

Microbiological tools to explore LAB–Propionibacteria synergy in view of dairy by-product valorisation

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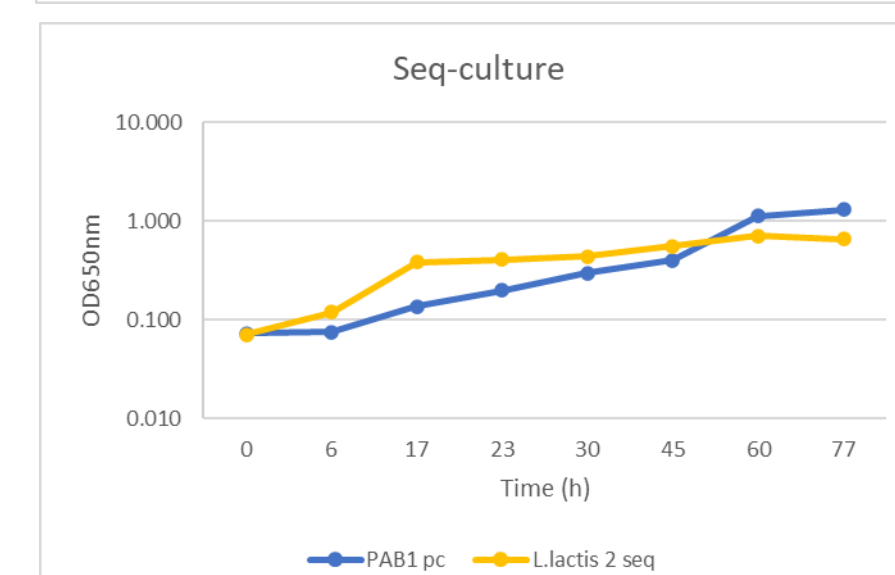
