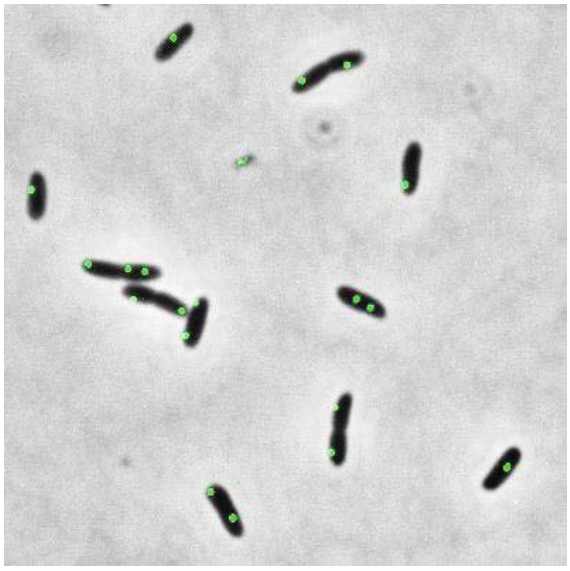
Massey University

DNA Replication Asynchrony in *Pseudomonas fluorescens* SBW25



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Abstract

Bacterial growth rate is largely dependent on the availability of nutrients in the environment. Past studies have shown that bacterial species such as *E. coli* can significantly increase their growth rate in nutrient-rich environments by initiating multiple cycles of DNA replication simultaneously. As a result, offspring cells not only inherit the full chromosome, but also an additional, partially replicated chromosome. However, studies have found that this multi-fork replication does not occur in all organisms such as *Caulobacter crescentus*. Detailed investigations of replication fork dynamics have thus far been limited to only a small number of bacterial species.

In this study, the cell cycle and DNA replication dynamics of *Pseudomonas fluorescens* SBW25, a gram-negative, plant-associated bacterium, is investigated. The study involves incorporating arrays of repeated operator regions bound by their fluorescently-labelled cognate repressors. A single array appears as a fluorescent focus upon live-cell imaging. An origin proximal array can therefore be visualized to follow the chromosome replication and segregation process in single living cells. Nutrient concentrations were varied in order to learn if multi-fork replication occurs in the model organism.

Results from this study show evidence for concurrent DNA replication cycles in *P. fluorescens* SBW25. This process appears to be exacerbated in nutrient-rich media (LB) as opposed to cells grown in nutrient-poor media (M9-glycerol). Moreover, asynchronous DNA replication initiations were also observed. This more stochastic initiation appears to be a common phenomenon when cells are grown in nutrient-rich media but not in nutrient-poor media. This study sheds light on a key cellular process in the *Pseudomonads*, a genus where DNA replication has not been studied extensively.

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