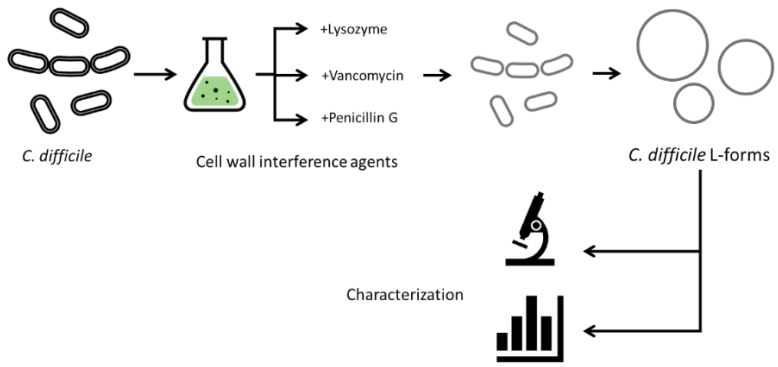
Induction of *Clostridioides difficile* L-form

Anchuleerat Maleehuan [1], Puey Ounjai [1], Phurt Harnvoravongchai [1], Tavan Janvilisri [2], and Surang Chankhamhaengdecha [1]*

1 Department of Biology, Faculty of Science, Mahidol University; anchuleerat.mal@gmail.com (A.M.); puey.oun@mahidol.ac.th (P.O.); phurt.har@mahidol.ac.th (P.H.)
2 Department of Biochemistry, Faculty of Science, Mahidol University; tavan.jan@mahidol.ac.th
* Correspondence: Department of Biology, Faculty of Science, Mahidol University; surang.cha@mahidol.ac.th

Abstract: L-forms are cell wall deficient variants of conventional bacteria that are capable to proliferate. Although L-forms were discovered for several decades, little is known about their general properties, mainly due to the lack of reliable methods for generating such bacteria. This work aims to create and characterize L-forms of *Clostridioides difficile* using cell wall interfering agents, including penicillin G, vancomycin, and lysozyme at various concentrations on osmoprotective brain heart infusion agar. The results revealed that *C. difficile* L-forms could be induced by 100 µg/mL penicillin G and 250 µg/mL vancomycin. The induced L-forms showed distinguished water drop-like colony morphology on agar plate. In addition, the abnormal shape or pleomorphic cells in induced condition was observed for L-forms by phase contrast microscopy. Muramic acid assay showed the significantly decrease of peptidoglycan abundance in L-form under the treatment of penicillin G. This study provides new investigation and characterization of the *C. difficile* L-form, which would be initiate a valuable research tool for gene delivery and biotechnology in *Clostridioides spp.*

Graphical abstract:



Keywords: L-forms; wall-less proliferation; *Clostridioides difficile*

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