains L244 and CIRM-BIA 1759 at 10<sup>7</sup> UFC/ml



+

**Addition of single trace elements at 10 times the concentration encountered in yogurt \***

Fermentation  
4 - 6 h at 42°C  
pH 5.0



**Antifungal assay**  
(visual evaluation, time to visible growth)

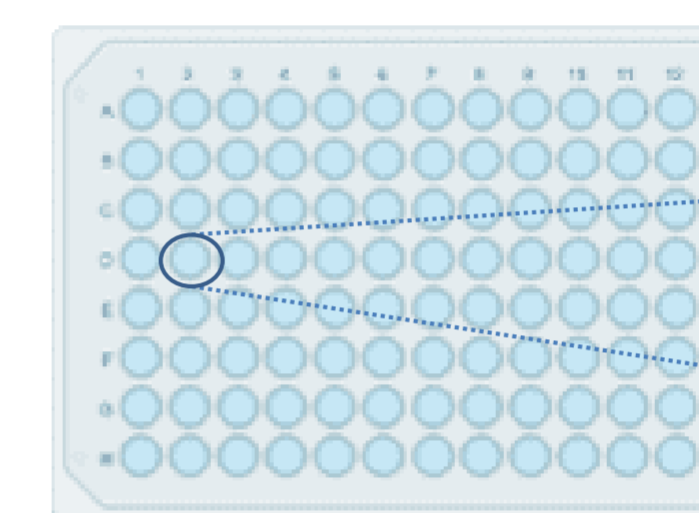
Contamination of the yogurt surface (50 spores or cells) :

- Mucor racemosus*
- Penicillium bifforme*
- Galactomyces geotrichum*
- Yarrowia lipolytica*

### 2 – Scavenging of trace elements in yogurt by antifungal LAB

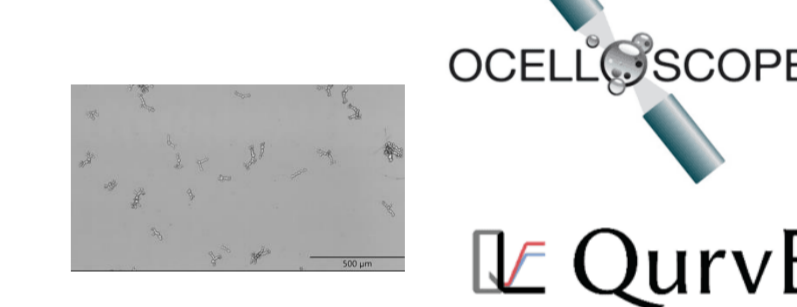
Quantification of trace elements by ICP-MS in lactoserum obtained from control yogurts and yogurt made with antifungal LAB after storage for 1 week (10 replicates per condition).

### 3 – Growth kinetics of fungal targets in lactoserum



Cell-free lactoserum from yogurts prepared +/- antifungal LAB +/- 10X Mn added after fermentation + 10<sup>3</sup> spores or cells/ml fungi

