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DETERMINING THE ROLE OF CDR20291_0493 SPORULATION INITIATION IN CLOSTRIDIUM DIFFICILE

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Introduction

Clostridium difficile is an anaerobic, gram positive, spore-forming bacteria. In the United States, *Clostridium difficile* is the most frequently reported nosocomial pathogen by Centers for Disease Control and Prevention. *Clostridium difficile* resides in the large intestine and releases two exotoxins (TcdA and TcdB). When high risk patients with weakened immune systems take antibiotics, the microbiome in their guts are disturbed, and the bacteria is able to establish an infection. Symptoms of *C. difficile* infection may vary from a mild diarrhea to a life threatening inflammation of the colon.

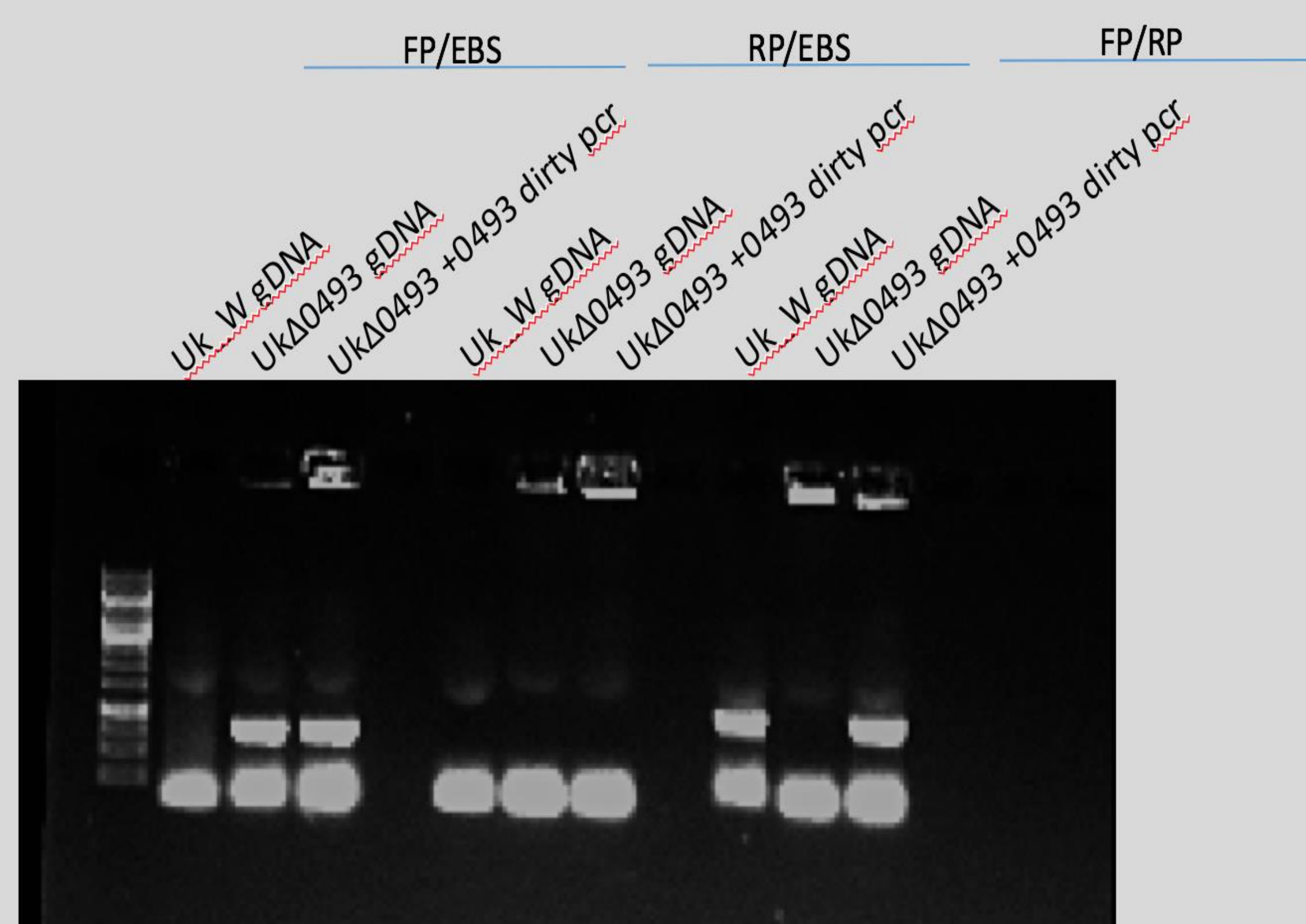
The infection is transmitted by spores, which are resistant to heat, acidity and antibiotics. Spores are the major reason of the disease transmission; however, the gene regulatory network that regulates gene sporulation and the environmental signals that trigger sporulation are not completely understood. This is why it is important to understand how these spores are formed so drugs can be targeted to formation.

