

## BACKGROUND

- Bacterial vaginosis (BV), a risk factor for preterm birth, is a common female genital tract infection characterised by significant growth of anaerobic bacteria such as *Mobiluncus* spp., and depletion of *Lactobacillus* spp.<sup>1</sup>
- However, *L. iners* is sometimes linked with transition from normal vaginal microbiota (VMB) to BV.<sup>1</sup>

## OBJECTIVES

- We investigated and compared the metabolic profiles of *Mobiluncus curtisii* and *L. iners* cultured in the same media to determine the possible physicochemical mechanisms by which they promote abnormal VMB (dysbiosis) that leads to BV.

## METHODS

- L. iners* (ATCC 55195) and *M. curtisii* (ATCC 35241) in 100 µl suspension were cultured separately in 800 µl tryptic soy broth (TSB, supplemented with 5% defibrinated sheep blood) and 100 µl of 100 mM <sup>13</sup>C<sub>u</sub>-glucose (Fig. 1).
- Bacterial growth curves were determined (Fig. 2).
- Control samples - bacterial species in media without <sup>13</sup>C-substrate; media and <sup>13</sup>C-substrate only (no bacteria), and media with phosphate buffered saline only were also incubated (Fig. 1).
- After incubation in anaerobic conditions at 37° C for 24 hours, the media from both species and control samples were analysed by <sup>13</sup>C-nuclear magnetic resonance spectroscopy (<sup>13</sup>C-NMR) at 9.4T to detect metabolites produced by bacterial metabolism (Fig. 3).



Figure 1. Bacterial species incubated in Whitley DG250 anaerobic workstation. -ve control – no bacteria.

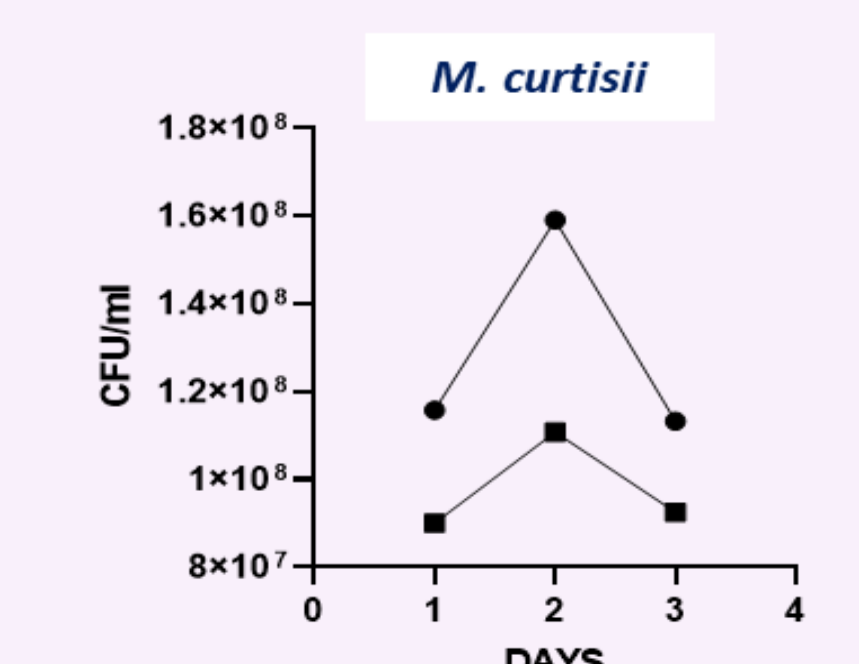
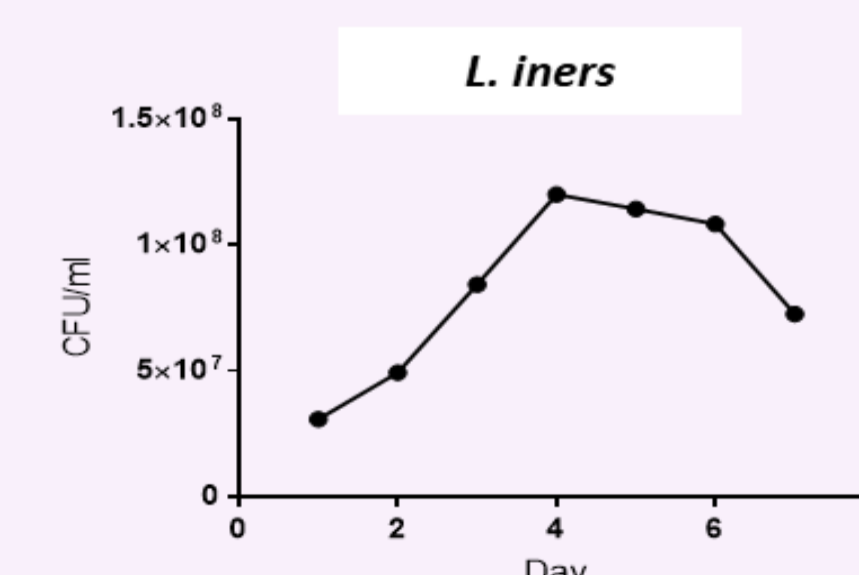


