Basic characterization of BopA, a homolog of the Shigella effector IcsB

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Abstract

The annual death toll of *Shigella* infection is of approximately one million, consisting of mostly children from developing countries. The effector protein IcsB plays a critical role in the pathogenesis of *Shigella* species by protecting it from the host defense system. Interestingly, BopA, a homolog of IcsB, plays a similar role in another pathogenic bacteria Burholderia pseudomallei. This study was designed to characterize the behaviour of BopA by comparing its toxicity in yeast with IcsB and its relationship with the host proteins that interact with IcsB. First, full length BopA was found to be toxic while two mutants constructed, Δ80 and Cys312Ala (C312A), relieved toxicity in yeast. Moreover, the expression of Δ80 was observed, but not for C312A by Western blotting. Taken together, these results correspond to the results obtained for IcsB. As the BopA mutants did not auto-activate reporter genes, it is possible to detect interactions with prey proteins by yeast-two-hybrid (Y2H). We are currently testing if host proteins SGT1, DDX3X, and FANCL that were found to interact with IcsB are also capable of interacting with BopA according to the Y2H. We also hope to construct additional BopA mutants to test if the domain that is critical for interacting with the prey proteins is also required by BopA. The findings of this study will facilitate the understanding of the molecular behaviours of BopA and IcsB, which can be harnessed to develop novel therapeutic strategies.

Introduction

- *Shigella flexneri* is the causative agent of shigellosis characterized by abdominal pain and diarrhea with or without blood or mucus [1].
- IcsB, the effector protein secreted by the Type III Secretion Apparatus (T3SA), has shown to facilitate the escape from ATG8/LC3 positive vacuoles formed during cell-to-cell spreading by the host immune system [1, 2].
- However, the underlying molecular mechanisms of IcsB and its host target proteins remain largely unknown.

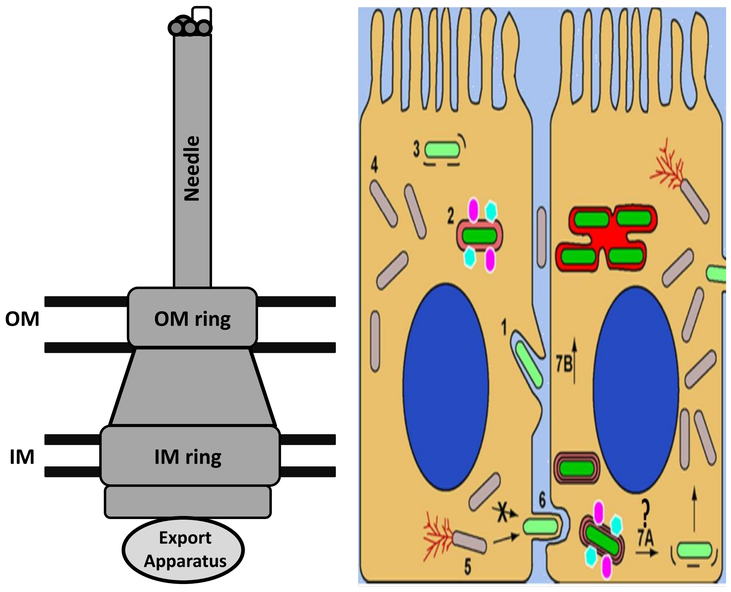


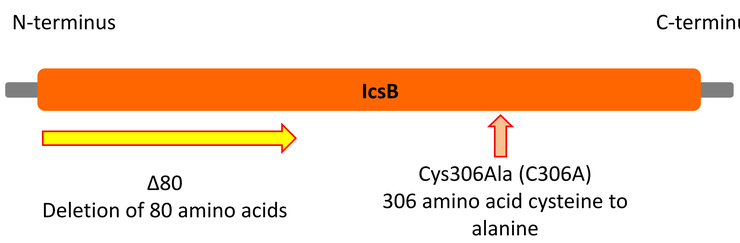
Figure 1. (A) A visual representation of the Type Three Secretion apparatus adapted from Campbell-Valois and Pontier, 2016. (B) A visual representation of the life cycle of *Shigella flexneri* adapted from Campbell-Valois et al, 2015.

Approach and hypothesis

- BopA from *Burkholderia pseudomallei* is a homolog of IcsB and has shown to perform a similar function to IcsB[3].
- Characterization of the behavior of BopA by comparing its toxicity in yeast with IcsB and its relationship with the host proteins that interact with IcsB will allow a deeper understanding of functions of IcsB and BopA.

Methodology

IcsB mutants:



BopA mutants:

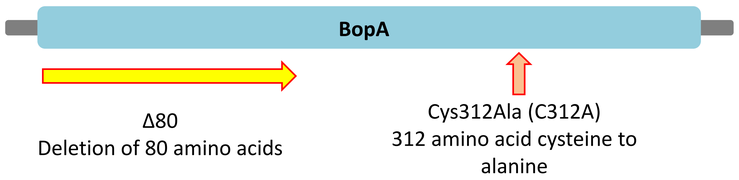


Figure 2. A visual representation of the IcsB mutants and homologous mutations in BopA constructed using PCR mutagenesis. Δ80 : deletion of part of the chaperone binding domain. C312A/C306A: catalytic cysteine to alanine.

