Bacterial morphogenesis vis-à-vis cell size in the various stages of biofilm development

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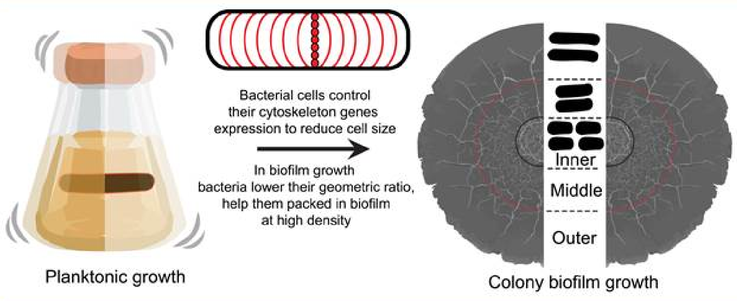
Abstract

Bacterial cell division is regulated by the bacterial cytoskeletal protein FtsZ/ RodZ and the level of Z ring assembly. MreB actin protein is found to regulate the cell wall component peptidoglycan synthesis and insertion. The cytokinesis process also is regulated during the biofilm stage at nutrient limiting conditions. However, if the nutrients are available during normal growth of life, the cell division rates and cytokinesis will be faster to reach to the optimum density to exhibit the density dependent gene network. Besides, bacterial pleomorphism was found to occur in biofilm under overcrowding condition to facilitate the cells to escape and settle at a new substratum. Rod shaped cells align their orientations with nearby cells and colonize at the base and edges, whereas coccoid cells dominate the upper surfaces in a biofilm. Phenotypic heterogeneity helps to optimize the interactions with cells and the attached surfaces. Cell morphology, aggregation and arrangement within biofilm were studied in *Bacillus subtilis 121* and *Escherichia coli 1610* and found that the biofilm development was undergoing pleomorphic changes. Rod cells tremendously reduced their size to pack with high density within biofilm. Cytoskeleton genes were downregulated as compared to planktonic growth to reduce bacterial cell size. Coccoid cells preferred to live in biofilm mode, whereas the transformed rods acquired the flagellar motility. It is hypothesized that the biofilm stage of bacterial cells are regulated by the genes which regulate cytokinesis, morphology and cell wall synthesis for morphogenesis.

Graphical Abstract



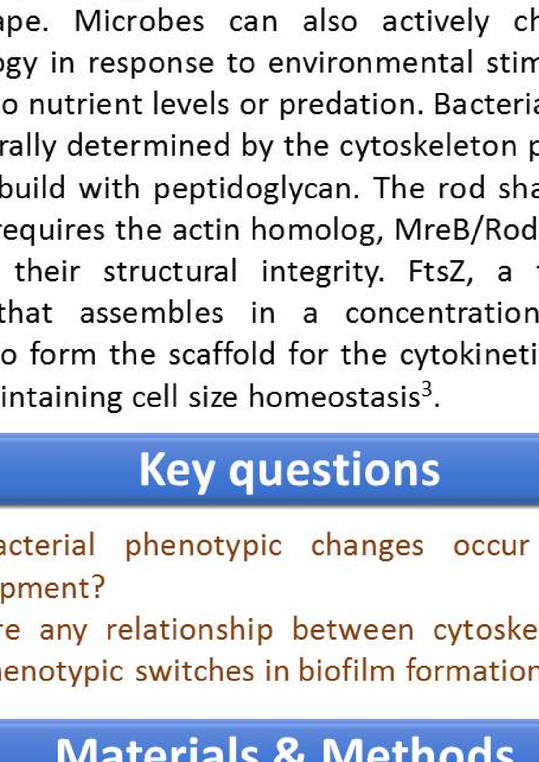
Introduction

Morphogenesis is a highly important theme in both biology and elemental structure. In nature, self-organization can lead to significant selective advantages for living organisms and also found in biofilm forming bacteria[1]. Biofilms are incredibly heterogeneous at the microscopic level and look very much like cities bustling with micro colonies, connected by channels through which water, nutrients, and oxygen can diffuse[2]. An important unexplored consequence of the formation of high-density bacterial colonies is spatial organization caused by the "contact biomechanics" arising from cellular growth and division. The main phenotypic characteristic of a microbial cell is their shape. Microbes can also actively change their morphology in response to environmental stimuli, such as changes to nutrient levels or predation. Bacterial cell shape is structurally determined by the cytoskeleton proteins and cell wall build with peptidoglycan. The rod shape of most bacteria requires the actin homolog, MreB/RodZ protein to maintain their structural integrity. FtsZ, a tubulin like protein that assembles in a concentration-dependent manner to form the scaffold for the cytokinetic ring helps in the maintaining cell size homeostasis[3].