Figure 2. Bacterial growth curves. CFU, colony forming unit; TSB, tryptic soy broth; BHI, brain heart infusion broth

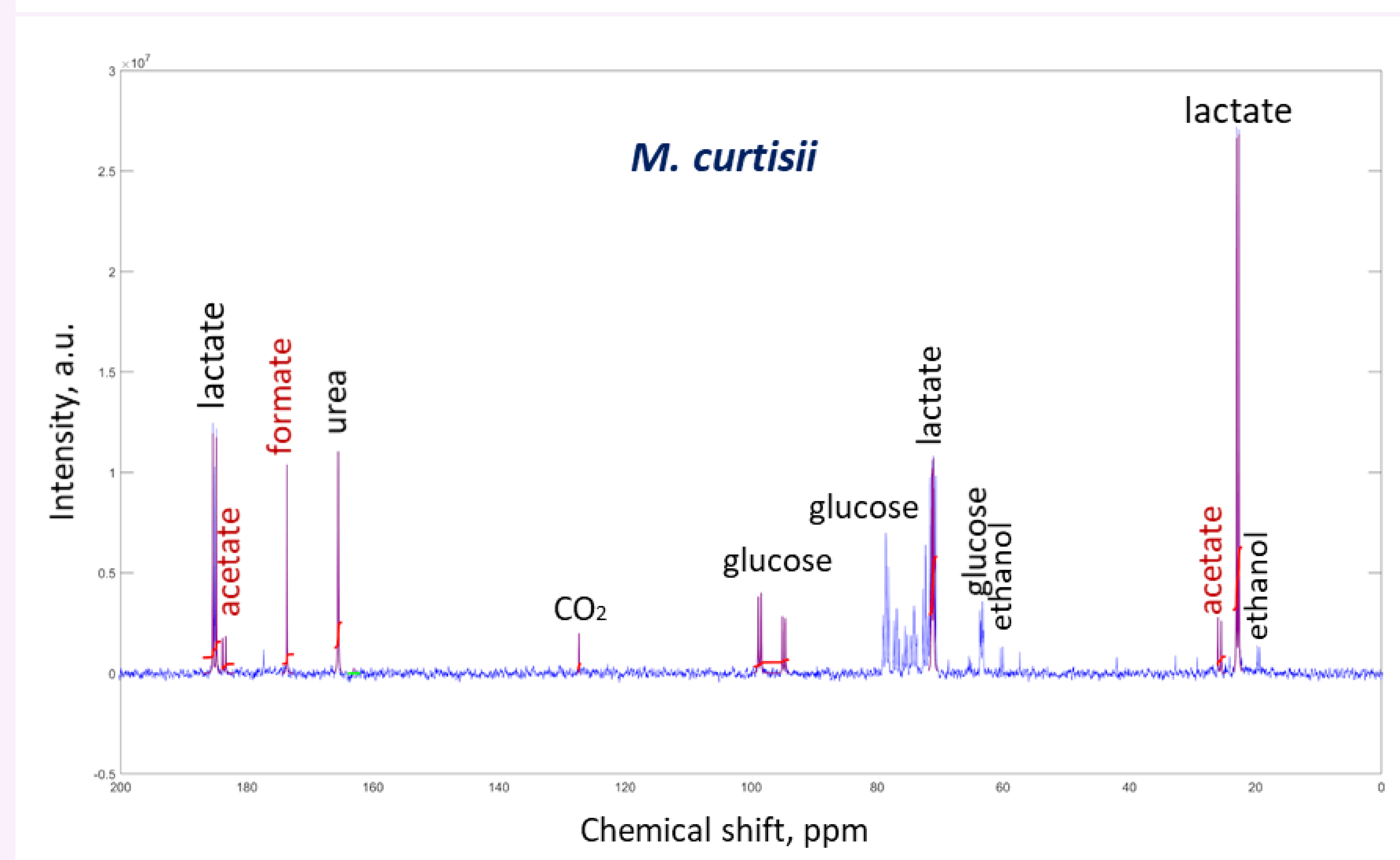
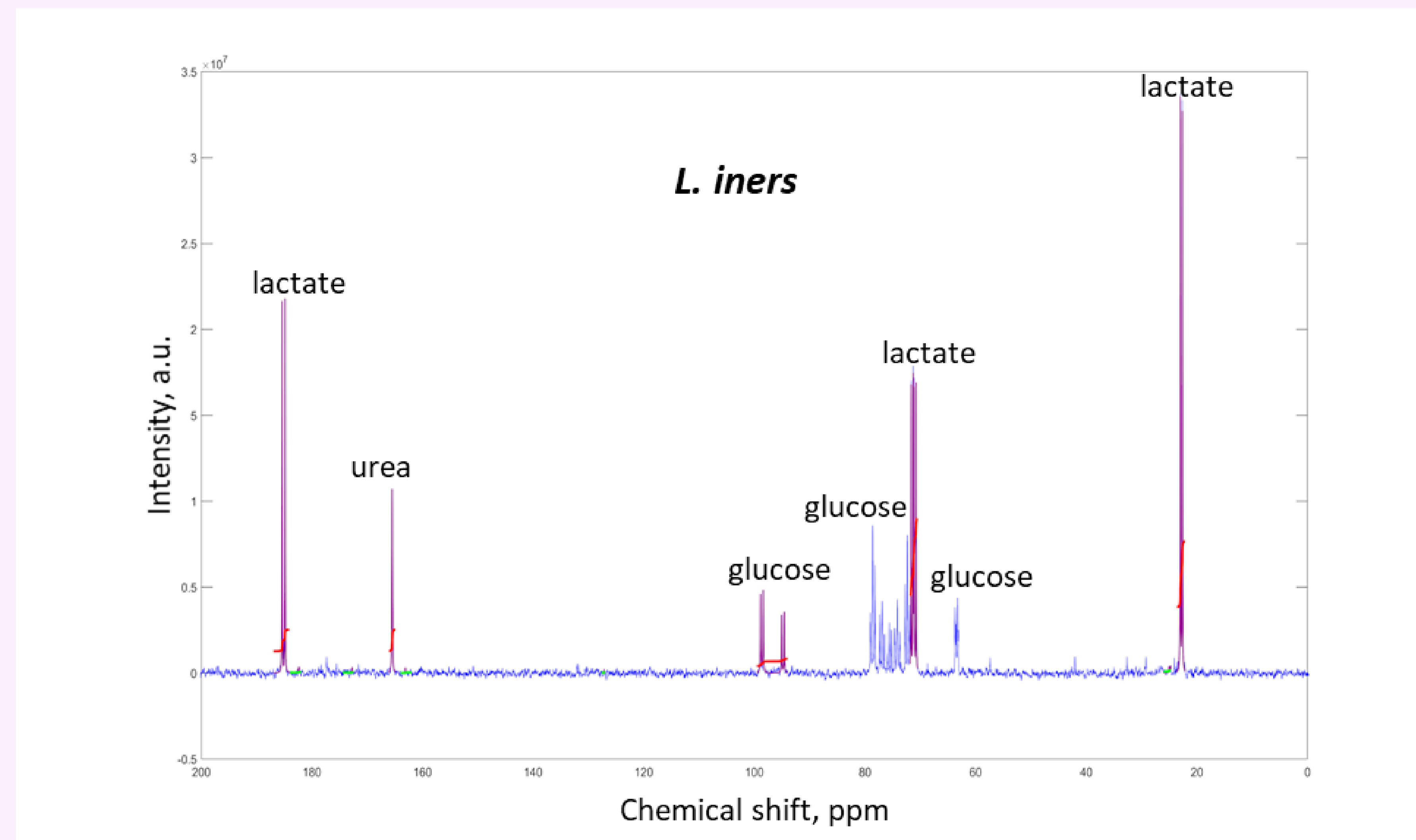