Result

Figure 3. The IcsB and BopA mutants yeast transformation result comparison. WT: wildtype. Positive control used: empty pGBKT7. Negative control used: yeast with no added DNA.

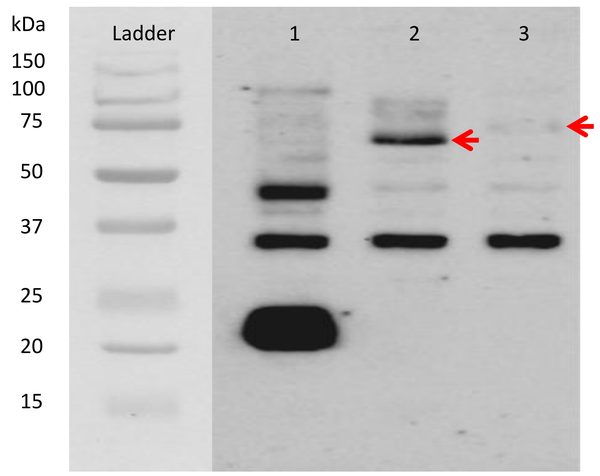


Figure 4. The BopA mutants western blot result. 1: Empty pGBKT7 (control). 2: pGBKT7_BopA_ Δ80. 3: pGBKT7_BopA_C312A. Red arrows indicate the protein expression of each mutant.

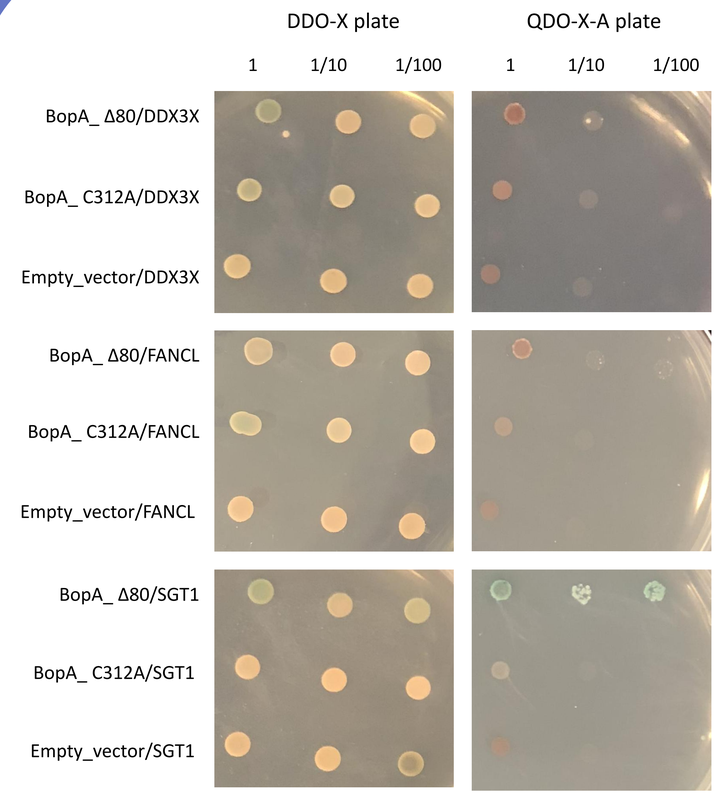


Figure 5. The yeast mating result for pGBKT7_BopA_Δ80 and C312A. Host proteins: DDX3X, FANCL, and SGT1. Positive control: empty pGBKT7.

Conclusion

- The wildtype BopA was found to be toxic in yeast.
- Both BopA mutants relieved toxicity but Δ80 was able to reduce more than C312A and the Western blot validated this result.
- This closely resembles the result obtained for IcsB supporting that IcsB and BopA serve similar functions and C306/C312 are indeed part of the catalytic site.
- Yeast mating result seems to confirm the interaction between SGT1 and BopA but not DDX3X or FANCL suggesting SGT1 as the leading candidate that interacts with IcsB and BopA.

Future directions

- Since FANCL interacted stronger with IcsB_Δ80_C306A than IcsB_Δ80, BopA_Δ80_C312A should be constructed to further characterize its interaction with FANCL.
- The *Salmonella* effector SspH2 has shown to interact with SGT1 and subsequently with Nod1 to subvert the immune response [4]. This suggests a possible interaction model for IcsB.

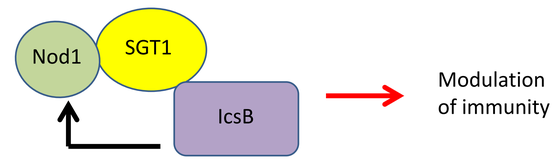


Figure 6. A visual representation of possible relationship between SGT1, IcsB and Nod1. Black arrow indicates that IcsB can also act on Nod1 by binding to SGT1.

References

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