Deciphering the inner workings of unique Bacteroides pore-forming toxins

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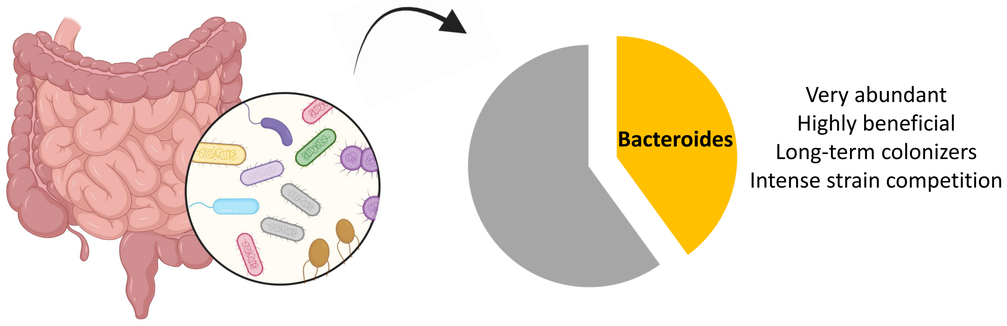
The human gut is a highly competitive environment

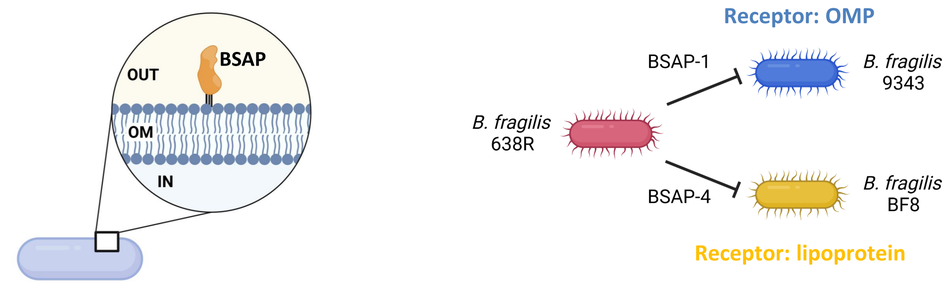


Bacteroides strain selection over time

What drives Bacteroides intra-species competition?

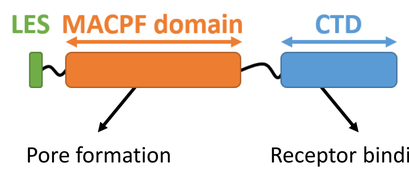
1. BSAPs: a novel class of bacterial pore-forming toxins

Bacteroidales-Secreted Antimicrobial Proteins



BSAPs are bactericidal, surface exposed lipoproteins

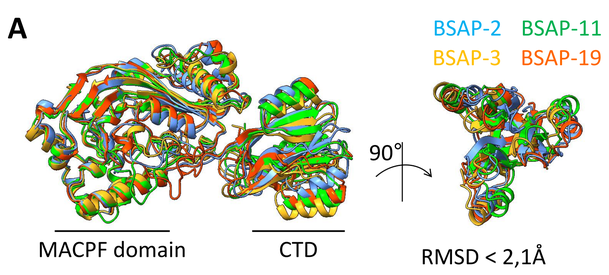
BSAPs are crucial for intra-species competition and are highly specific to their receptors



BSAPs possess a Lipoprotein Export Signal (**LES**) for surface localization, a **MACPF** domain for lytic pore formation, and one to several C-terminal domains (**CTDs**) of unknown function.