- Growth measurement for 7 d at 10°C using oCelloScope

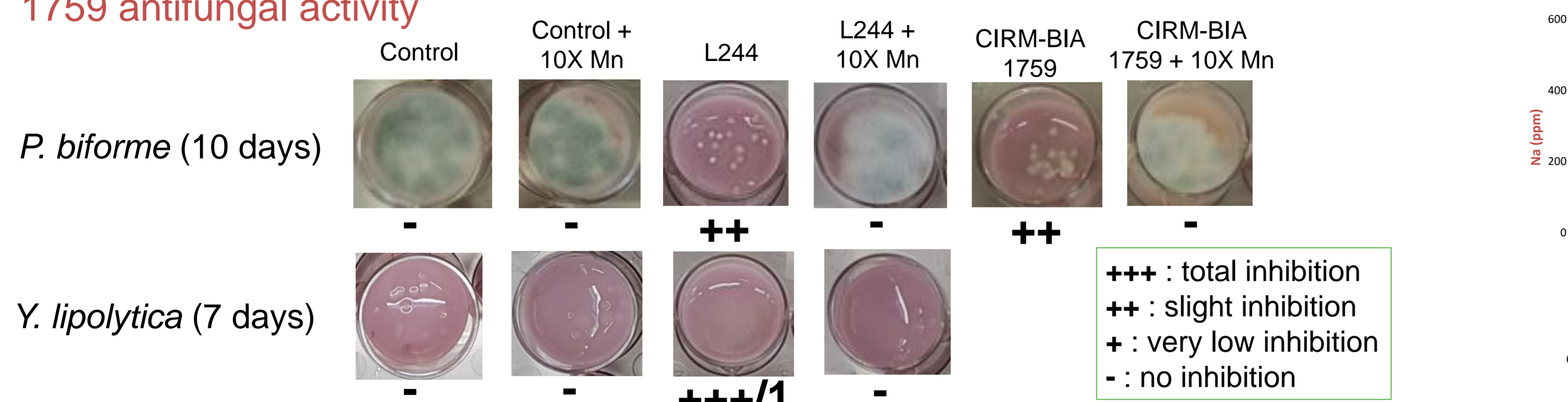


- Modelization of growth parameters using QuivE

\* 6 metals : cobalt, copper, iron, manganese, nickel and zinc  
12 vitamins : vitamins B1, B2, B3, B5, B6, B8, B9, B12, C, D, E and K1

