Mechanistic Insights Into (p)ppGpp Mediated Survival of *Neisseria meningitidis* Under Nutrient Stress

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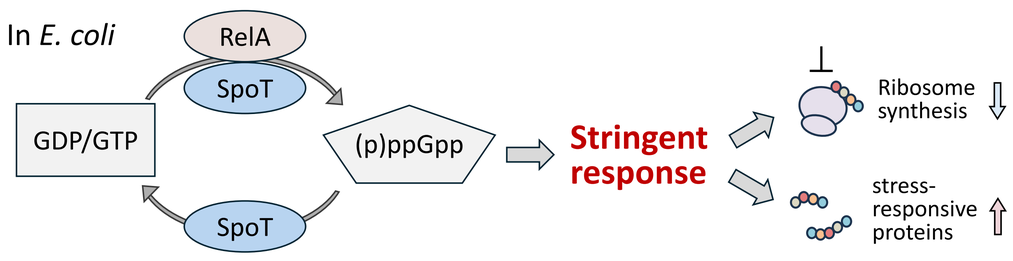
Background

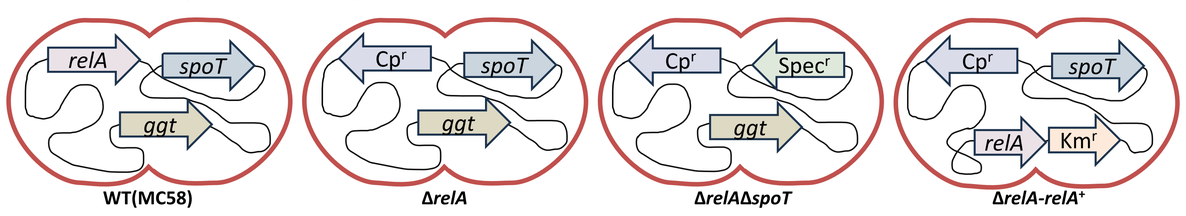
Invasive meningococcal disease caused by *Neisseria meningitidis* is associated with a high mortality rate. During infection, *N. meningitidis* encounters a range of microenvironments involving fluctuations in the availability of carbon and nitrogen source(1) such as respiratory mucosa. It must survive and colonize in such environments. Therefore, **the bacterial response to nutrient stress** is essential. One of the well-known stress adaptation systems in bacteria is the **stringent response**, mediated by the second messenger (p)ppGpp. In *E. coli*, stress tolerance is enhanced by (p)ppGpp through a **global resource re-allocation** from ribosome synthesis to the synthesis of stress-responsive proteins(2). However, many aspects in *N. meningitidis* remain unrevealed. This study aimed to investigate how **(p)ppGpp contributes to the survival** of *N. meningitidis* under **amino acid starving conditions**.



Materials and Methods

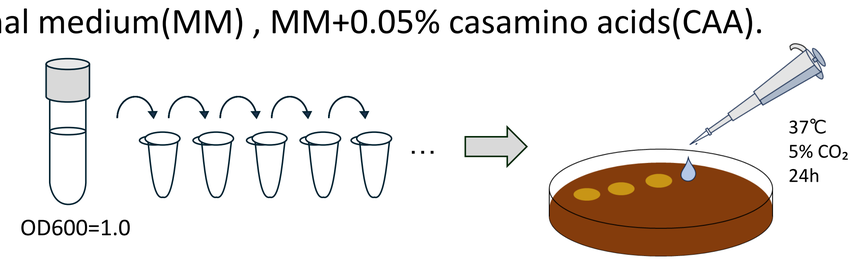
Bacterial strains

N. meningitidis strain MC58 was used as a parental strain. A (p)ppGpp synthase gene deleted mutant (Δ*relA*), a double mutant lacking both the synthase and hydrolase genes (Δ*relA*Δ*spoT*), and a Δ*relA* complemented with *relA*$^+$ strain (Δ*relA*-*relA*$^+$) were constructed.



