The Research and Application of Microbial Secondary Metabolites in Drug Design and Development

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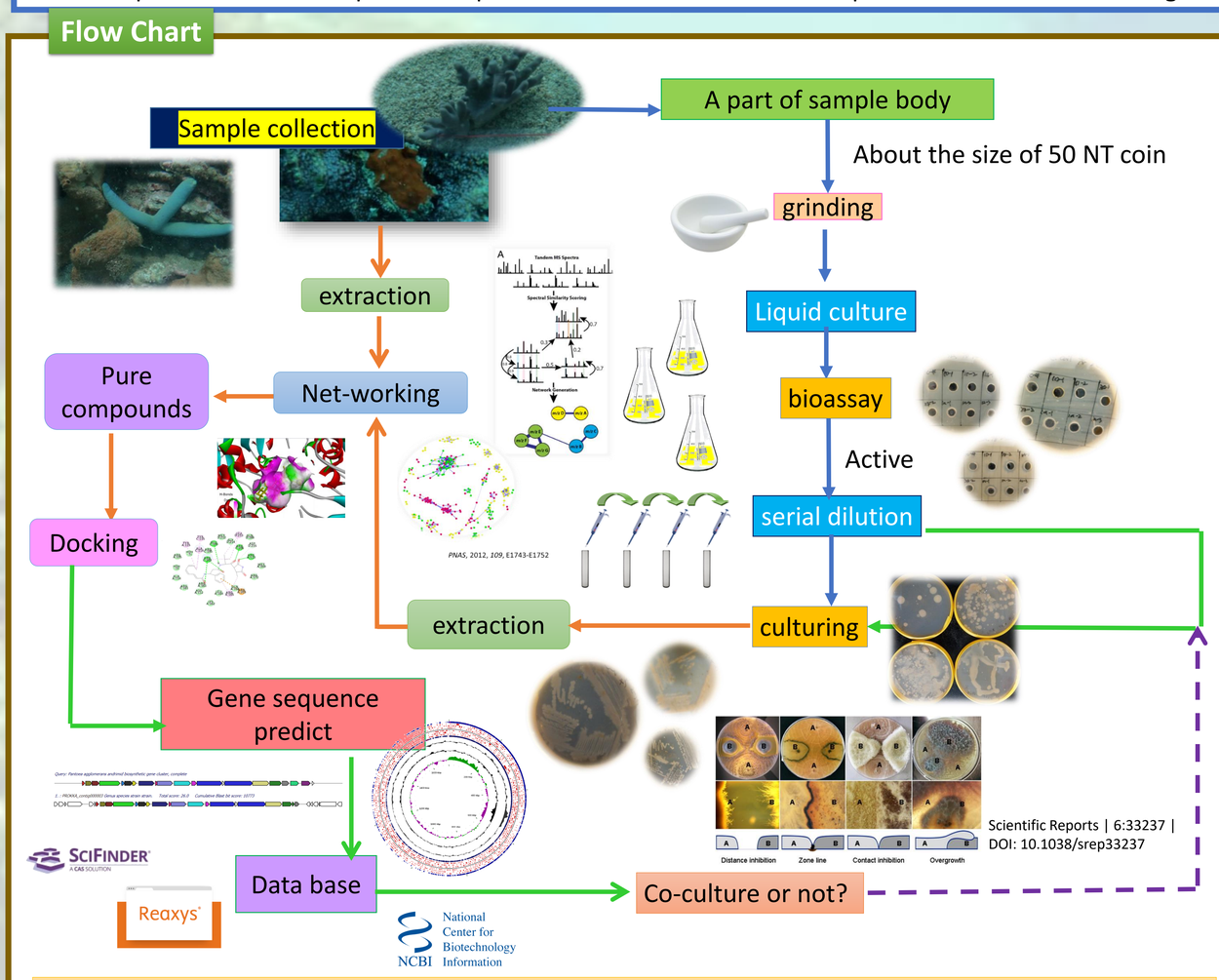
Abtract