Objectives

Our objective is to understand the role of CDR20291_0493 gene in sporulation initiation of *C.difficile*.

Results

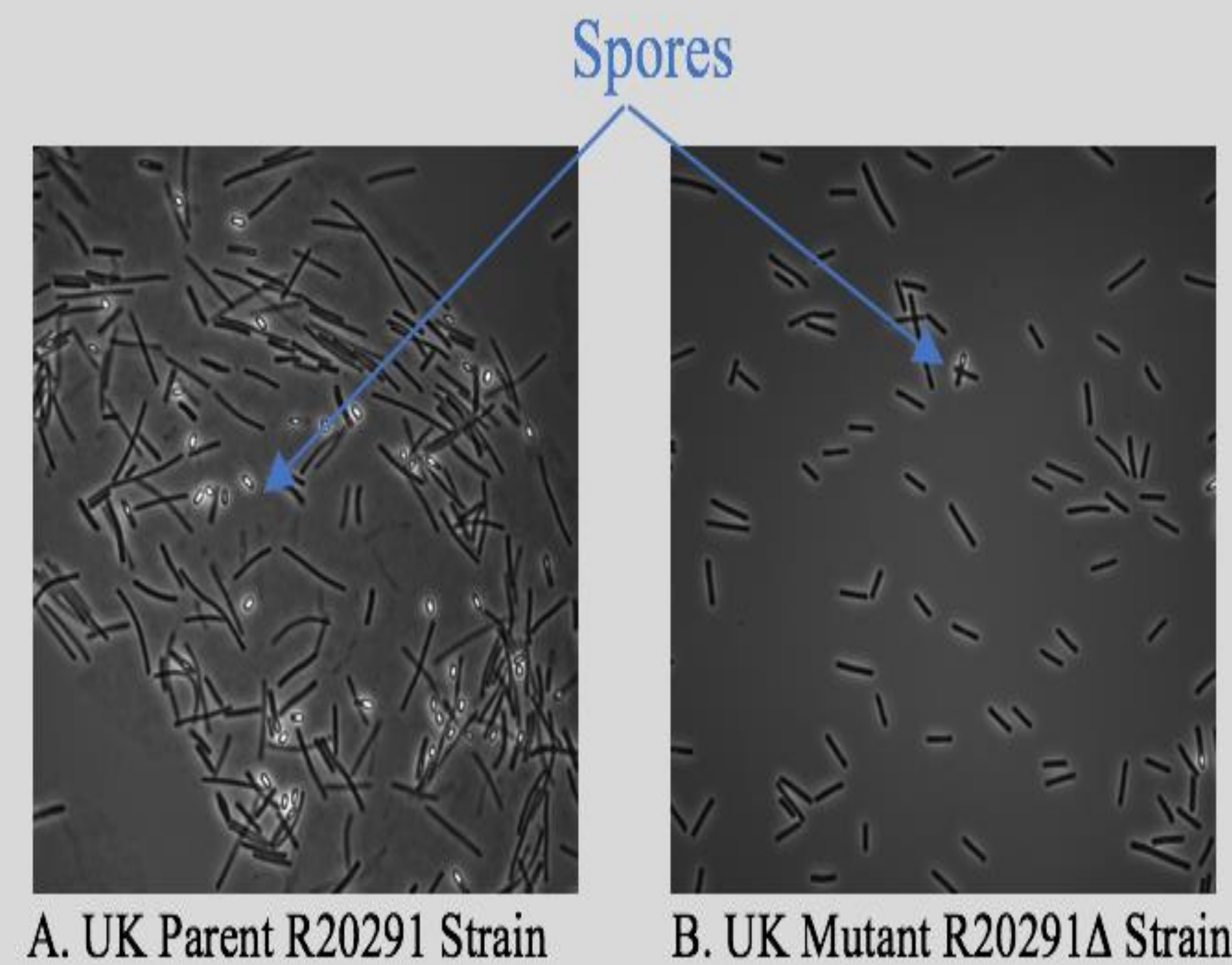
Construction and confirmation of Δ 0493 mutant by PCR