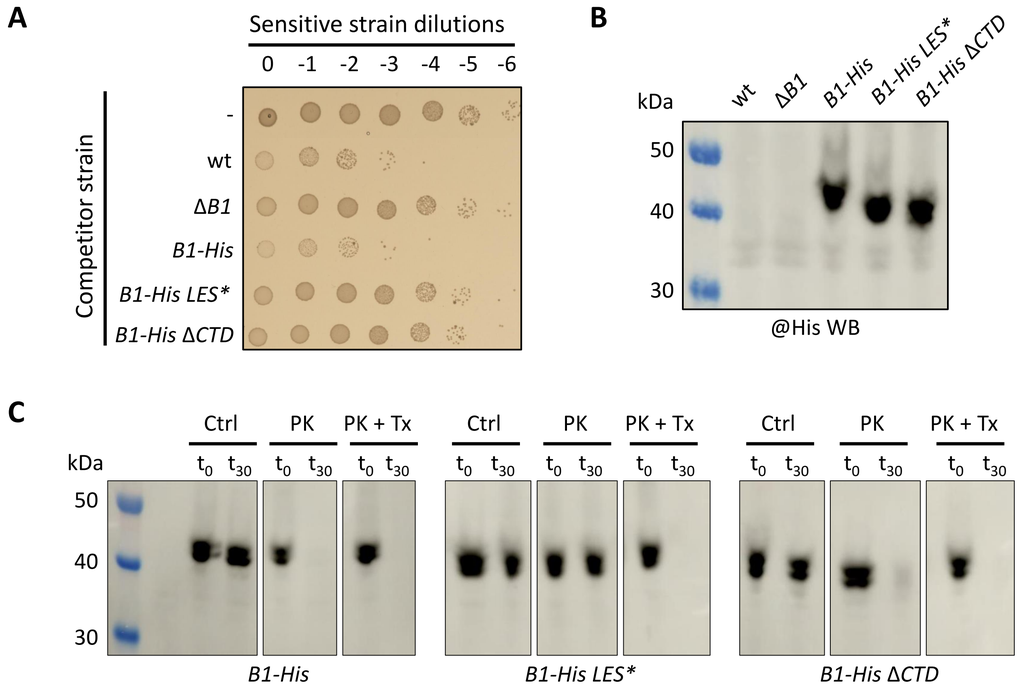
How do BSAPs bind their receptors with high specificity?