Key questions

- Do bacterial phenotypic changes occur in biofilm development?
- Is there any relationship between cytoskeleton genes and phenotypic switches in biofilm formation?

Materials & Methods



Results

Fig. 1. Development of Colony biofilm. *Bacillus subtilis* 121 showed three distinguished wrinkle zones (outer, middle, and inner) 6 h onwards, where as no such zone observed in *Escherichia coli* 1610 biofilm up to 24 h.

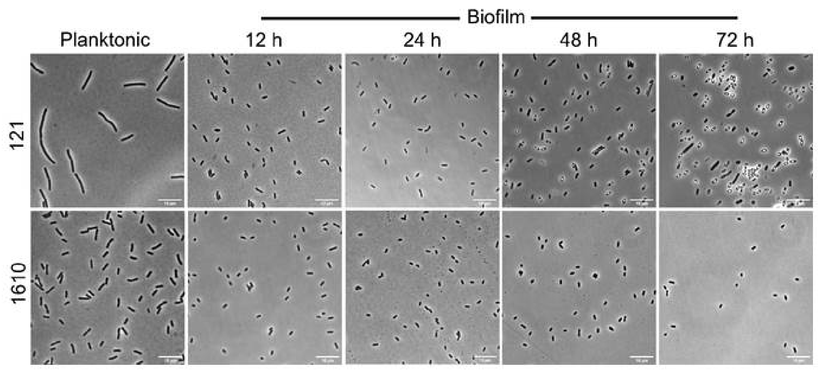


Fig. 2. Phenotypic variation of bacterial cells in planktonic and biofilm mode of growth. Tremendous reduction in cells size was observed when bacteria grown in biofilm mode. Cells size of *Bacillus subtilis* 121 and *Escherichia coli* 1610 in biofilm growth was reduced significantly compared to the planktonic growth.

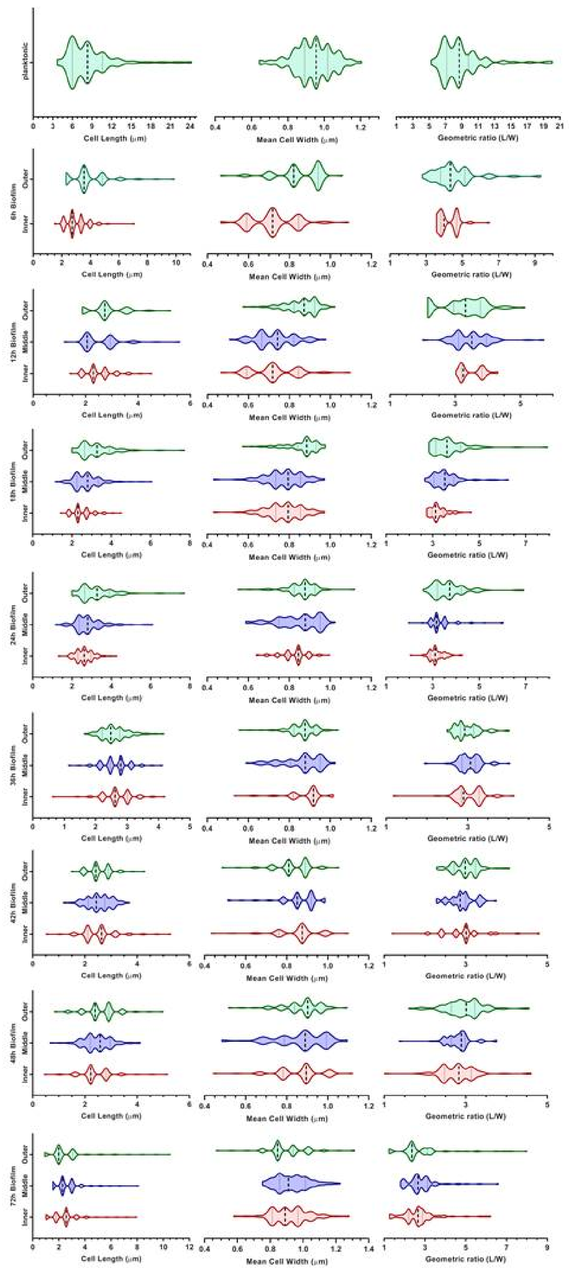


Fig. 3. Geometric variation of *Bacillus subtilis* 121 in biofilm development. Cells phenotypes were measured through Oufti software[4]. The geometric ratio of round (coccus) shape is 1. Geometric ratio revealed that with the progression of biofilm development, cell shape was changed from rod to coccus due to requirement of high cell density and packing in limited area. Hence, inner core showed much more smaller cells than middle and outer zone.

