# Another way to perceive the pathogenicity of *Shigella flexneri* by taking into account biosurfactants.

**Abstract**

All previous research on biosurfactants has focused on bioremediation, biorestoration, and food biopreservation. They have never been linked to *Shigella* *flexneri* pathogenicity. Biosurfactants are biomolecules involved in several phenomena in the microbial world. This review in no way contradicts the previous work done on the shigella model. It aims to give an opinion on two recent published history regarding the roles played by biosurfactants in the pathogenicity of *Shigella*.

**Introduction**

Shigella flexneri is a Gram-negative facultatively intracellular pathogen responsible for bacillary dysentery in humans. More than one million deaths occur yearly due to infections with Shigella spp. and the victims are mostly children of the developing world. The pathogenesis of Shigella centres on the ability of this organism to invade the colonic epithelium where it induces severe mucosal inflammation. Much information that we have gained concerning the pathogenesis of Shigella has been derived from the study of in vitro models of infection. Using these techniques, a number of the molecular mechanisms by which Shigella invades epithelial cells and macrophages have been identified (Jennison & Verma, 2004).

***Shigella* bacteria and the secretion of Biosurfactants**

Biosurfactants are molecules with surfactant properties [1, 2]. These molecules, well known in bacteria of the genus *P. aeruginosa* [3, 4], *B. subtilis* [5], and *B. lichenifornis* [6], have been widely studied and used in soil decontamination [7]. A recent study conducted in our laboratory clearly shows that *Shigella* strains are capable of producing and secreting biosurfactants [8, 9]. Similarly, we also find that *Shigella* isolated in a clinical setting were also capable of emulsifying hydrocarbons and therefore of producing biosurfactants.

Biosurfactants can be bound to the bacteria as in *Salmonella* thanks to its LPS or excreted into the extracellular environment as in *B. subtilis* or *P. aeruginosa* [10]. Biosurfactant molecules are excreted into the extracellular environment to perform very specific functions that are supposed to be vital for bacteria [11].

In *S. flexneri*, pathogenicity is determined by its type III secretion apparatus which has the power to secrete effector proteins into the target cell [12] to initiate its pathogenesis [13]. Indeed, in the absence of cellular contact, the secretion apparatus of Shigella is not functional, however some proteins are secreted in escape conditions [13-19]. T3SS is only functional when there is cell contact[12]. Indeed, in experimental conditions, cell contact is mimicked by the use of Congo red. The secretion of biosurfactants is under the control of T3SS [8].

Since the type III secretion apparatus is essential in the invasion mechanism of epithelial cells [20], it therefore appears clearly that these molecules would have a determining role to play at the level of membrane interfaces at the time of invasion, depending on their amphiphilic nature. Unlike the Shigella virulence proteins encoded by the ipa-ipg operon [21, 22], biosurfactants are found in the medium. By monitoring biosurfactant production, works have been demonstrated that the biosurfactant production of Shigella species was highly dependent on bacterial culture density involving the Quorum Sensing (QS) [9].

From this point of view, the fact that *Shigella* spp. emulsifies hydrocarbons during normal culture [8, 9] supports the hypothesis that biosurfactants are part of the 5% of Shigella effectors secreted during escape in the absence of any T3SS activity . Furthermore, the fact that Congo red induction shows an emulsifying power, would mean that during invasion or cell contact between the epithelial cell and the T3SS, the biosurfactant(s) secreted by *Shigella* is (are) also secreted and would probably play a primary role in the mechanism of *Shigella* pathogenicity as Rafts can trigger contact-mediated secretion of bacterial effectors via a lipid-based mechanism [23]. Based on recent publications, it seems difficult to accept that biosurfactants play no role in the pathogenicity of Shigella bacteria. Many host factors involved in the Shigella-prompted actin rearrangements remain elusive.

**Shigella's mobility and pathogenicity link with Biosurfactants**

Biosurfactants biomolecules have been shown to be at the origin of several multicellular phenomena such as "swarming" described for the first time in *P. aeruginosa* [24-29] and then in many other species such as *B. subtilis* [30-33], *Serratia marcescens* [34, 35], *Salmonella enterica* serovar Typhimurium [36], *Vibrio cholerae* [37] and *Proteus mirabilis* [38, 39]. Indeed, swarming is a collective swarming movement of bacteria favored by the secretion of biosurfactants on a semi-solid surface. The recent study by Kinavouidi et al, 2020 highlighted this phenomenon in all reference and clinical strains of *Shigella* [8, 9].

