



Bioprospecting of culturable marine biofilm bacteria for novel antimicrobial peptides

Shen Fan¹, Peng Qin¹, Jie Lu¹, Shuaitao Wang¹, Jie Zhang¹, Wei Ding^{2*}, Weipeng Zhang^{1*}



¹MOE Key Laboratory of Evolution & Marine Biodiversity and Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao, China

²MOE Key Laboratory of Marine Genetics & Breeding and College of Marine Life Sciences, Ocean University of China, Qingdao, China

³College of Pulmonary & Critical Care Medicine, Chinese PLA General Hospital, Beijing, China

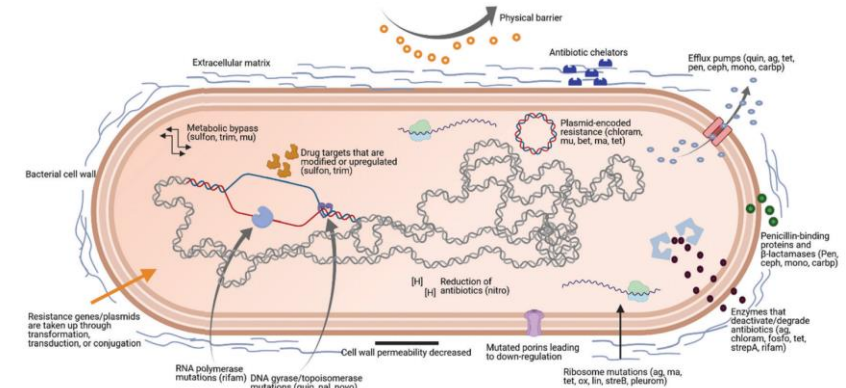
⁴MOE Key Laboratory of Marine Genetics & Breeding and College of Marine Life Sciences, Ocean University of China, Qingdao, China

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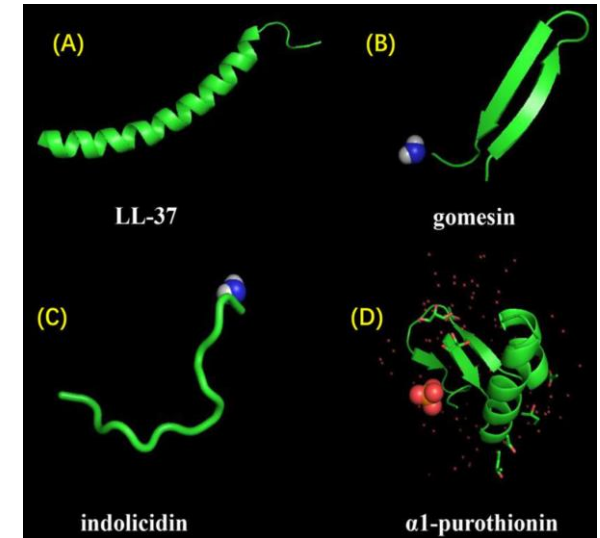
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Application potential of AMPs

- The curative effect of traditional antibiotics on drug-resistant bacteria is declining, and antibiotic resistance has become a major public health threat.
- Antimicrobial peptide (AMPs) is an amphipathic and broad-spectrum peptide, which is usually insensitive to the drug resistance of pathogens.
- AMP is mainly modified by non-ribosome synthesis or ribosome synthesis, and some ribosome synthetic peptides can function without modification, which makes it possible to identify AMPs directly from microbial genome.
- Deep learning can identify the sequence characteristics by self-learning sequence characteristics, which can solve the problem that short sequences limited by AMPs and low identity can't achieve accurate prediction. It provides a theoretical method for extracting AMPs from a large number of sequences.



