The DNA cytosine methylome of *Streptomyces coelicolor*

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DNA methylation is the most common epigenetic modification detected in the genomic DNA of both prokaryotes and eukaryotes. In eukaryotes, 5-methyl-cytosine (m5C) plays an important role in the regulation of many relevant cellular processes. In prokaryotes, its role in gene expression was less investigated. Recently it was demonstrated that DNA cytosine methylation triggers morphological and physiological differentiation of *Streptomyces coelicolor* in both liquid and solid rich media (Pisciotta *et al.*, 2018). Previous results were obtained using GYM and R5A media.

Streptomyces coelicolor is a Gram-positive microorganism considered as a model of bacterial physiological and morphological differentiation (Fig. 1) in streptomycetes, prolific producers of secondary metabolites with important biological activities.

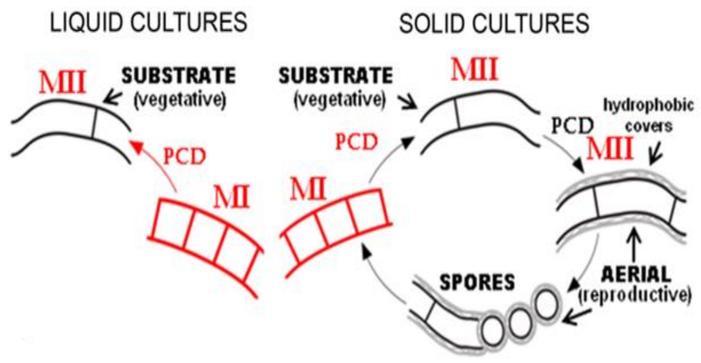
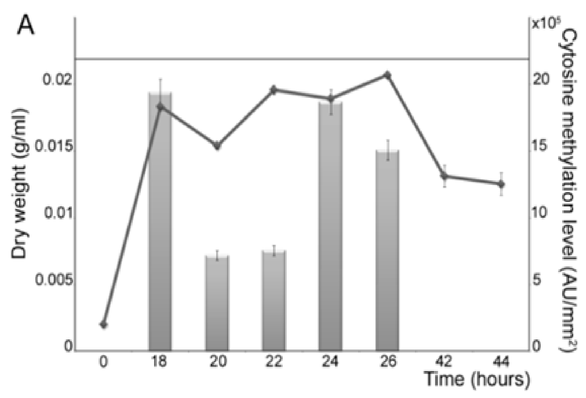


Figure 1: Life cycle of *S. coelicolor* M145 A3(2) in the liquid R5A and on solid GYM (Yagüe *et al.* 2013).

Aims: (i) to investigate DNA cytosine methylation levels in the defined liquid medium containing Maltose and Glutamate (MG),
(ii) to evaluate the effect of cytosine demethylation on growth and antibiotic production and
(iii) to map cytosine methylome using the whole genome sequencing after bisulfite treatment (BS sequencing).

DNA cytosine methylation along growth in MG

Dot blot analysis using the antibody against 5-MeC demonstrated that the level of methylated cytosines changed along the growth: it is higher at 18h and 24h (Figure 2A). CLSM analysis showed that cells are in MII phase both at 18 and 24h of growth (Figure 2B-C).



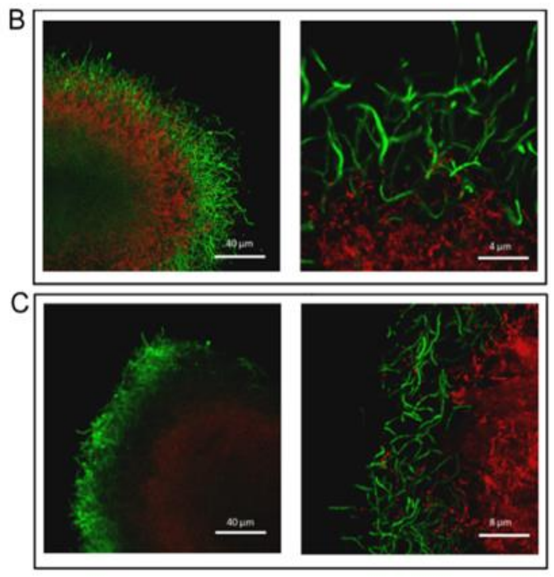
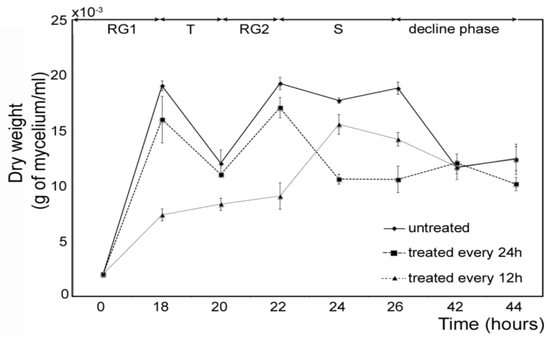