Indeed, bacteria belonging to the genus *Shigella* are constitutionally immobile, due to the absence of a flagellum. Shigella flexneri uses elements of the host cell cytoskeleton to move within cells and from cell to cell [40]. However recent woks showed that *Shigella* are capable of swarming in semi-solid medium. This phenomenon has been considered as typical of motile bacteria [8, 9].. Despite the absence of flagellum in *Shigella* spp. and the proven immobility of Shigella spp. under microscopy, these bacteria exhibited swarming mobility [8, 9]. Thus, one could conjecture that by secreting the biosurfactant during its pathogenicity, *Shigella* would approach the epithelial cells to establish the invasion mechanisms. Until writing of this paper, intra- and intercellular bacterial motility of *S. flexneri* was limited only to the recruitment of actin filaments inside eukaryotic cells during the invasion mechanism [40-44], to the Browmian movement driven by intestinal peristaltic movements and especially bacterial interactions with lipid rafts [23, 45]. Many studies have shown that biosurfactant production and secretion is associated either with resistance to antiseptics in *Salmonella* *enterica* serovar Typhimurium (Rossi et al., 2016), or with the virulence of P. aeruginosa [18, 28] and the formation of biofilms in *P. aeruginosa* [46, 47] and *B. subtilis* [48], it seems reasonable to assume that *Shigella* would use this same mechanism to colonize epithelial cells. These indices tend to confirm the hypothesis that the mechanism of cell invasion by *Shigella* is also due to the production and secretion of biosurfactants. The recent work rhymes with the role of a host protein receptor for activated C kinase 1 (RACK1) that promotes *Shigella flexneri* actin-mediated invasion, motility, and cell-to-cell spreading [42]. The movements of bacteria inside the cell are modulated by the IcsA (VirG) protein, which recruits and activates, through its N-terminal domain, cellular factors, including the N-WASP (Wiskott-Aldrich Syndrome Protein) and the Arp2/3 complex [41, 43, 44, 49]. This multiprotein complex allows the nucleation of actin and catalyzes the elongation of an actin tail that propels Shigella through the cytoplasm of the host epithelial cell. EPEC, *S. flexneri* and *S. enterica* can alter the phosphorylation status and distribution of occludin and zonula occludens (ZO1), allowing the bacteria to disrupt epithelial barrier function.

Indeed, in light of the work carried out by Kinavouidi et al., 2020 [8], it seems more than obvious that this effector is none other than the biosurfactant secreted by *Shigella* spp. because as mentioned above, the surfactant properties of its molecules make them more capable of destabilizing cell tight junctions as previously demonstrated with P. aeruginosa [24, 25].

This supports the fact that one of the underlying mechanisms of *Shigella* spp. would be the production of biosurfactants that accompany this movement. In fact, IcsA mutants are capable of entering epithelial cells with the same efficiency as the wild-type strain [40]. However, they are unable to move in the cell cytoplasm, form protrusions, or disseminate in a cell monolayer. This observation sufficiently shows that these mechanisms are interdependent. Indeed, the recruitment of actin filaments would be the guide, the cytoskeleton the support and the biosurfactant the sliding agent allowing the bacteria to slide on the one hand, to form protrusions on the other, but also to destabilize the tight/membrane junctions and promote the tissue diffusion of the infection.

**Biosurfactants and autophagy escape**

Autophagy is an intracellular host defense process that results in the lysosomal degradation of organelles and large protein complexes [50-52]. It is induced during stress, nutrient deprivation, bacterial or viral infection. In *Shigella* spp., the icsB gene encodes an effector protein that plays an important role in the mechanisms of protection against autophagy [50-52]. However, IcsB is not the only Shigella effector to have a direct or indirect role in the activation or protection against this process. It has been showed that the binding capacity of the IcsB protein to cholesterol allowed the bacteria to escape from the autophagosome formed by the host cell to destroy the bacteria. Therefore, deletion of the CBD (Cholesterol Biding Domain) significantly increased the formation of autophagosomes. IcsB protein interacted with the IcsA protein to escape autophagy [51]. These observations suggest two possibilities: i) the IcsB protein would have a biosurfactant binding domain that would potentiate the action of this protein in vivo, ii) IcsA would have in addition to the interaction domain with IcsB, a biosurfactant binding or interaction domain that would potentiate the escape of the bacteria from autophagy (by destabilizing the autophagosome) and concomitantly with bacterial dissemination. Still, the simple fact that icsB mutants are unable to disseminate suggests that biosurfactant is not directly responsible for autophagy escape, first because it is established that both IcsB and Atg5 have a common IcsA/VirG binding domain, so this protective role would be essential to mix with biosurfactant like molecule. Second, it seems more obvious that this entire multiprotein complex is required to destabilize the autophagosome.

**Conclusion**

The versatile role of biosurfactants should not be limited to bioremediation and the food biopreservation. The pathogenicity aspects of biosurfactants have not yet been elucidated. Several ambitious studies should be conducted, especially those concerning intracellular pathogenic bacteria including *Shigella*, *Salmonella* and many others. These studies will make it possible to know the mechanisms of pathogenicity of these bacteria.

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