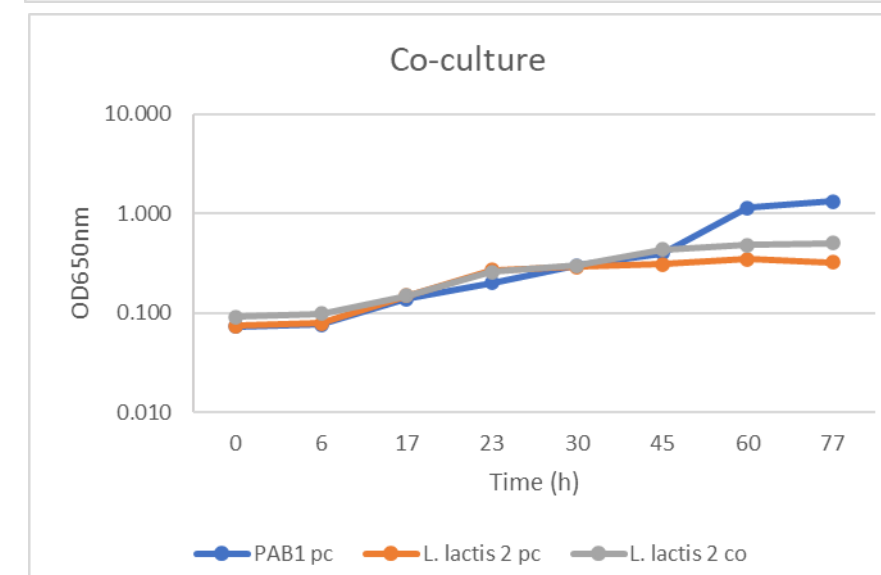
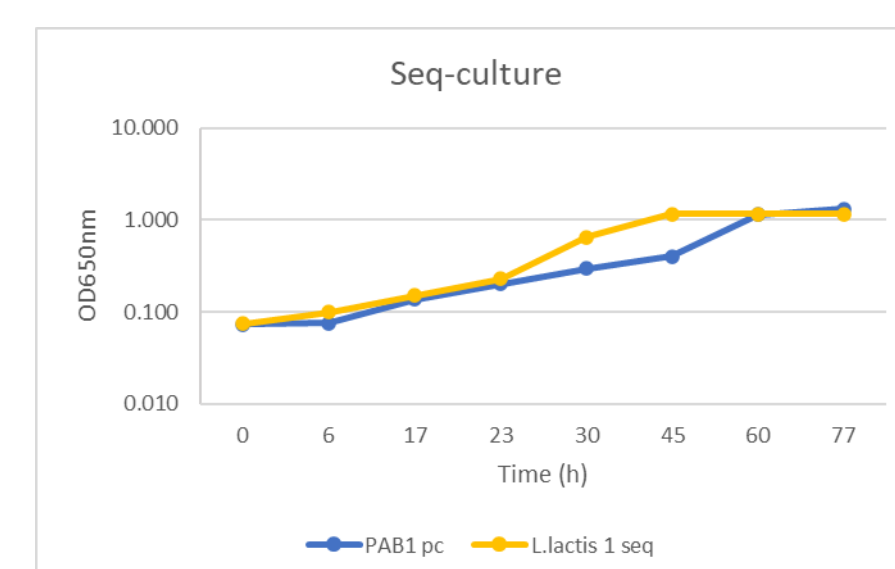
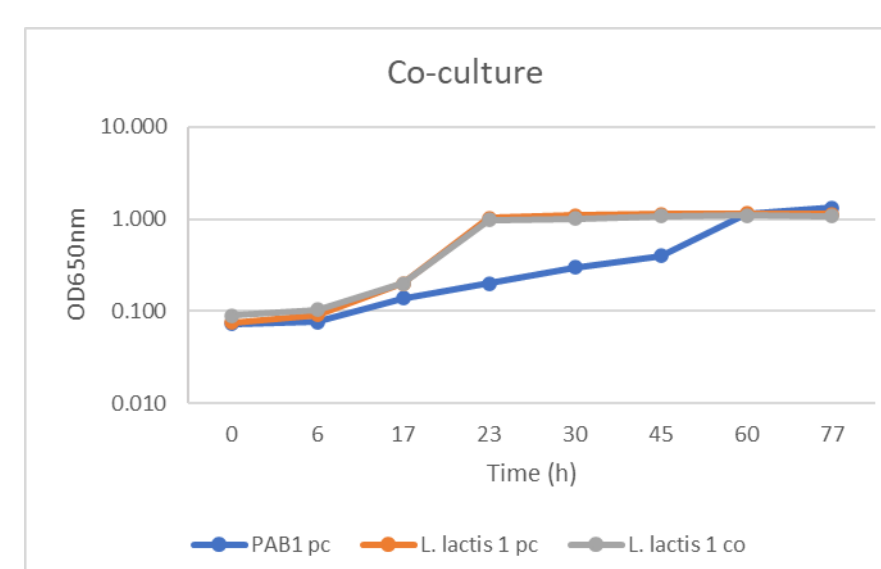
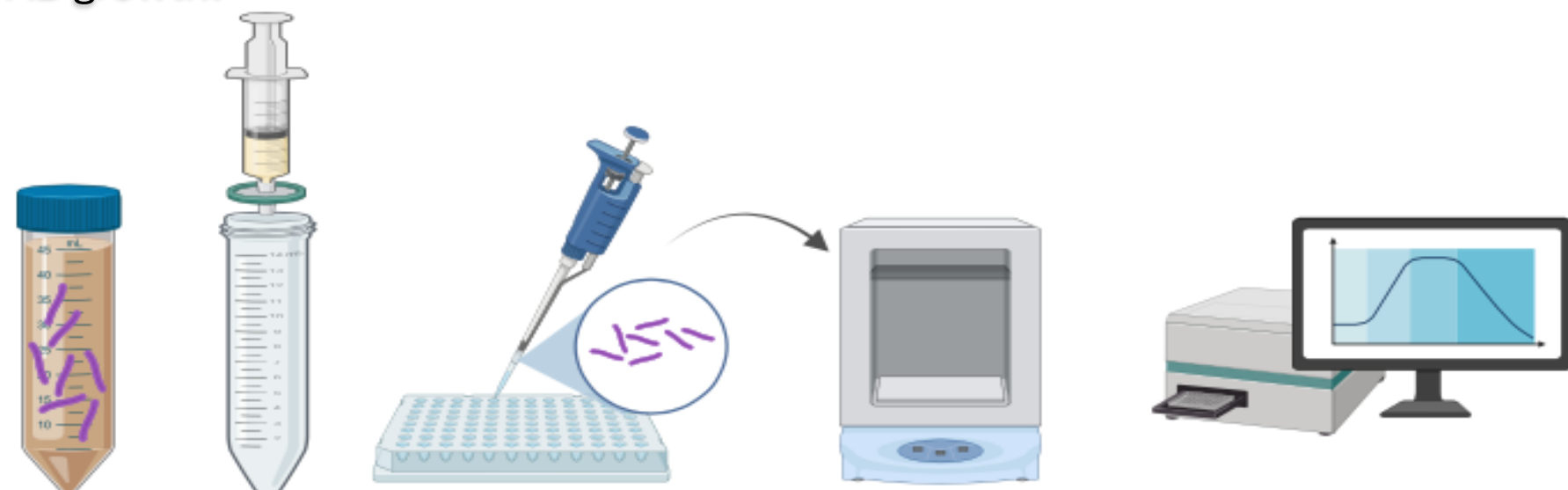
INTRODUCTION & AIM