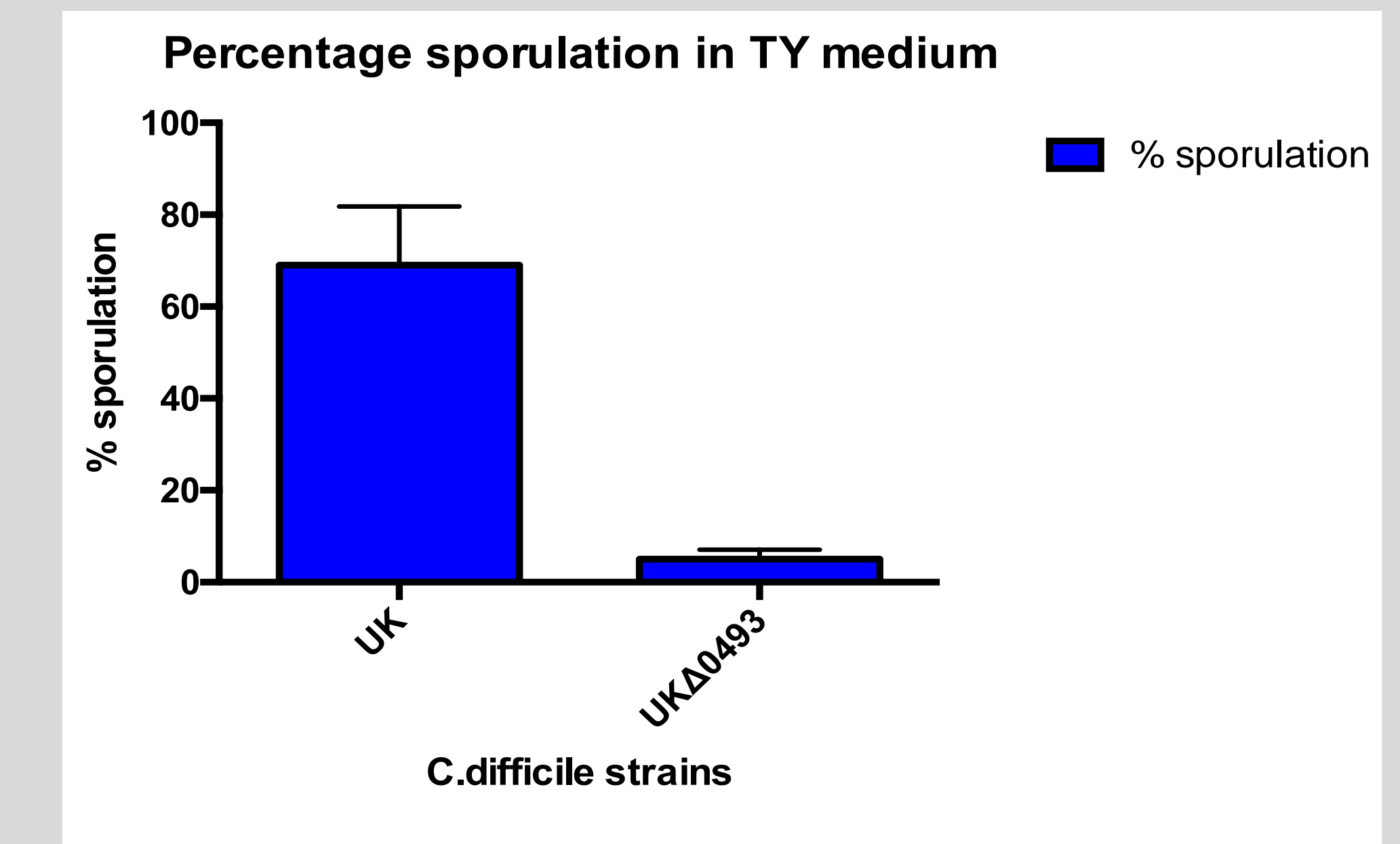
Mutation confirmed with primers specific for introns (FP/EBS, RP/EBS) and gene FP/RP