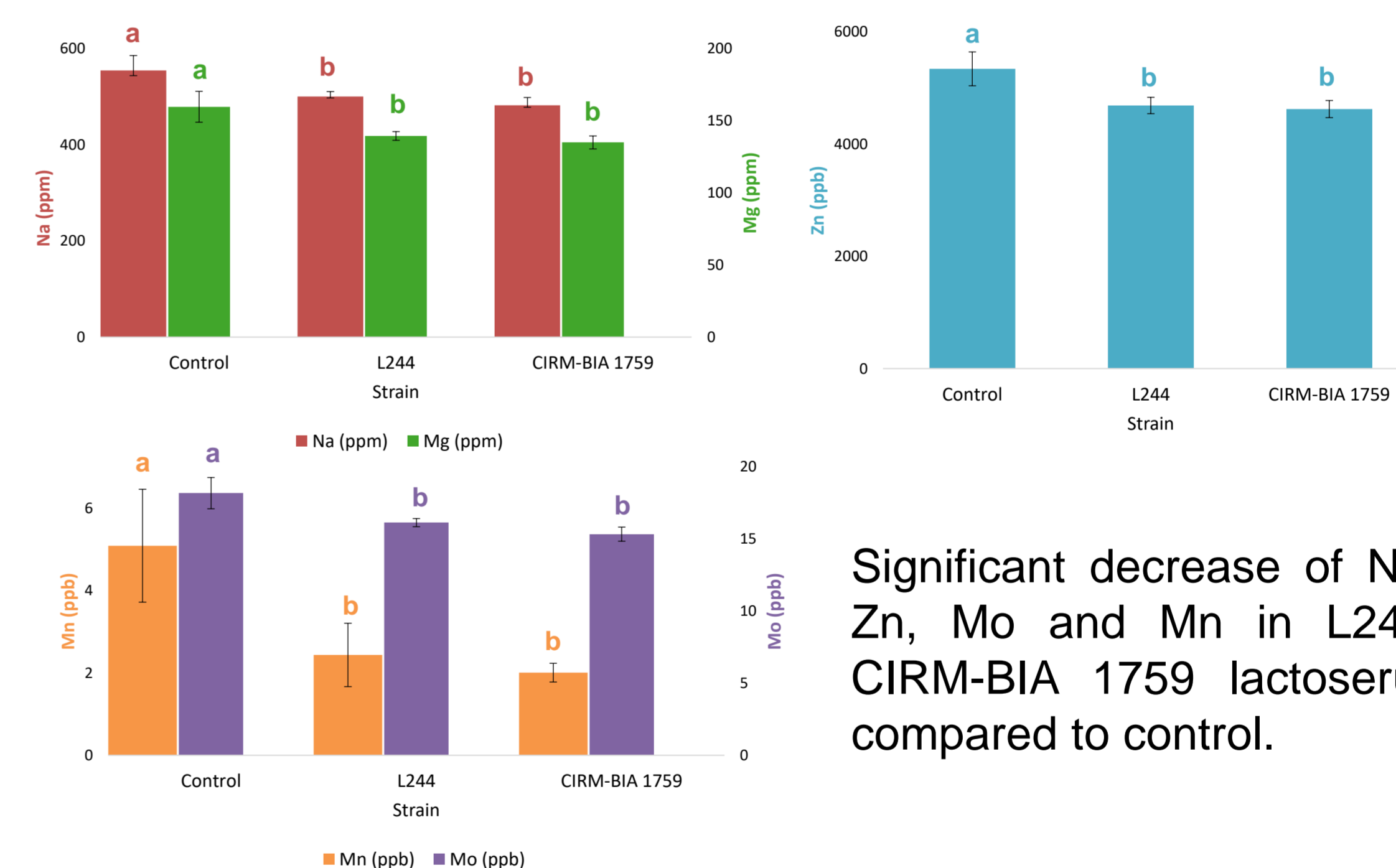
## Results

### 1 – Effect of trace element addition before fermentation on L244 and CIRM-BIA 1759 antifungal activity



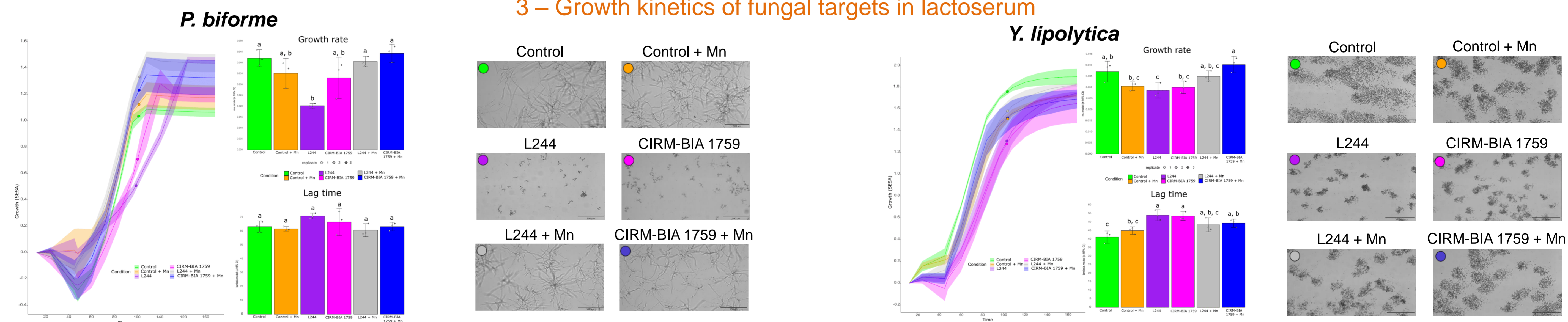
- Suppression of growth inhibition by L244 and CIRM-BIA 1759 strains after the addition of Mn at 10X against *P. bifforme* and *Y. lipolytica*.
- No observed antifungal activity against *G. geotrichum* and *M. racemosus* (L244 and CIRM-BIA 1759), and *Y. lipolytica* (CIRM-BIA 1759).
- No effect after addition of other trace elements on L244 and CIRM-BIA 1759 antifungal activity.

### 2 – Scavenging of trace element in yogurt by antifungal LAB



Significant decrease of Na, Mg, Zn, Mo and Mn in L244 and CIRM-BIA 1759 lactoserum as compared to control.

### 3 – Growth kinetics of fungal targets in lactoserum



- Mn addition in antifungal lactoserum restored growth (eg, lag time and/or growth rate) of fungal targets.

## Conclusions

- Suppression of *P. bifforme* and/or *Y. lipolytica* growth inhibition by L244 and CIRM-BIA 1759 was observed in yogurt after the addition of 10X Mn but not of other trace metals or vitamins. L244 and CIRM-BIA 1759 did not show any antifungal activity against the 2 other fungal targets, *G. geotrichum* and *M. racemosus*.
- Increased Mn scavenging but also other trace elements was shown using ICP-MS in the presence of antifungal strains. Among these, Mn was the most limiting one (< 3 ppb), confirming the first results.
- Growth rates of *P. bifforme* and *Y. lipolytica* decreased in L244 and CIRM-BIA 1759 lactoserum, but were equivalent to controls levels after the addition of Mn. In addition, lag times increased in L244 and CIRM-BIA 1759 lactoserum and were equivalent to controls after the addition of Mn.
- Competition-exclusion for manganese is a major mechanism in L244 and CIRM-BIA 1759 antifungal activity against *P. bifforme* and *Y. lipolytica* but Mn scavenging has no effect against *M. racemosus* and *G. geotrichum*.**

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#### References :

- Siedler *et al.*, 2020 <https://doi.org/10.1128/AEM.02312-19>
- Leyva Salas *et al.*, 2018 <https://doi.org/10.3389/fmicb.2018.01787>