Lactic acid bacteria (LAB) are known to enhance both growth rate and biomass production of Propionibacteria (PAB) (Piveteau et al., 1995). Accordingly, lactate addition to a lactose-containing medium shortens the lag phase and increases the carrying capacity of selected PAB strains (Coronas et al., 2023). Based on this evidence, and within the framework of a project aiming to improve the fermentative performance of PAB in lactose-rich dairy by-products, this study focused on the development of simple microbiological tool to evaluate LAB/PAB interactions.



Which inoculation strategy enhances PAB growth?

PAB and LAB were either co-inoculated or sequentially inoculated in YELactose. For sequential inoculation LAB were grown in YELactose until the early stationary phase. After cell removal, the supernatant was adjusted to pH 6.0. PAB were then inoculated into the LAB-spent medium, and growth was monitored by OD₆₅₀. While in co-culture, the growth kinetics of the mixed culture overlapped with those of LAB in pure culture, making it difficult to evaluate any effect of LAB on PAB, in sequential culture, LAB clearly reduced the lag phase of PAB. Thus, sequential inoculation was identified as the most suitable inoculation strategy to enhance PAB growth.



Which medium permits differential enumeration of LAB and PAB in mixed culture?

Mixed cultures (Lactococcus lactis + PAB) were inoculated in Second Cheese Whey (SCW), and viable plate counts were performed on MRS + 4% NaCl, YELactose, and YELactate. As reported by Thierry and Gagnaire (2011), LAB grew on MRS + 4% NaCl while PAB did not. On the contrary, YELactate and YELactose supported the growth of both PAB and LAB. However, while on YELactate a clear morphological differentiation between PAB and LAB colonies was observed, on YELactose LAB colonies closely resembled those of PAB, thus hampering their differentiation. Overall, YELactate was identified as the most suitable medium for the selective enumeration of PAB and LAB in mixed cultures.



mixed culture on YELactate



mixed culture on YELactose



mixed culture on MRS+4% NaCl



Does thermal treatment affect SCW fermentability?

Sample	Condition	log CFU/mL (72h)	pH (72h)
SCW A	Heat-treated	9.4±0.06	4.05±0.1
SCW A	Untreated	9.3±0.04	4.38±0.03
SCW B	Heat-treated	10.0±0.03	4.08±0.02
SCW B	Untreated	9.3±0.03	4.22±0.01

No contamination were observed in heat treated SCW while visible in untreated SCW. PAB1 counts on heat treated samples were comparable to that obtained in untreated samples. pH decrease indicates that heat treatment does not affect fermentation. Thus, heat treatment can be applied to suppresses background microbiota with no detrimental effect on SCW fermentability.

Two SCW samples (A and B), differing in composition, were either heat-treated (110 °C, 5 min) or left untreated, and inoculated with the PAB1 strain at 6 log CFU/mL. After 72 h of incubation at 30 °C under anaerobic conditions, viable plate counts were performed on YELactate. The pH was also measured to monitor fermentation performance.



Inoculated with PAB



Not inoculated



Inoculated with PAB



Not inoculated

CONCLUSION

These findings provide practical tools for the design of mixed starter cultures and lay the groundwork for further research on LAB–PAB synergistic interactions, aimed at enhancing the valorization of dairy by-products through fermentation.

REFERENCES

Coronas et al. (2023) Propionibacteria as promising tools for the production of pro-bioactive scotta: A proof-of-concept study. *Frontiers in Microbiology* 14: 1223741.

Piveteau et al. (1995) Interactions between lactic and propionic acid bacteria *Lait*, 75 : 331-343
DOI: <https://doi.org/10.1051/lait:19954-525>

Thierry, A. & Gagnaire, V. (2011) Propionibacterium spp. and cheese ripening. In McSweeney, P. L. H. & Fox, P. F. (Eds.), *Cheese: Chemistry, Physics and Microbiology* (pp. 841–856). Academic Press.