Gan, B.H. et al. *Chemical Society reviews*. 2021

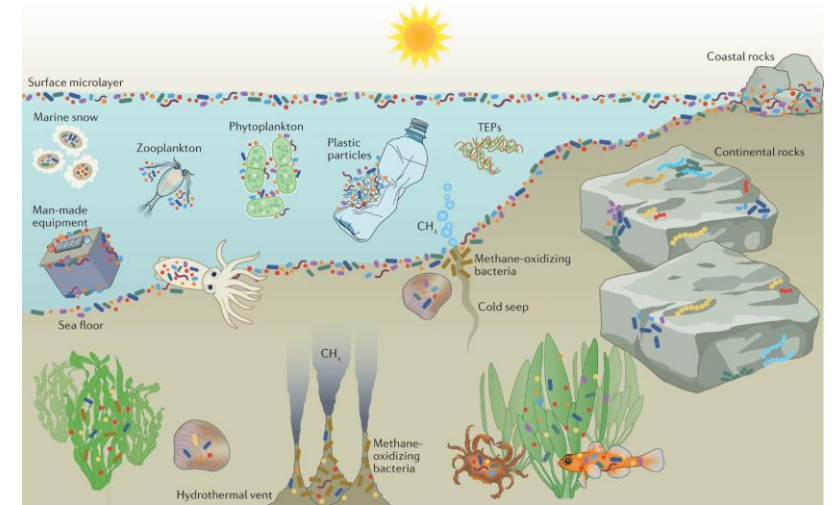


Huan Y. et al. *Front Microbiol*. 2020

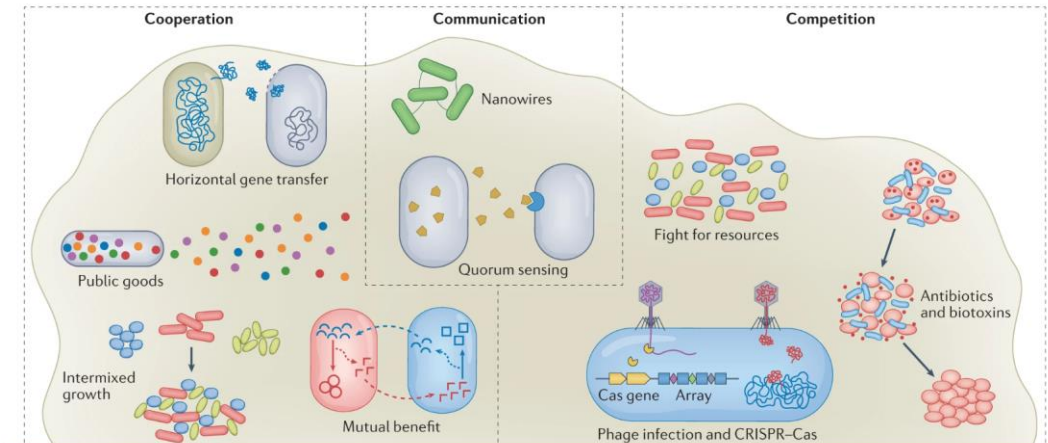


Biofilms are important reservoirs of natural products

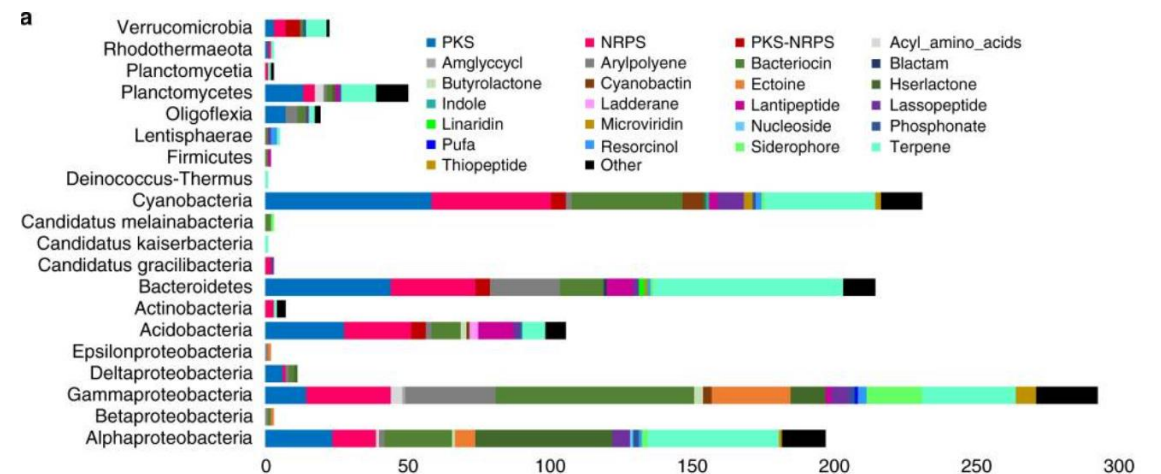
- Bacteria-derived AMPs account for only 12% of the APD3 database, while nearly 40% of AMPs are of human origin.
- Biofilms are widely present in marine environments, with a species diversity far exceeding that of free-living microbial.
- Biofilm-associated bacteria may produce various metabolites during competition, and up to 60% of gene functions in these bacteria remain unknown.
- This indicates a great potential for discovering new AMPs, making biofilms a promising reservoir of natural products and potential drug candidates.



Qian P.Y. et al. *Nat Rev Microbiol.* 2022



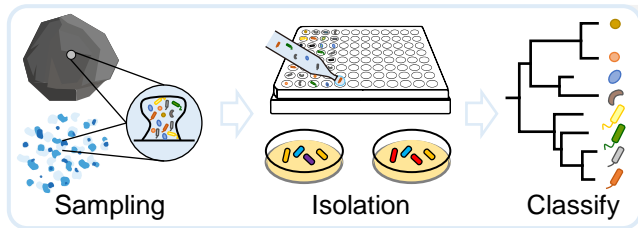
Qian P.Y. et al. *Nat Rev Microbiol.* 2022