Results

Phase contrast Microscopy



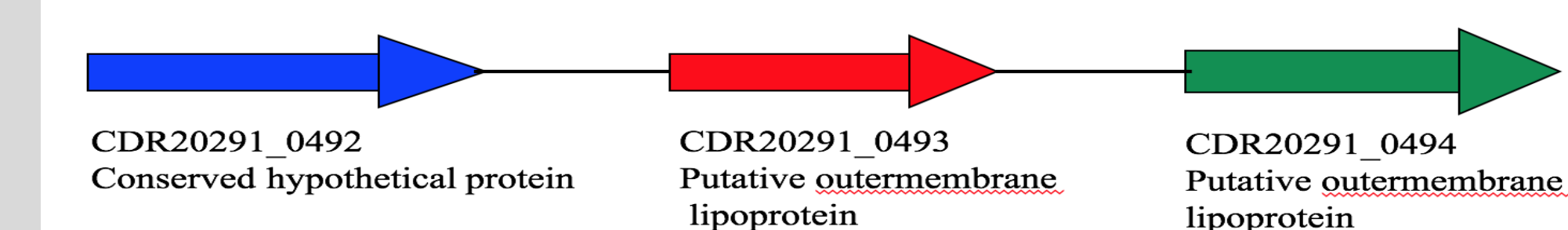
Sporulation Assay



Summary

- R20291 Δ mutant produced less spores compared to the parent R20291 strain.
- After the CDR20291_0493 complementation more sporulation was expected however, sporulation did not come back, because of this, gene CDR20291_0493 is predicted to be in an operon associated to downstream gene CDR20291_0494