2. BSAPs possess a CTD-structure / receptor type relationship



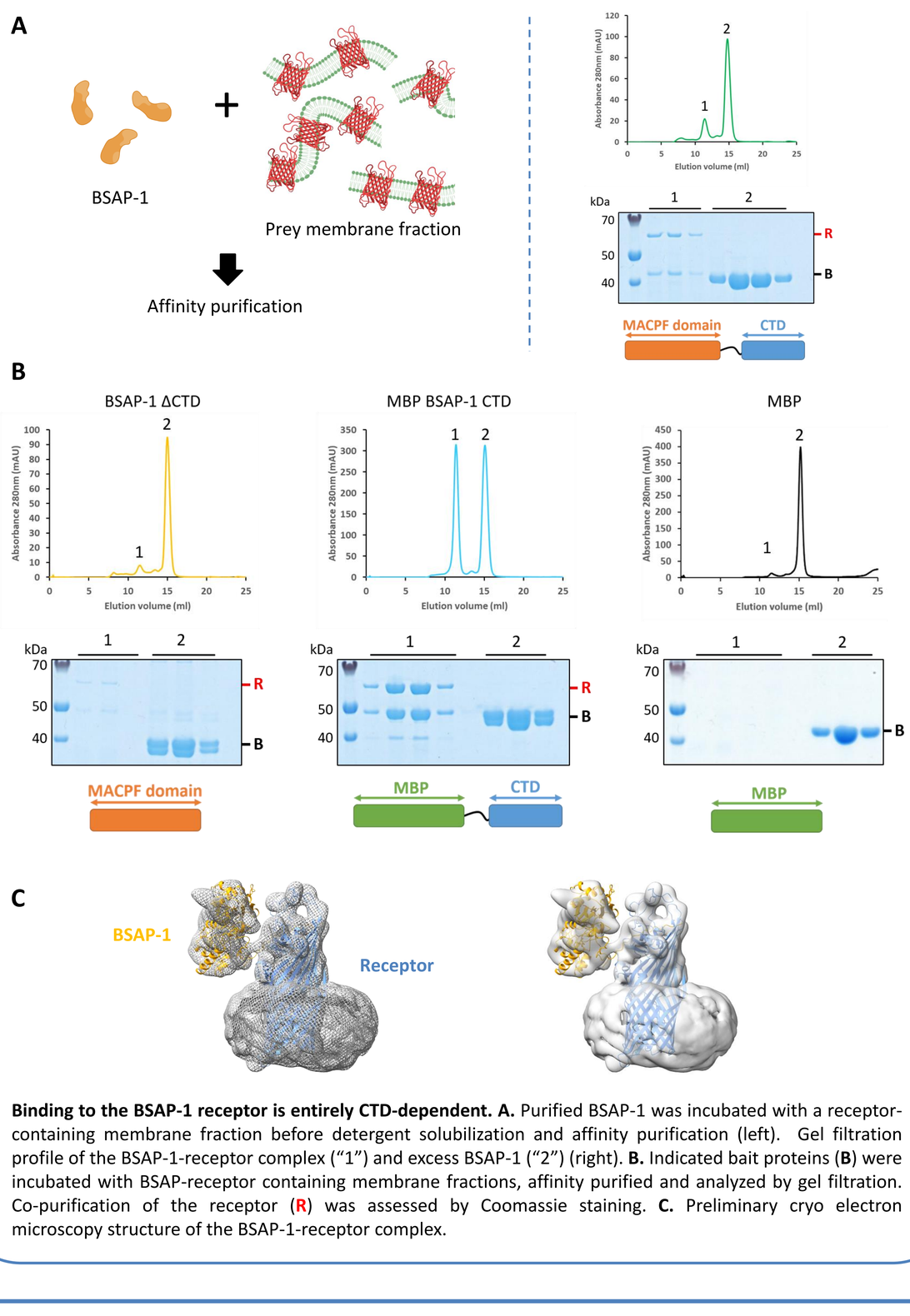
Structural classification of BSAP CTDs. A. Structural comparison of experimentally validated LPS-targeting BSAPs. **B.** The CTDs of 175 non-redundant BSAPs were retrieved, structurally predicted via AlphaFold2[1], and clustered following all-vs-all comparison. The putative receptor type is indicated for each cluster.

3. The BSAP-1 CTD is essential for bactericidal activity



Deleting the CTD of the model protein BSAP-1 prevents its bactericidal activity *in vivo*. A. Survival analysis of a BSAP-1 sensitive strain following co-culture with competitor strains expressing the indicated BSAP-1 derivatives. **B.** Immunoblot analysis of total cell extracts of competitor strains used in A. **C.** Cell surface exposure of BSAP-1 derivatives assessed by Proteinase K shaving of intact cells. Representative results are shown for all experiments.

4. The BSAP-1 CTD drives receptor complex formation



Binding to the BSAP-1 receptor is entirely CTD-dependent. A. Purified BSAP-1 was incubated with a receptor-containing membrane fraction before detergent solubilization and affinity purification (left). Gel filtration profile of the BSAP-1-receptor complex ("1") and excess BSAP-1 ("2") (right). **B.** Indicated bait proteins (**B**) were incubated with BSAP-receptor containing membrane fractions, affinity purified and analyzed by gel filtration. Co-purification of the receptor (**R**) was assessed by Coomassie staining. **C.** Preliminary cryo electron microscopy structure of the BSAP-1-receptor complex.

Conclusions

- The specificity of BSAPs is entirely provided by their CTD
- The CTD is sufficient for receptor recognition and formation of a stable complex
- BSAPs can be classified according to their CTDs and exhibit a clear CTD-structure/receptor relationship

Reference
[1] Jumper, J. *et al*. Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).

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