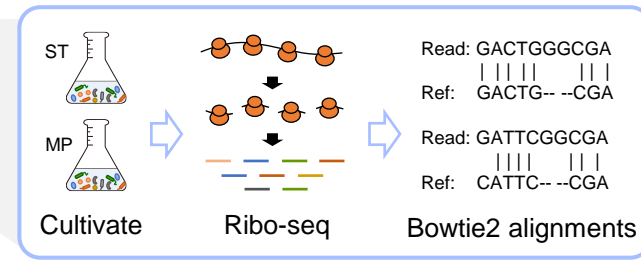
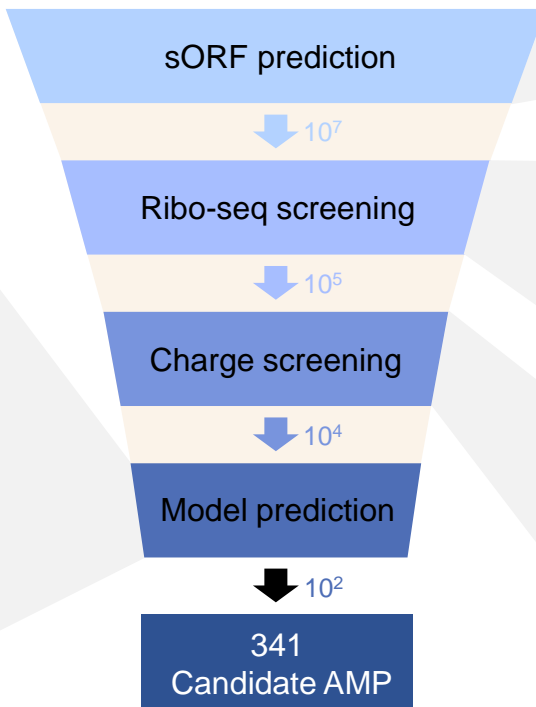
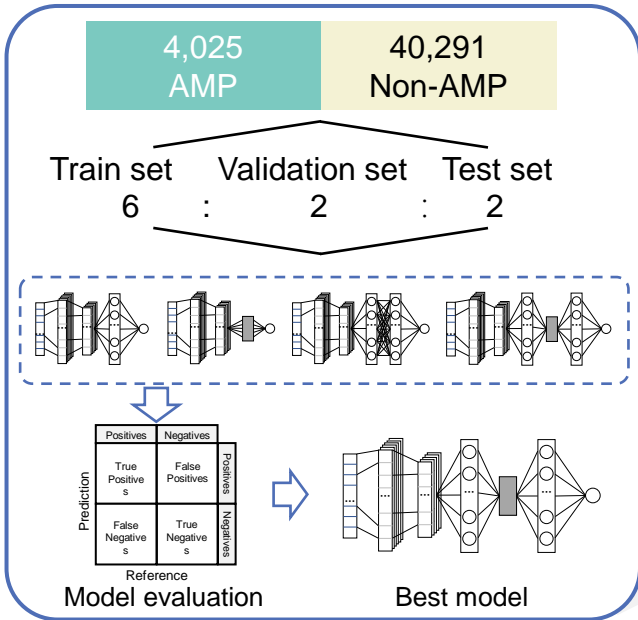
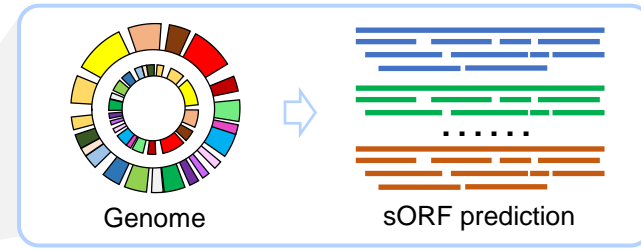
Zhang W.P. et al. *Nat Rev Commun.* 2019



AMPs identification workflow



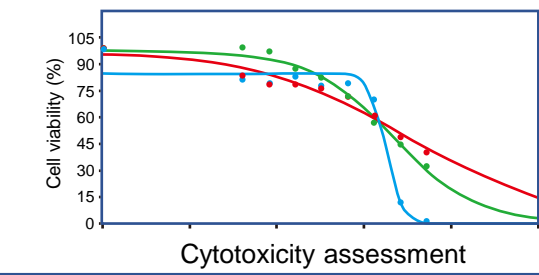
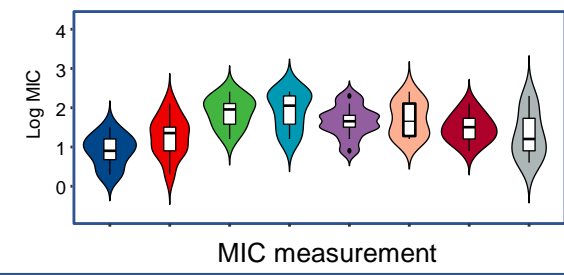
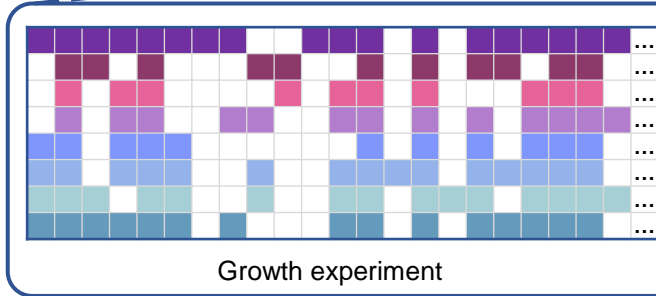
713 strains and genomes



Sequence	Len	Charge	MW	...
RKFHEKHHSHRGRYR	14	8	1875	...
GLFDIVKKVVGALGSL	16	1	1616	...
LLPNLLKSL	10	1	1123	...
FVQWFSKFLGRIL	13	2	1641	...
...