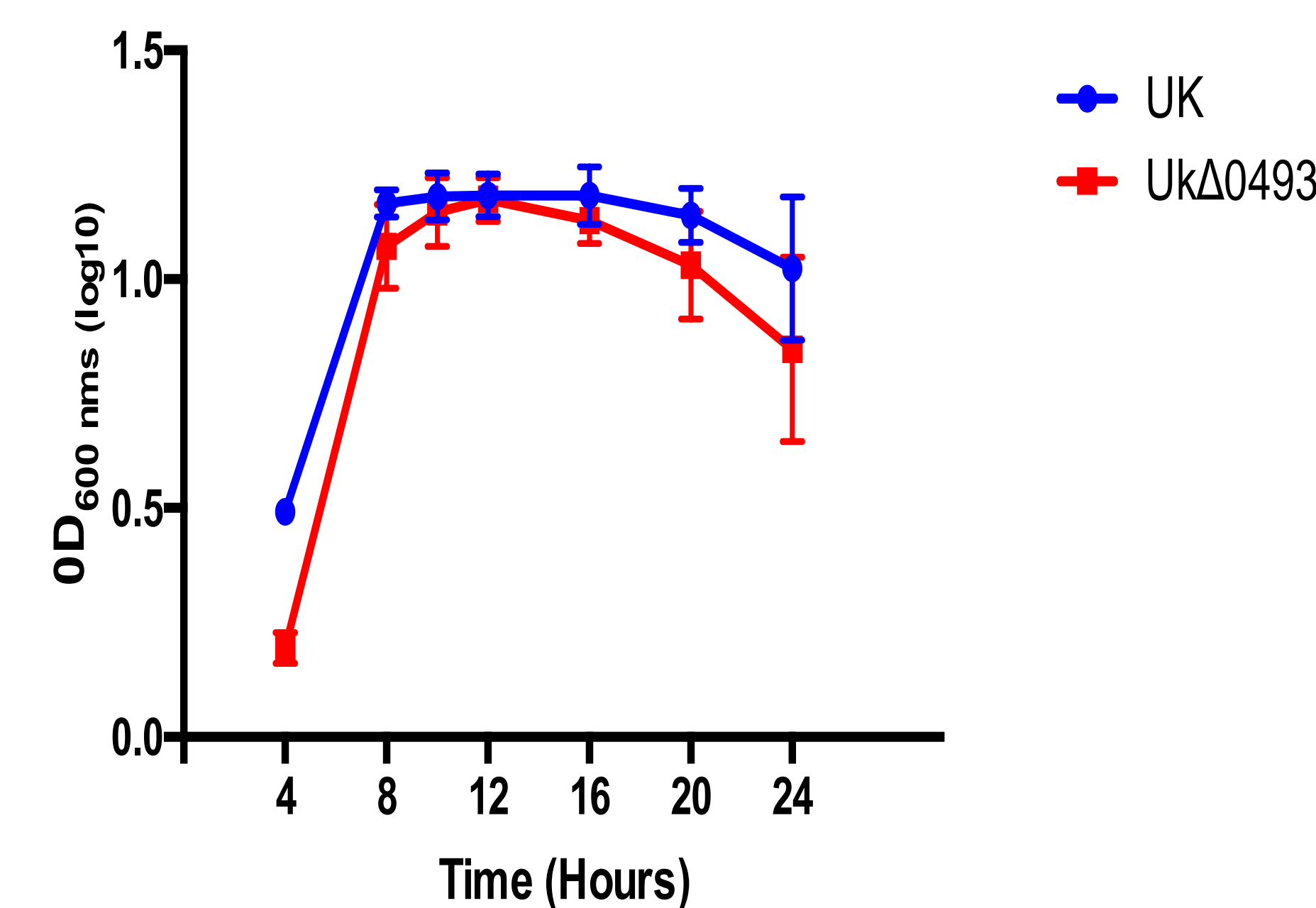
Gene locus of CDR20291_0493



- Our predictions are:
 - Complementation of CDR20291_0493 Δ mutant with both CDR20291_0493 and CDR20291_0494 will rescue the sporulation phenotype.
 - CDR20291_0493 and CDR20291_0494 are in an operon and both CDR20291_0493 and CDR20291_0494 have a role in sporulation initiation in *C.difficile*.

Growth profile of UK parent and UK Δ 0493 mutant

Growth curve of UK parent and UK Δ 0493 in TY medium



Future Studies

- To explain why sporulation did not come back after a complementation was performed to the mutant strain.
- To complement both CDR20291_0493 and CDR20291_0494 by cloning them in a same vector.
- Sporulation assay of CDR20291_0493 Δ and CDR20291_0494 Δ complemented strain

References

- Girinathan, Brintha P, et al. "Effect of *TcdR* Mutation on Sporulation in the Epidemic *Clostridium Difficile* Strain R20291." *MSphere*, American Society for Microbiology, 15 Feb. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5311115/.
- Girinathan, Brintha Parasumanna, et al. "Pleiotropic Roles of *Clostridium Difficile* Sin Locus." *PLoS Pathogens*, Public Library of Science, 12 Mar. 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC5864091/.