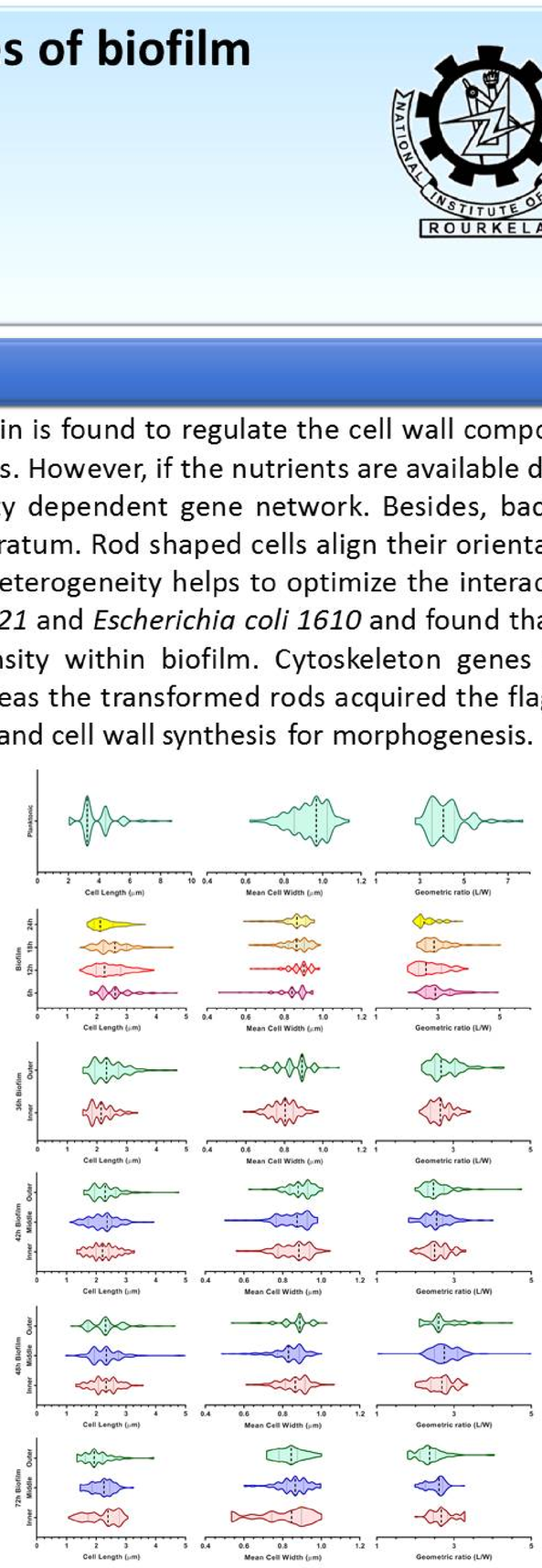


Fig. 4. Geometric variation of *Escherichia coli* 1610 in biofilm development.

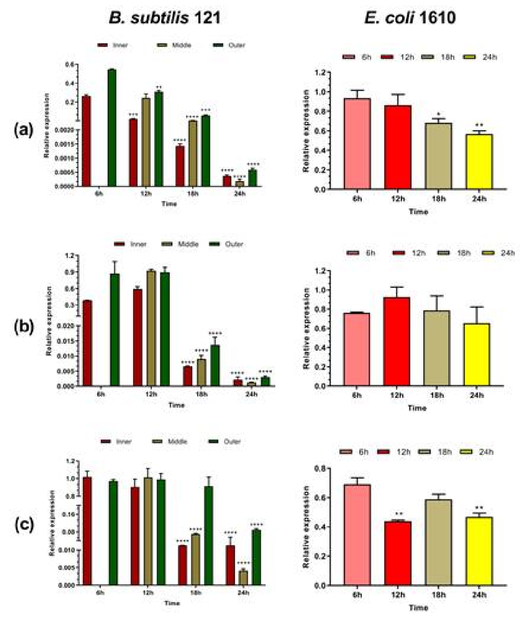


Fig. 5. Cytoskeleton genes (a) *rodZ* (b) *mreB* (c) *ftsZ* expression study in biofilm development. In biofilm mode, relative expressions of cytoskeleton genes were calibrated with planktonic growth. Relative expressions of cytoskeleton genes were significantly down regulated with respect to time ($p<0.05$; One way ANOVA followed by Tukey's multiple comparison test). Down regulation of *rodZ* and *mreB* helps to reduction in cell size, which indirectly support bacteria to spatially organized in biofilm.

Conclusion

- Phenotypic variation of *Bacillus subtilis* 121 and *Escherichia coli* 1610 in biofilm mode is the indication of the involvement of cytoskeleton protein in biofilm development and maintenance
- The reduction of cell size in biofilm development is maintain by gradually lower expression of *rodZ* and *mreB*
- The regulator of cytoskeleton genes expression and biofilm development will be studied further

Acknowledgement

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References

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