Charge > 2+ → True (Retain) / False (Abandon)

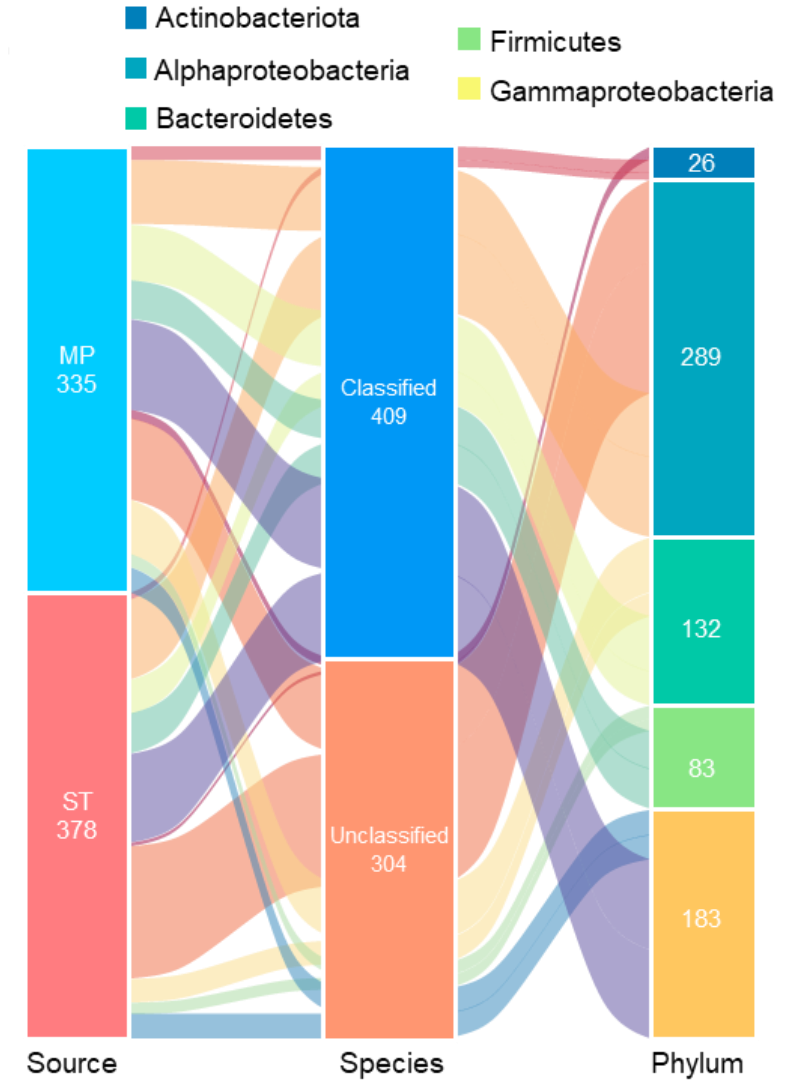
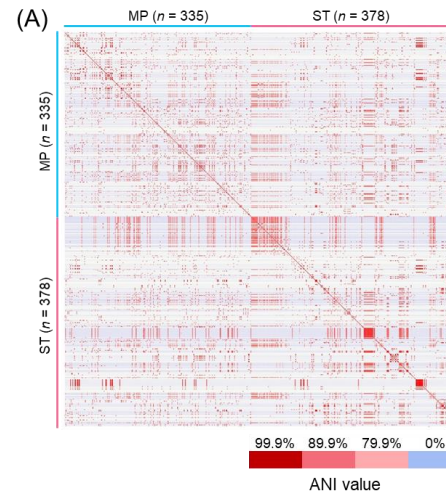
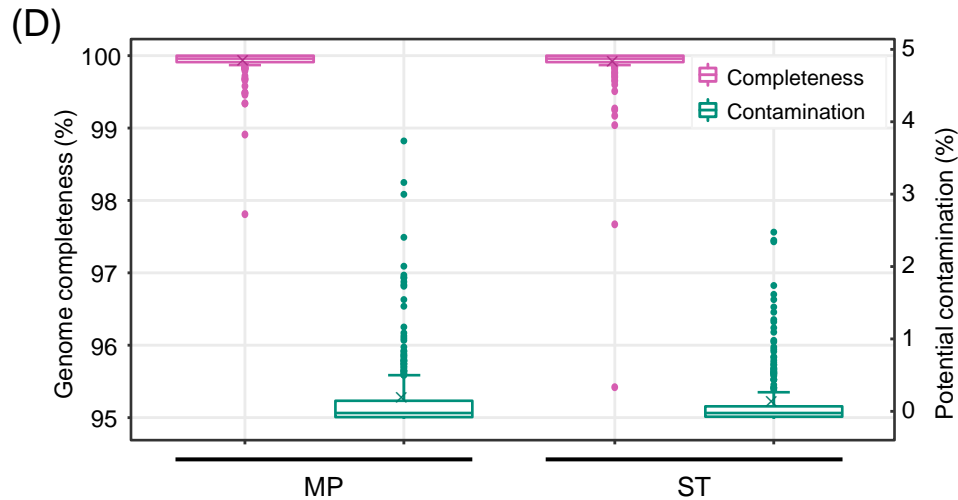
Activity verification





Marine biofilm species diversity

- A total of 713 non-redundant high-quality genomes were obtained (MP: 335; ST: 378).
- ANI analysis revealed that 93.4% of genome pairs have ANI values below 76%, indicating significant genomic differences.
- A total of 304 genomes (accounting for 42.6% of all strains) could not be classified at the species level.