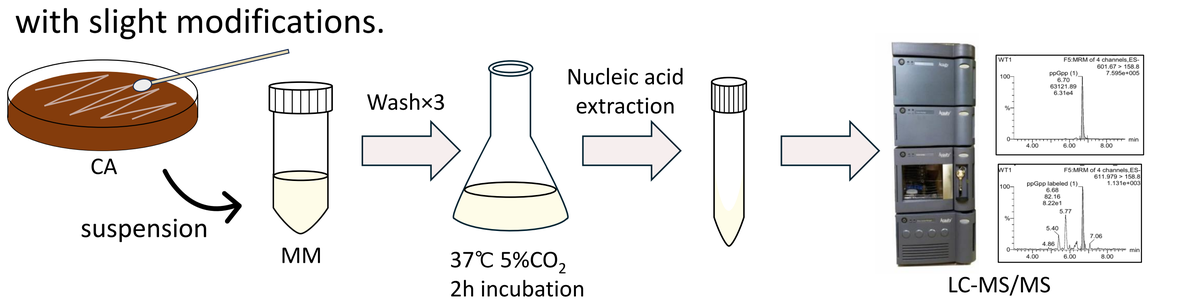
Growth kinetics

A serial dilution of the bacteria was dropped onto Chocolate agar(CA), Minimal medium(MM) , MM+0.05% casamino acids(CAA).



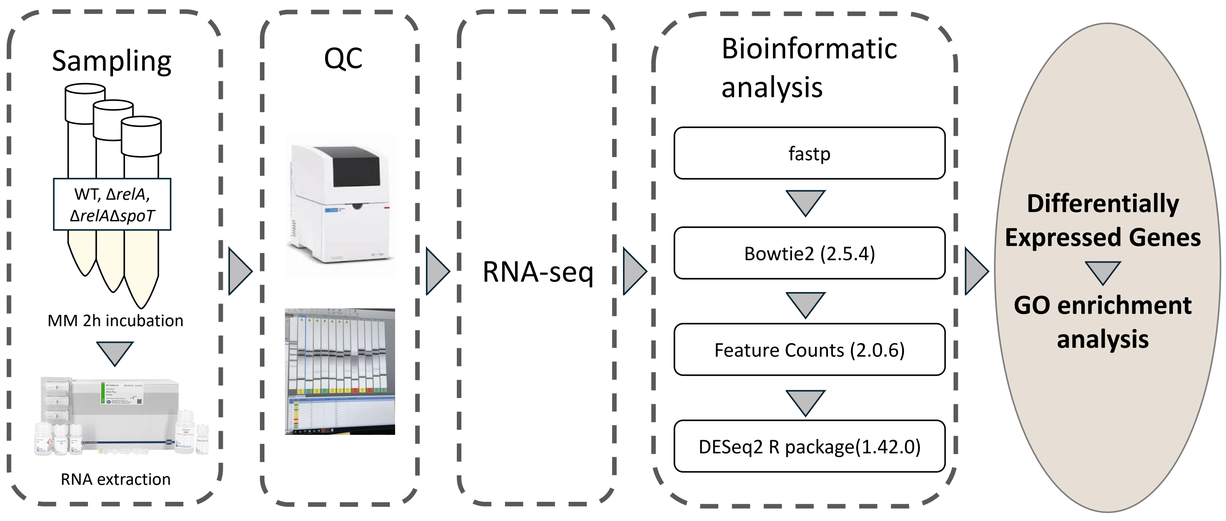
ppGpp quantification

ppGpp extraction and quantification were performed as previously reported(3), with slight modifications.