Figure 3. <sup>13</sup>C-NMR spectra of metabolites produced by *Lactobacillus iners* (top) and *Mobiluncus curtisii* (bottom) after anaerobic incubation in tryptic soy broth (TSB) supplemented with 5% defibrinated sheep blood and 100 µl of 100 mM <sup>13</sup>C<sub>u</sub>-glucose for 24 hours at 37° C. Bacterial vaginosis- and preterm birth-associated metabolites are highlighted in red. <sup>13</sup>C-urea added as a chemical shift and concentration reference.

## RESULTS

- At the peak of the exponential growth phase, the concentrations of both bacterial species did not differ significantly (*L. iners*:  $1.73 \pm 0.32 \times 10^8$  CFU/ml, n=4 vs *M. curtisii*:  $1.42 \pm 0.241 \times 10^8$  CFU/ml, n=2,  $p = 0.27$ ).
- M. curtisii* produced acetate, formate, carbon dioxide and ethanol that were either absent or negligible in the *L. iners* media (Fig. 3).
- Both bacterial species produced lactate, which was confirmed by enzyme-based spectrophotometry to be L-lactate, and ~3-fold higher in *L. iners* compared to *M. curtisii* media (Fig. 4).

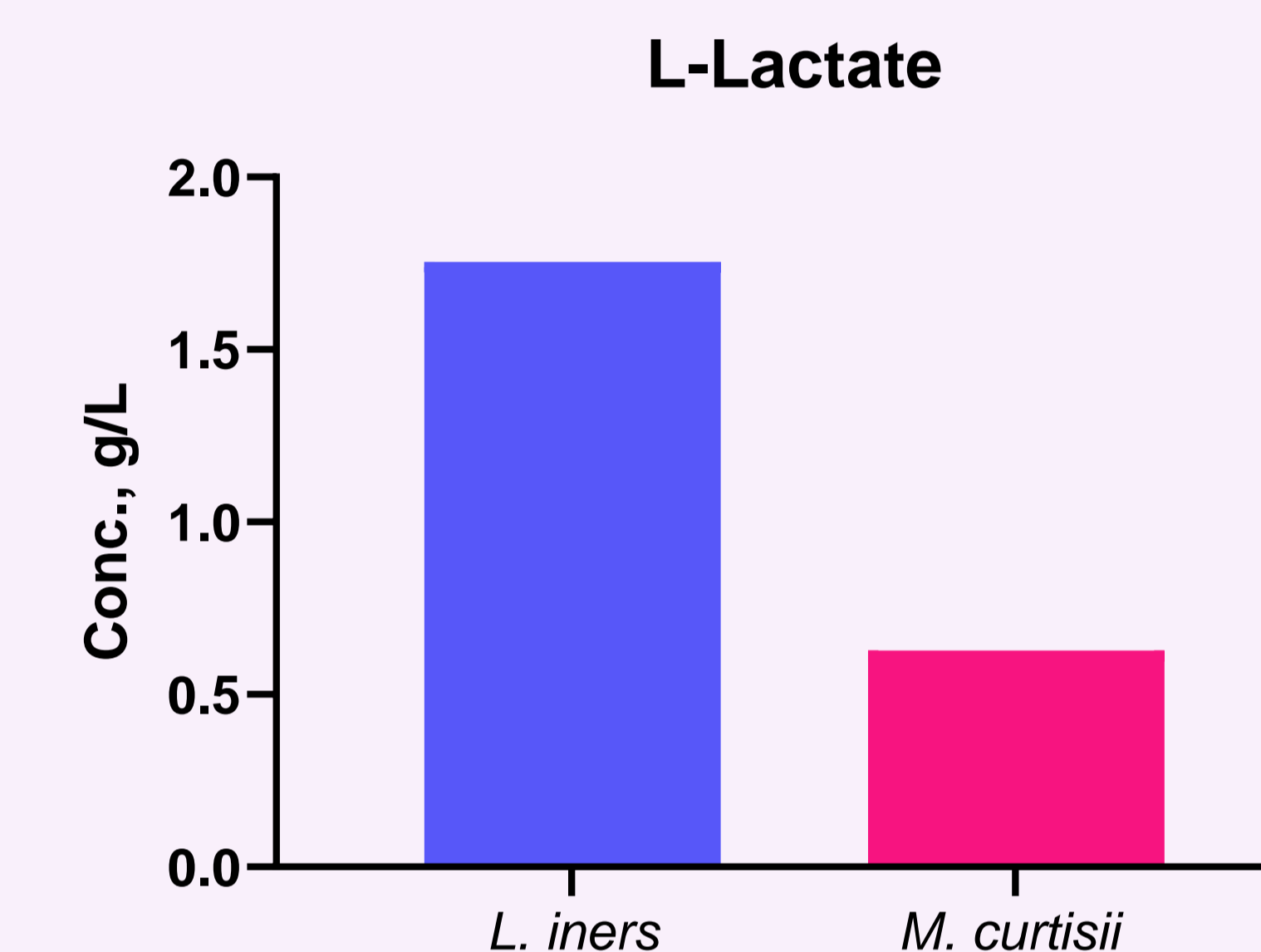


Figure 4. L-lactate produced by bacterial species after incubation in tryptic soy broth supplemented with 5% sheep blood and 100 mM <sup>13</sup>C<sub>u</sub>-glucose for 24 hours at 37° C (n=1). No significant difference in bacterial concentration.

## CONCLUSION

- These findings show that *M. curtisii* can be cultured in TSB. It could contribute to the propagation of BV through the production of metabolites, plausibly by increasing vaginal pH and inhibiting neutrophil chemotaxis.
- Some of these metabolites such as acetate are predictive of spontaneous preterm birth especially in symptomatic pregnant women.<sup>2</sup>
- Conversely, *L. iners* does not produce significant amounts of BV-associated metabolites, instead may promote eubiosis by producing significant amounts of L-lactate.

## REFERENCES

- [1] Amabebe & Anumba. *Font Med* 2018. 5:818.  
 [2] Amabebe et al. *PLoS One* 2019. 14:e0222455