Figure 2: Growth curve and DNA cytosine methylation pattern (grey bars) during growth in MG (A). CLSM analysis of mycelia after 18h and 24h of growth (B).

Effect of demethylation on *S. coelicolor*

5-aza-dC was added every 24h and 12h of growth. The treatment carried out every 12h displayed a stronger effect on the cell growth and antibiotic production (Figure 3).



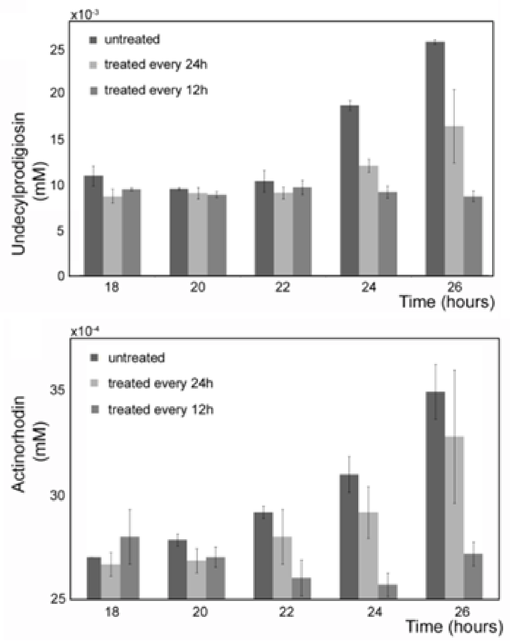


Figure 3: Effect of 5-aza-dC treatment every 12h and 24h on growth, undecylprodigiosin and actinorhodin production. Untreated culture (using DMSO) is indicated by continuous line.

Methylome map

BS sequencing results revealed a total of 3360 methylated cytosines of the genome (8.87 Mb) and two most recurring methylated sequences (Figure 4). 138 genes contain the CmG motif, 158 the CmHG motif and 29 the CmHH motif in their upstream region (Figure5). H stands for A, T or C.

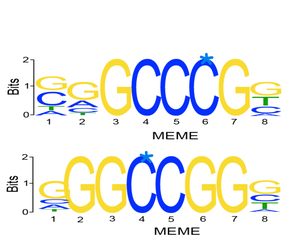


Figure 4: Methylated sequences. Asterisks indicate the mC.

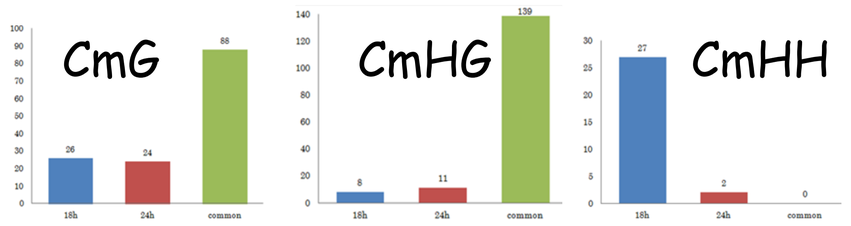


Figure 5: Number of genes containing in their upstream region the methylated motifs at 18h, 24h or both (indicated like 'common'). H stands for A, T or C.

Conclusion

This study reports for the first time the mapping of the cytosine methylome of *S. coelicolor*. Two methylated sequences were detected present upstream 325 genes.

References

Yagüe P, et. al. PLoS One. 2013 e 10., 8(3):e60665.

Pisciotta et al., Sci Rep. 2018 12;8(1):13686