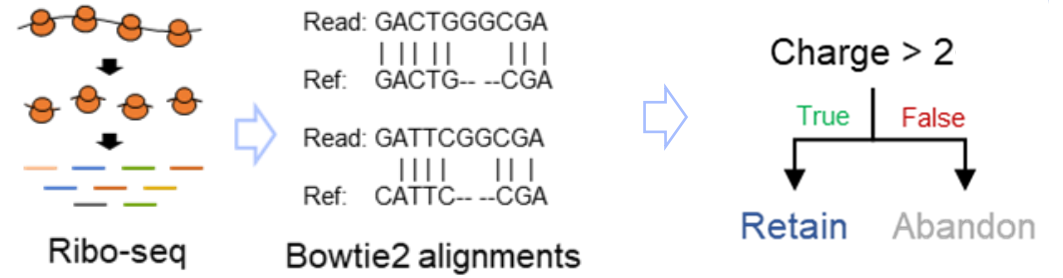
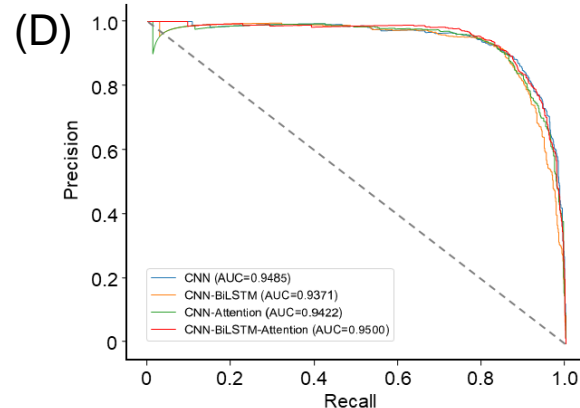
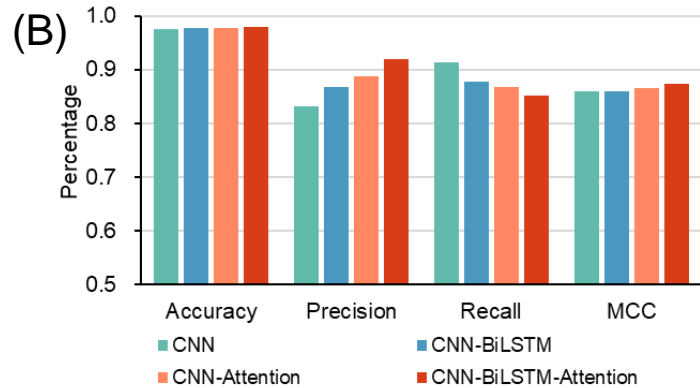
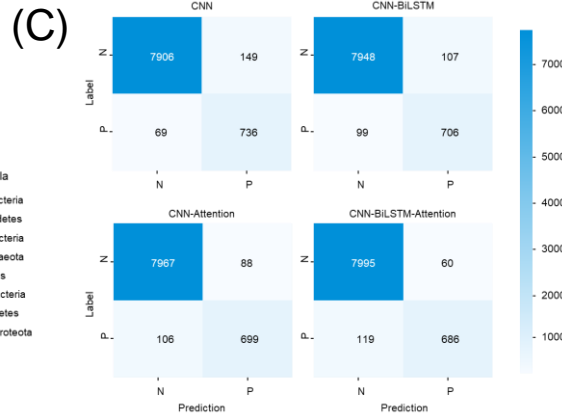
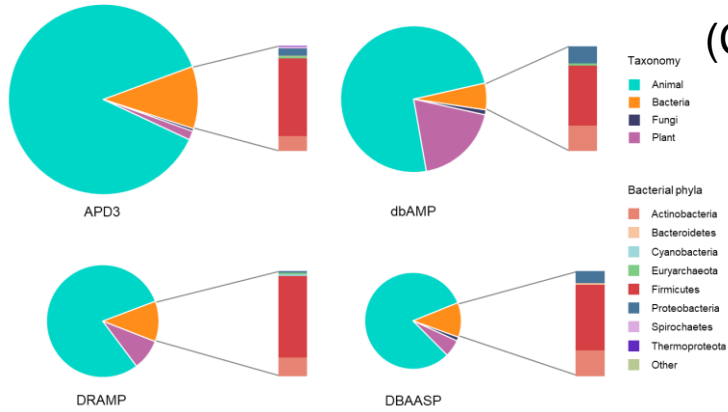


The results indicate that marine biofilm communities possess rich species diversity and novelty.



Model prediction performance

➤ Using Ribo-seq data mapped to sORFs predicted from the genome, along with net charge screening, 80,430 candidate AMPs were identified.



➤ Four deep learning models were developed using 4,025 non-redundant AMP sequences collected from public databases.

