Therapeutic efficacy of bacteriophage cocktail against multidrug resistant *Streptococcus agalactiae* infection in *Oreochromis niloticus*

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Background

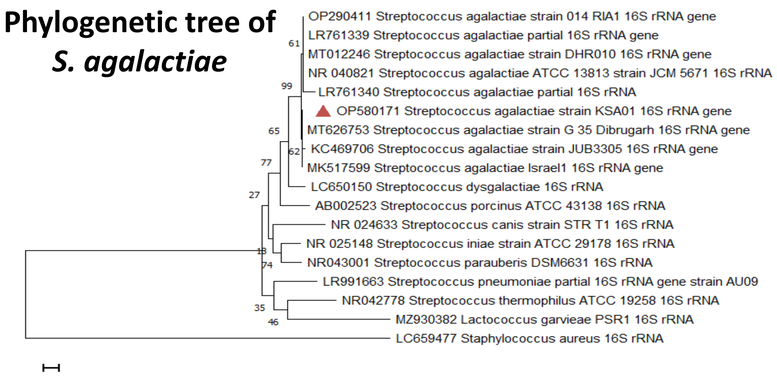
- In aquaculture, **bacterial infections** continue to be a serious economic issue around the world.
- Among the bacterial infections, Streptococcosis caused by ***Streptococcus agalactiae*** is a major one affecting aquaculture worldwide.
- Propelled by the overuse and abuse of **antibiotics** in the aquaculture systems, there is a wide spread of **antibiotic resistant bacteria** in both human and environment, reaching through food chain and recreational activities.
- Aquaculture systems have been identified as the hotspots for **multi-drug resistant (MDR)** bacteria and **antimicrobial resistant (AMR) genes** due to the extensive and indiscriminate use of antibiotics against bacterial pathogens.
- In this scenario, the current aquaculture sector is in urge for an alternate **biocontrol agent**, which helps in supporting the fish health and a healthy environment.
- **Bacteriophage therapy** is a promising alternative strategy to antibiotic resistance and is getting more magnetism due its abundance, host specificity and self-limiting ability
- However, one of the obstacles to phage therapy is **phage resistance**, and it can be acquired through genetic mutations, followed by consequences of phenotypic variations
- **Phage cocktail** composed of two or more bacteriophages can be used for broader spectra of activity and to achieve effectiveness under a greater diversity of conditions and to suppress the emergence of anti-phage resistance in bacteria.
- This study aims to formulate a phage cocktail of five lytic phages (previously isolated and characterize d phages) and to evaluate its **therapeutic potential (*in vivo*)** against *Streptococcus agalactiae* in tilapia (*Oreochromis niloticus*).

Methodology

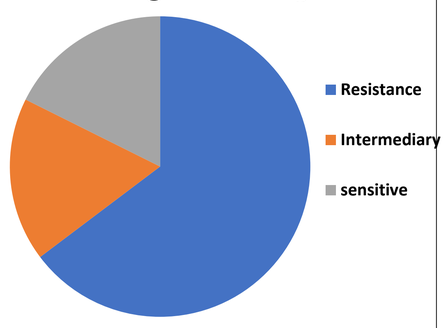
1. **Isolation and molecular confirmation** of *S. agalactiae* from infected tilapia farm, Kerala, India
   - Screening of **antibiotic susceptibility** of *S. agalactiiae* (CLSI, 2021)
2. **Isolation and purification of bacteriophage** against *S. agalactiae* (Adams, 1959)
   - Primary host *Streptococcus agalactiae* KSA/01 (Genbank Acc. OP580171)
3. **Characterization**
   - Growth kinetics- one step growth curve, thermal and pH stability tests
   - Morphology- High resolution Transmission Electron Microscopy (HR-TEM)
   - Genetic diversity- Random Amplified Polymorphic DNA Fingerprinting- RAPD (Shivu et al., 2007)
4. ***In vitro* lytic potential of phages-** monophage and phage cocktail- Turbidity reduction method (Kusradze et al., 2016)- UV-Vis Spectrophotometric measurement (600 nm)
5. **Challenge experiment in tilapia** (*Oreochromis niloticus*) (Khalifa et al., 2018)
   - Fish control (Group A), Bacterial control (Group B), Phage therapy (Group C), Phage control (Group D)
   - Bath therapy- 200 µl/l water
   - (Ethical clearance- 174/ac/08/CPCSEA)
6. **Relative Percent Survival (RPS)**= [1 − (% mortality in test group/% mortality in control group)] × 100