Transcriptomic analysis

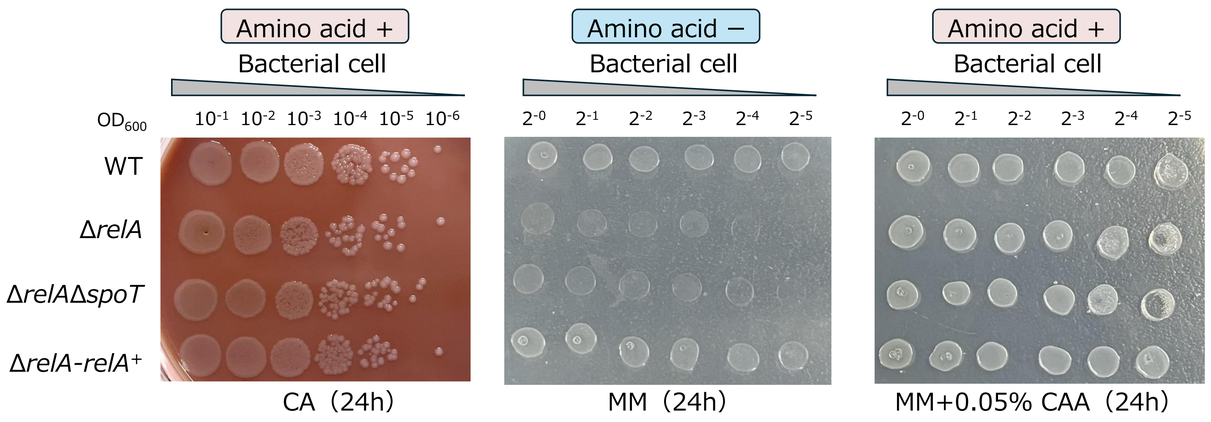
The experiment was performed according to the workflow shown below.



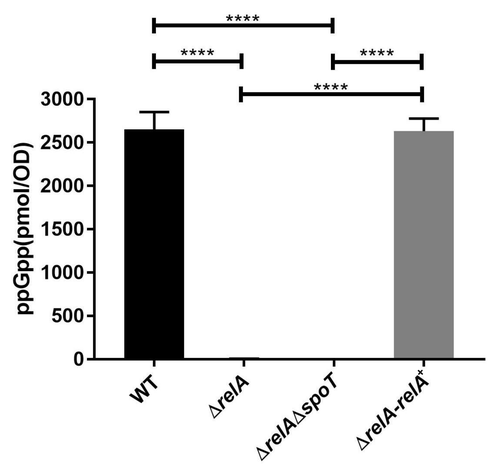
Results

Growth kinetics

Both Δ*relA* and Δ*relA*Δ*spoT* mutants exhibited **growth defects** under amino acid starving conditions.



ppGpp quantification

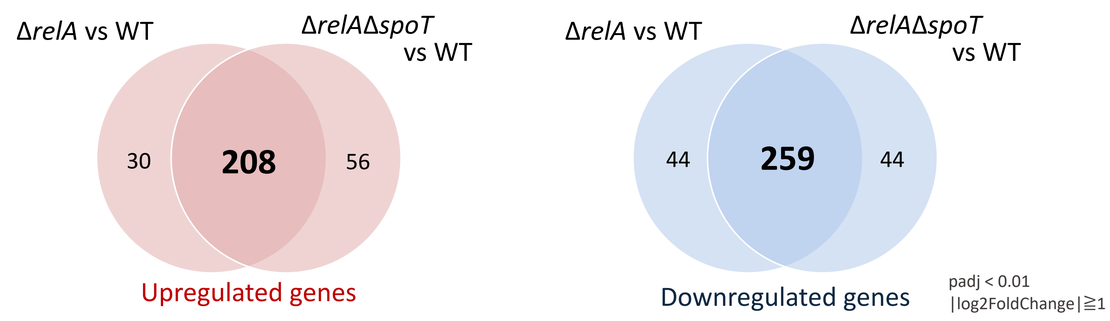


Both Δ*relA* and Δ*relA*Δ*spoT* mutants exhibited significantly **reduced ppGpp levels** under amino acid starving conditions.