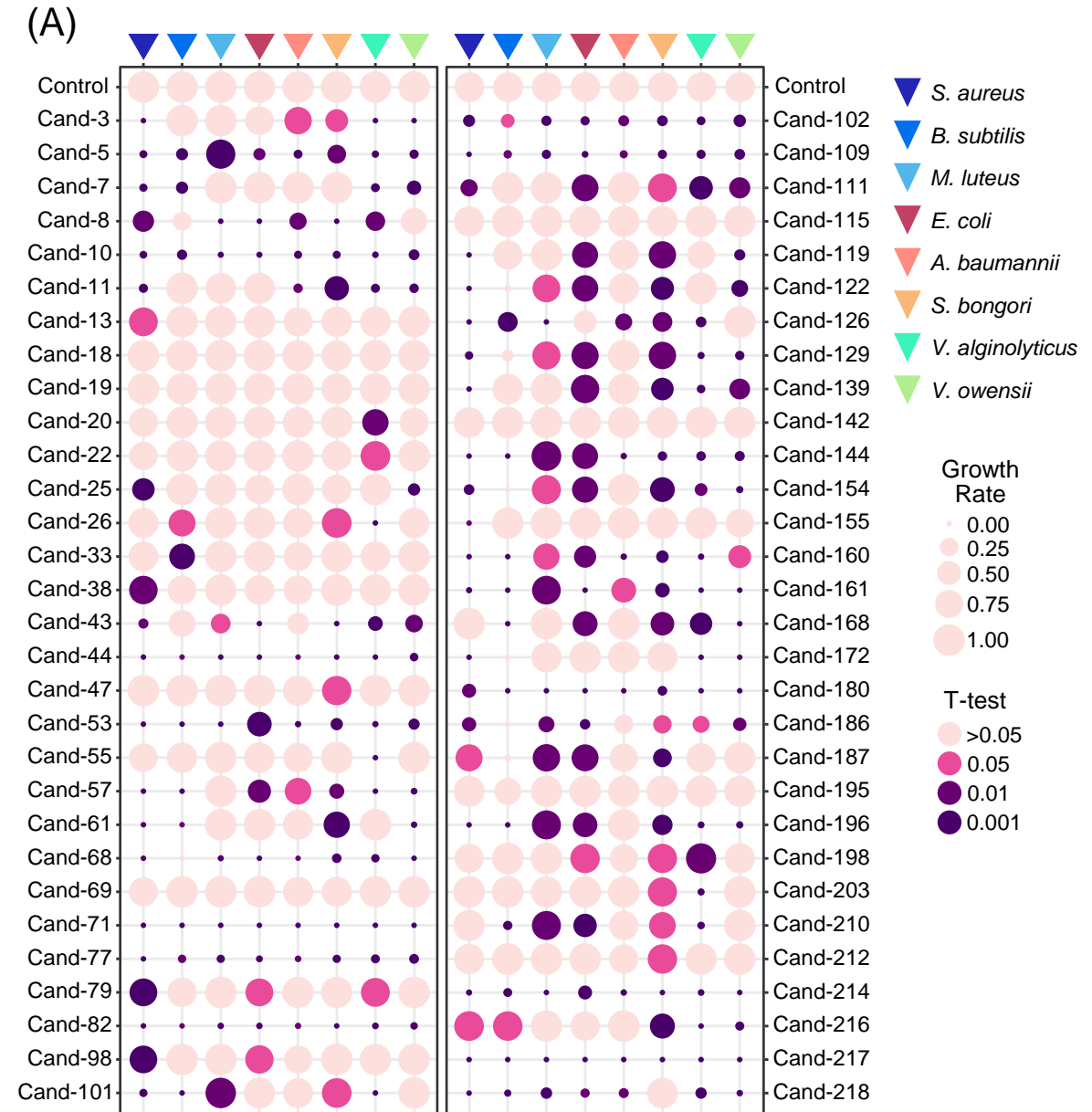
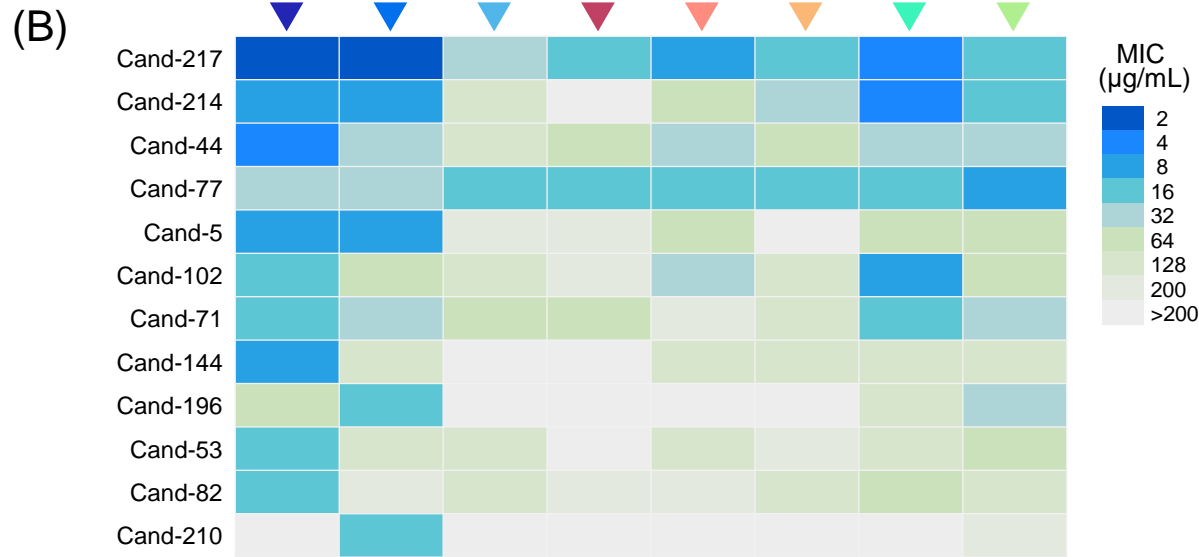
➤ the CNN-BiLSTM-Attention model achieved the highest accuracy (92%) and MCC (87%), striking a balance between precision and recall, and demonstrating the best overall performance.

