Correlation between Swarm and Swim Assay with Biofilm Formation in Salmonella Typhi

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Abstract

Salmonella Typhi (S. Typhi) is the etiologic agent of typhoid fever, a systemic illness characterized by high fever, nausea, and muscle pain, which infects only human. The main aim of this study was to identify possible variation in the swarming and swimming patterns, as well as the biofilm forming capability among fifty-five selected strains of S. Typhi. Correlation between motility and biofilm formation was then determined. Swarming and swimming motility were performed with swarm plate and swim plate assays; while biofilm formation was determined by growing the bacterial strains for 48 hours in 96-well microtitre plates to allow biofilm formation which was then stained with 0.5% crystal violet. Out of 55 S. Typhi strains, two strains (3.6%) were unable to swim in the swim plate assay; while only one strain (1.8%) failed to show any swarming capability in the assay. None of the strains was found negative in the swarm plate and swim plate assays. Interestingly, a high degree of variation was observed in the swimming and swarming motility patterns among the strains. We are unclear if this observation of variable swimming and swarming motility patterns would be associated with the pathogenicity or virulence of the strains. Further study is required to investigate this association. We did not observe clear correlation between motility and biofilm formation capability. Nonetheless, three strains which demonstrated the highest motility capability also showed the highest capability in biofilm formation; whereas the weakest biofilm former was motility-impaired. In conclusion, this study demonstrated that S. Typhi strains examined were highly variable in the motility and biofilm forming capability. More studies are required before any definitive conclusion could be made.

Introduction

S. Typhi causes typhoid fever only in humans. Some acute infections in humans will lead to asymptomatic carrier state, in which the pathogen colonizes and persists in the gall bladder and disseminates periodically via urine and faeces (Crawford et al., 2010). Recent study showed that S. Typhi is frequently associated with the presence of gallstones in asymptomatic human carrier (Crawford et al., 2010). Salmonellae was able to form bile-mediated biofilms on human gallstones and also cholesterol coated surfaces in an in vitro assay (Crawford et al., 2010). Bacterial biofilm is defined as a community of microorganisms attached to a solid surface using extracellular polymeric substances (Branda et al., 2005). However, motility is also one of the essential virulence factors of pathogens, particularly in S. Typhi. There are two types of motility patterns: swim and swarm. Swarming motility is operationally defined as a rapid multicellular bacterial surface movement powered by rotating flagella; whereas swimming motility is a mode of bacterial movement powered by rotating flagella but, unlike swarming motility, it takes place as individual cells moving in liquid environments (Kearns, 2010). Motility is essential for biofilm formation, probably for overcoming the electrostatic repulsion of cells and surfaces (Pratt and Kolter 1998; Walker et al., 2004). However, the role of motility on biofilm maturation and its effect on architecture have not been elucidated, as there is no a clear report that relates motility and mature biofilm formation (Lazazzera, 2005). This question, therefore led us to investigate the relationship between biofilm formation and surface motility.

Materials and Methods

Bacterial strains

A total of 55 human S. Typhi strains were studied. The strains were isolated from Malaysia, Indonesia, Vietnam, Papua New Guinea and Chile from 1980 to 2008 maintained in glycerol stocks at minus 20°C.

Swarming and swimming capability

One spot of an overnight culture was lightly touched in the middle of a swarm plate (Nutrient Broth [NB], 0.5% [wt/vol] glucose, 0.5% agar) or a swim plate (NB, 0.5% glucose, 0.25% agar) and plates were incubated at 37°C for 24h. The diameter of motility was measured and patterns of swimming and swimming on the agar plate were determined accordingly to Wook Kim et al. (2003).

Biofilm quantification of human Salmonella Typhi strains

The condition for the assay was LB broth as growth medium, incubation at 48 h, fixation at 80°C and staining with 0.5% crystal violet stain. Each strain was inoculated into at least 8 wells of 96-well plate. After incubation, unbound cells were removed by inversion of microtitre plate, followed by vigorous tapping on absorbent paper.
Subsequently, adhered cells were fixed for 30 min at 80°C. Adhered cells were stained by addition of 220 µl of crystal violet (0.5%) for 4 min. The stain was removed by thorough washing with distilled water. In order to quantify adhered cells, 220 µl of decolouring solution (ethanol/acetone, 80:20%) was added to each well for 15 min. The absorption of the eluted stain was measured at 590 nm (Agarwal et al., 2011). Based on the O.D. reading of S. Typhi biofilms, strains were classified into the following categories: no biofilm producers, weak, moderate or strong biofilm producers, as previously described (Stepanovic et al., 2004).

**Results and Discussion**

A total of 2 strains (3.6%) were non-swimmers: TP672/05 and TP142/05 whereas 1 strain (1.8%) showed non-swarming ability. Of 55 strains characterized, 10 strains (18.2%) did not show any pattern for swimming capability; 51 strains (92.7%) did not show any pattern for swarming across the agar surface in the assay. We have observed four swimming patterns of *Salmonella* Typhi in the semi-solid nutrient media: Bull’s eye (Figure 1A), featureless (Figure 1B), suppressor (Figure 1C) and vortex (Figure 1D). More than half of the Typhi strains tested demonstrated bull’s eye and featureless characteristics, followed by vortex (10.9%) and suppressor (1.8%) pattern.

Two strains swarm across the agar surface demonstrated 2 different patterns of swarming: suppressor (Figure 2A) and featureless (Figure 2B).

Majority of strains (21, 38.2%) were found to be weak biofilm producers, while 21 (38.2%) strains did not form any biofilm. 10 strains (18.2%) were found to be moderate biofilm producers and only 3 strains were strong biofilm producers: TP675/05, ST1165/87, ST309/91. We observed that there was a reciprocal relationship between the diameters of spread for the swarvers and swimmers; when the swarm assay gave lower diameter value such as TP675/05 (2.15, 1.8678), the swim assay gave higher swim diameter value such as TP675/05 (7.45, 1.8678). Three strains with strong biofilm forming capability swam well (TP675/05, ST309/91, STVC1679/83). Surface motility might affect biofilm formation (Verstraeten et al., 2008). It has now become clear that out of many pathways, motility also affect the formation of biofilms, surface attached bacterial colonies (Verstraeten et al., 2008). Decision-making between rapid colonization of a surface and biofilm formation is central to bacterial survival (Verstraeten et al., 2008). However, the link between motility and biofilm formation tends to be complex because both processes might involve similar components at certain stages and specific conditions.

**Conclusion**

The best biofilm-formers, TP675/05, ST309/91, and STVC1679/83 displayed the highest motility, whereas the worst biofilm former was motility-impaired. Surface motility of *Salmonella* Typhi contributes to biofilm formation. Hence, a correlation is observed.

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**References**


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