N=3, Mean±SEM,
One-way ANOVA, Tukey test,
****p<0.0001

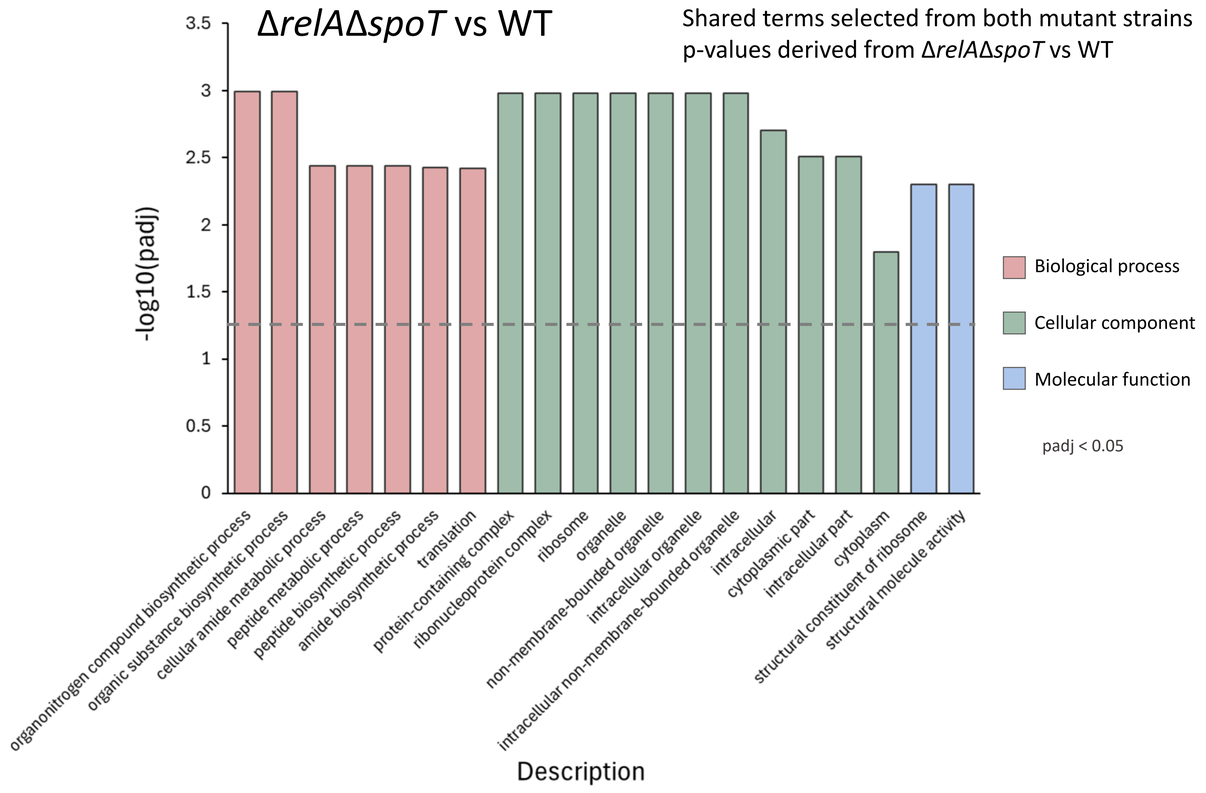
Differentially expressed genes

Transcriptomic analysis revealed 541 and 567 differentially expressed genes in the Δ*relA* and Δ*relA*Δ*spoT* mutants, respectively.



Transcriptomic analysis

Gene Ontology (GO) enrichment analysis showed significant enrichment of **protein translation-related GO terms** (e.g., cellular component, ribosome GO:0005840) in both mutants.



Conclusion

These findings suggest that *N. meningitidis* modulates metabolic processes via (p)ppGpp-dependent **regulation of protein translation** during amino acid starvation to **promote survival**.

References

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2. Zhu M, Mu H, Dai X. Integrated control of bacterial growth and stress response by (p)ppGpp in *Escherichia coli*: A seesaw fashion. iScience. 2024 Jan 9;27(2):108818.
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Conflicts of interest: The authors declare that there are no conflicts of interest.
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