We identified 341 candidate AMPs with distinct sequence characteristics from known AMPs.



Antibacterial effect of AMPs

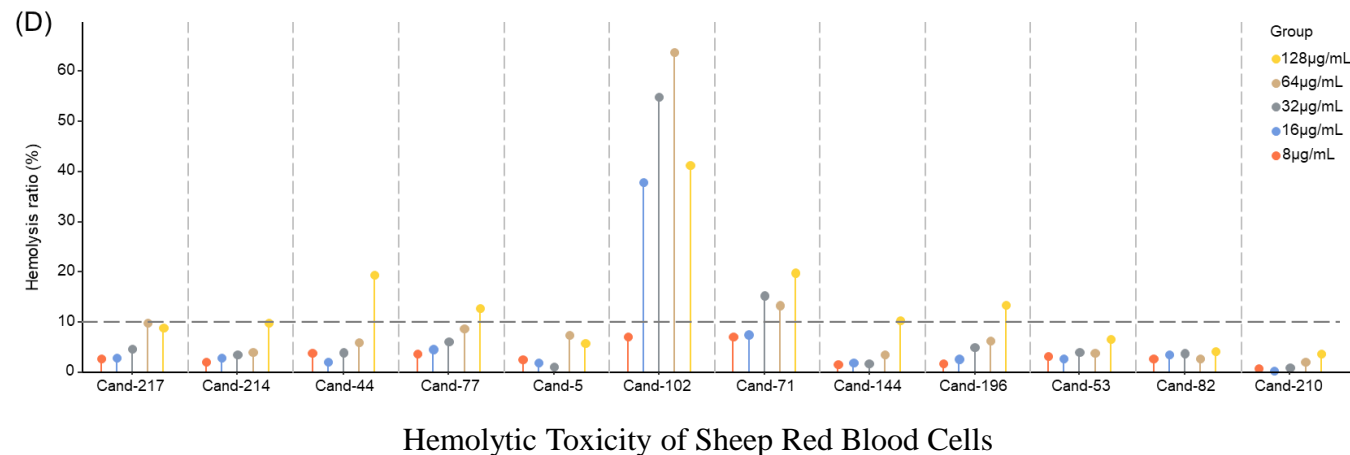
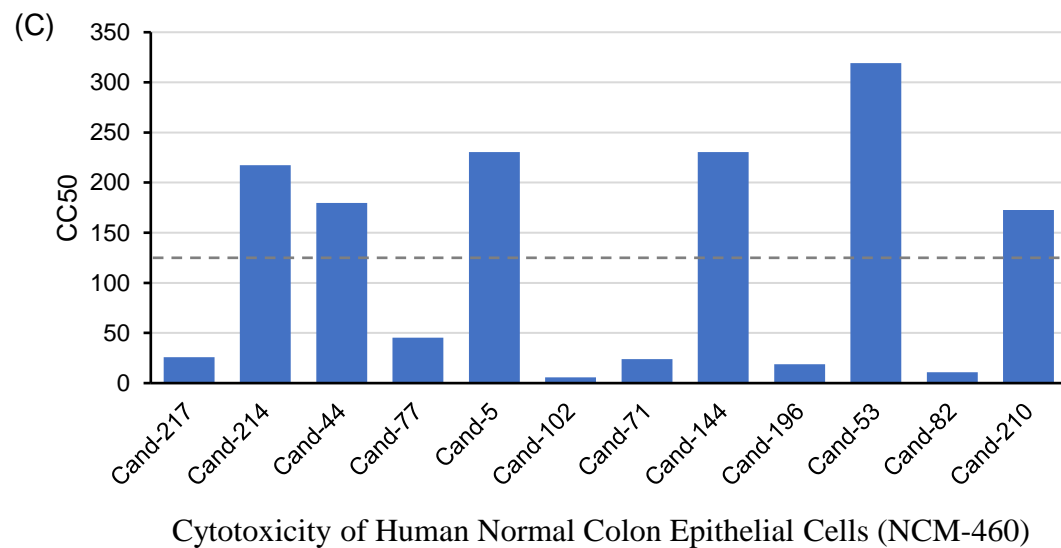
- Among the 60 candidate AMPs, 54 exhibited antimicrobial activity, resulting in an effectiveness rate of 90%.
- **MIC:** Each 12 AMPs demonstrated significant inhibitory effects against at least one bacterium ($MIC \leq 32 \mu\text{g/mL}$). 8 AMPs showed strong antibacterial activity against at least one pathogen ($MIC \leq 8 \mu\text{g/mL}$).