Results

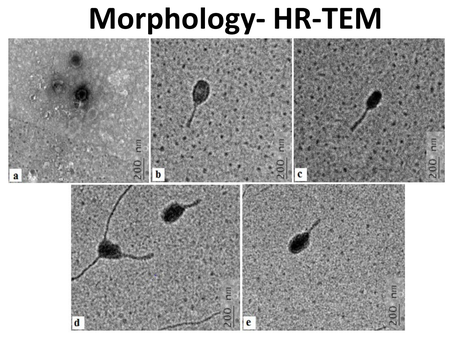
Phylogenetic tree of *S. agalactiae*



Antibiogram of *S. agalactiae*

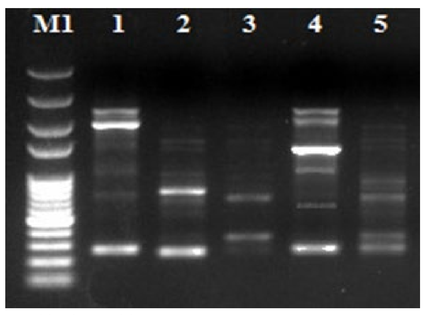


Morphology- HR-TEM



a- SAP1, b- SAP2, c- SAP3, d- SAP4, e- SAP5

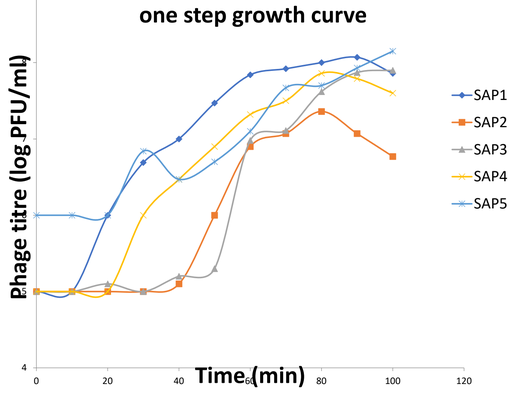
Genetic diversity- RAPD Fingerprinting



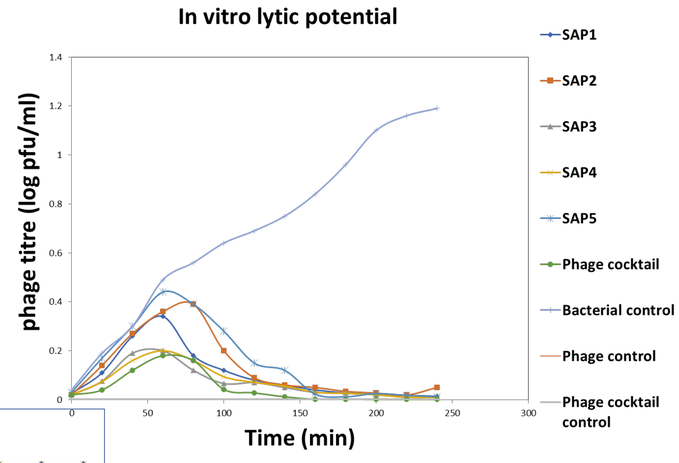
Lane: M1- 1kb plus Ladder, 1- SAP1, 2- SAP2, 3- SAP3, 4- SAP4, 5- SAP5

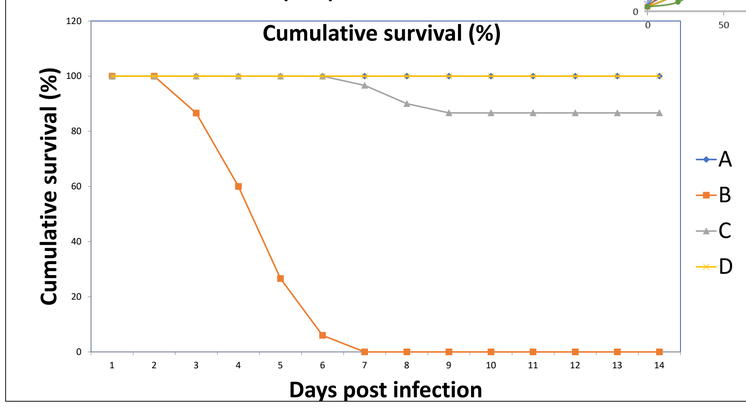
Phage	Transmission electron microscopy (TEM)				
	Head diameter (nm)	Tail length (nm)	Properties	Family	Order
SAP1	101.52±2	62.60±1	Isometric head and non-contractile tail	Myoviridae	Caudovirales
SAP2	83.34±0.2	128.73±0.1	Isometric head and contractile tail	Siphoviridae	Caudovirales
SAP3	66.61±0.01	152.03±0.1	Icosahedral head and contractile tail	Siphoviridae	Caudovirales
SAP4	71.07±0.21	161.63±0.2	Isometric head and contractile tail	Siphoviridae	Caudovirales
SAP5	79.88±0.3	98.26±0.1	Isometric head and non-contractile tail	Myoviridae	Caudovirales

one step growth curve



In vitro lytic potential





Challenge Experiment in *S. agalactiae* infected *Oreochromis niloticus*

Relative percent survival (%)- 86.68%

Conclusion

- Phage therapy, an alternative therapeutic measure is effective in controlling the bacterial infection and it will be **an effective and safe strategy** with **zero impact on both fish health and environment**.
- In this study, **five genetically diverse phages** were formulated as a **phage cocktail** against multidrug resistant *S. agalactiae*. In challenge study, the bacteria got eliminated from the host animal with a **significant reduction in mortality** in comparison with bacterial control
- Thus phage cocktail is found to be an effective therapeutic strategy to control the *S. agalactiae* infection in tilapia. In addition, phage cocktail was found to be safer as it provide **no possibility for the development of phage resistance in bacteria**.

Acknowledgements

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