Cytotoxicity and hemolytic toxicity of AMPs

- **Cytotoxicity:** Among the 12 AMPs, 6 exhibited low cytotoxicity ($CC_{50} > 128 \mu\text{g/mL}$).
- **Hemolytic Toxicity:** 10 AMPs exhibited a hemolysis rate of $<10\%$ at a concentration of $64 \mu\text{g/mL}$, while 6 showed a hemolysis rate of $<10\%$ at $128 \mu\text{g/mL}$.



The results indicate that the candidate AMPs exhibit low toxicity to animal cells, demonstrating good potential for drug development.

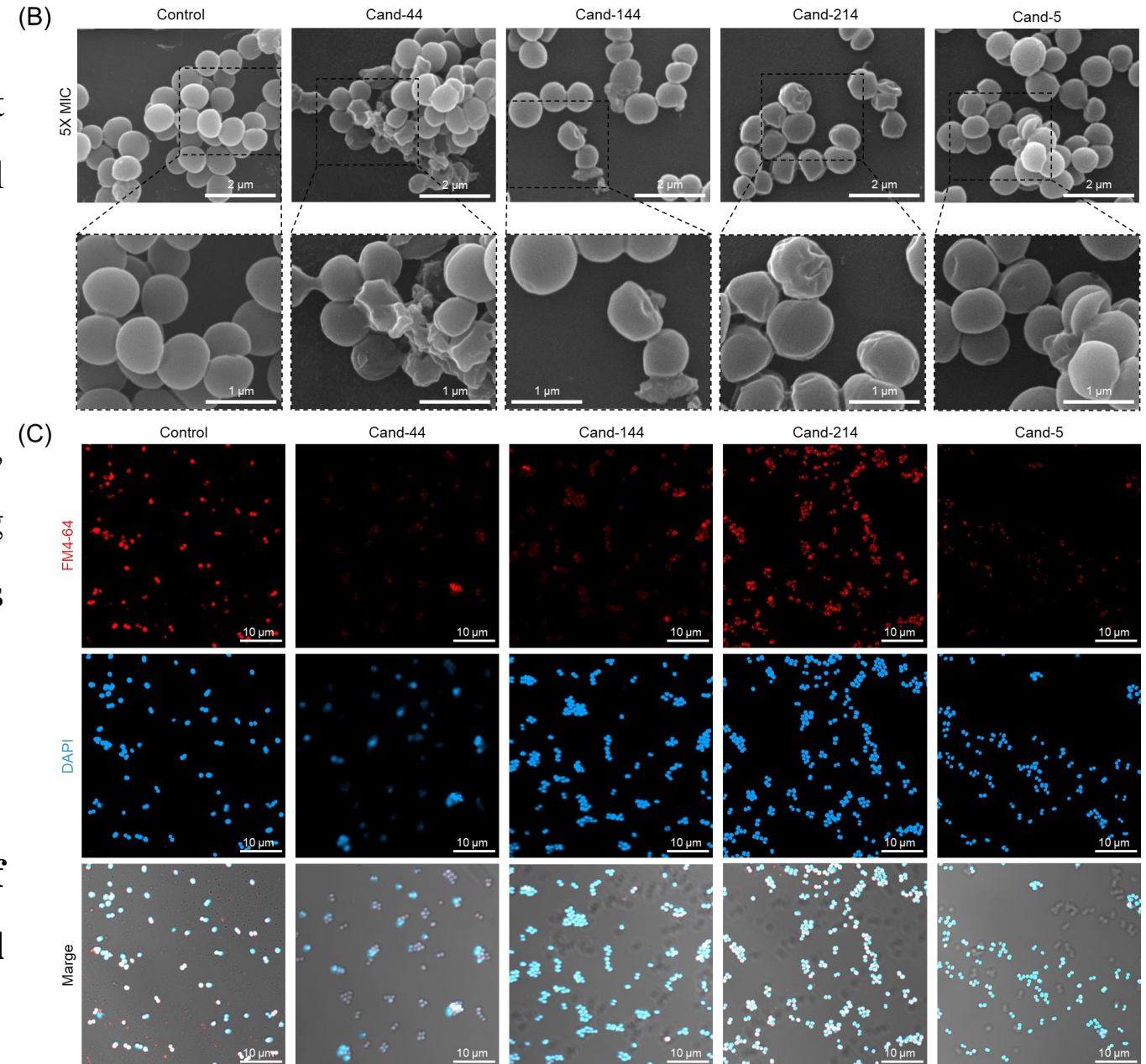


Mechanism of Action of AMPs

➤ **SEM:** Treatment with 4 AMPs caused significant shrinkage and damage to the bacterial cell membrane.

➤ **CLSM:** After treatment with four types of AMPs, there was no significant change in DAPI staining signals, while the FM4-64 staining signals (membrane dye) were significantly weakened.

The above results indicate that these 4 types of AMPs exert their effects by targeting the cell membrane of *S. aureus*.





Summary

- ❑ This study systematically explores AMPs from marine biofilms for the first time, expanding the AMP molecular library.
- ❑ The combination of deep learning and Ribo-seq analysis enables accurate and efficient prediction of AMPs, accelerating their development.
- ❑ Most AMPs in this study are highly effective against bacteria and have low toxicity, indicating their potential as antimicrobial agents.
- ❑ Marine biofilms may serve as a potential resource for developing treatments for antibiotic resistance.

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