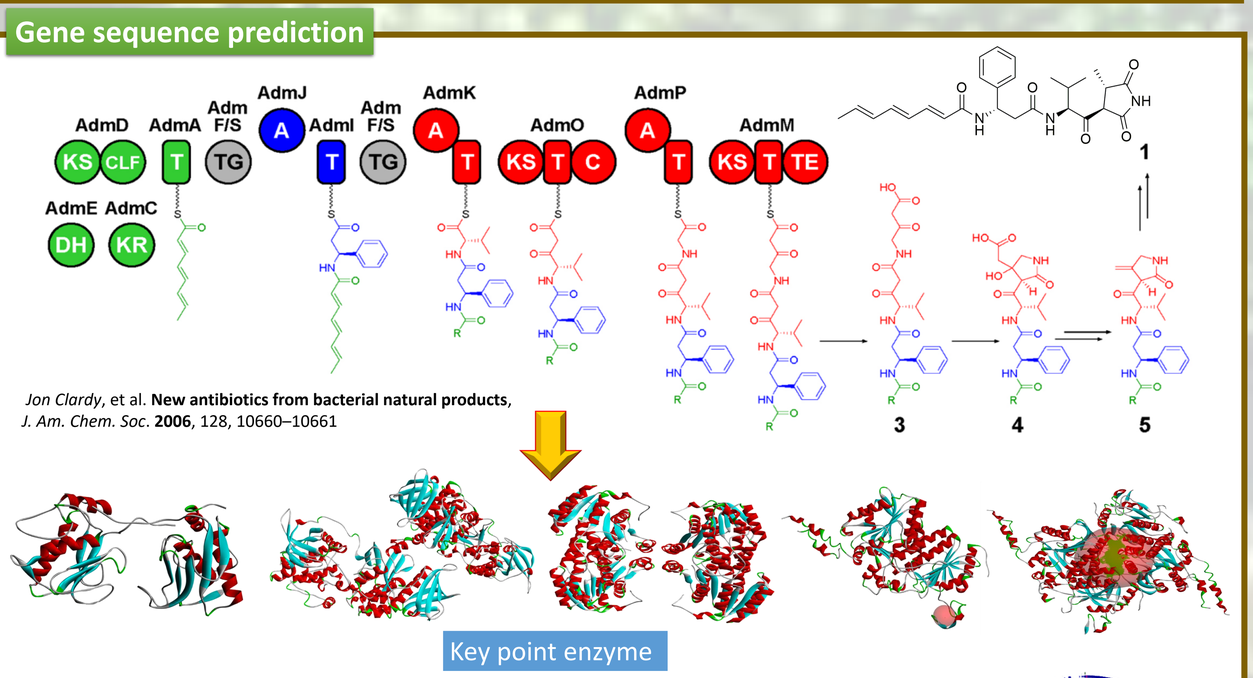
In the past study, we isolated a *Vibrio* strain (DJW-05-1) from *Agelas nemoechinata* and found it ethyl acetate extract had apparent antibacterial activity against *Staphylococcus aureus, Acinetobacter baumannii, Salmonella typhimurium,* and *Escherichia coli.* According to the mass spectral molecular network analysis, the bioactive extract contains lots of pseudopeptide compounds, such as andrimid and moiramide B. Our previous study show how to isolate such pseudopeptides, which is a good lead for developing new type of antibiotics. However, the pseudopeptides are very hard to purify because of their similar polarity and skeleton. In order to accelerate the design of the lead, we tried to modify the isolation process of the active bacterial strains, by combining with bioassay-guided fractionation isolation, molecular network analysis, and molecular modeling (docking) technology. Based on the new method, we hoped to save the time on the isolation of active bacterial strains and found the relationship between these bacteria and their host. Following the prediction the type of bioactive compounds by molecular networking, we might further search the key enzymes of their biosynthesis pathway and DNA sequence sets by bioinformatic data mining to predict/identify the bacterial species. It will be helpful to adapt the culture medium for the suspected bacteria for searching bioactive compounds.

Flow Chart

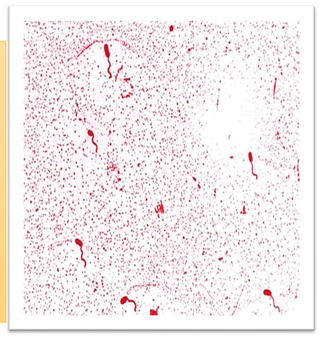


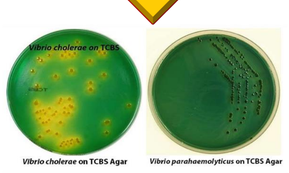
In the past, we would isolate the bacteria from the marine sample after making sure their antibacterial active. However, it would consume much resource on culturing the microorganism even if they didn't have any activity against the indicator bacteria. For saving these resource, we would directly culture the bacteria in the broth when we got the tissue of the marine sample. If these broth have antibacterial activity, we just isolate the bacteria from them. According to the result of net-working, we can know which kinds of compounds may be in the bacterial extracts, and predict the possible gene sequence for searching the possible species. By the information above, we can find the best culturing condition for this bacterial easily. Some active compounds produced from the microorganism might only have a few amount or be difficult to isolation. So as that, we need the molecular net-working to predict possible compounds from bacteria. These structure can be used to build model of molecular docking. The model would be helpful to make the SAR for new drug development.

Gene sequence prediction



After we isolated or predicted the bioactive compounds by molecular network analysis, we can dig out genome sequences of the key enzymes of biosynthesis pathway. Such DNA sequences could be compared with the data base to find what kind of bacteria possesses similar sequence. Then, we can modify the medium compositions for the culture of such bacteria.



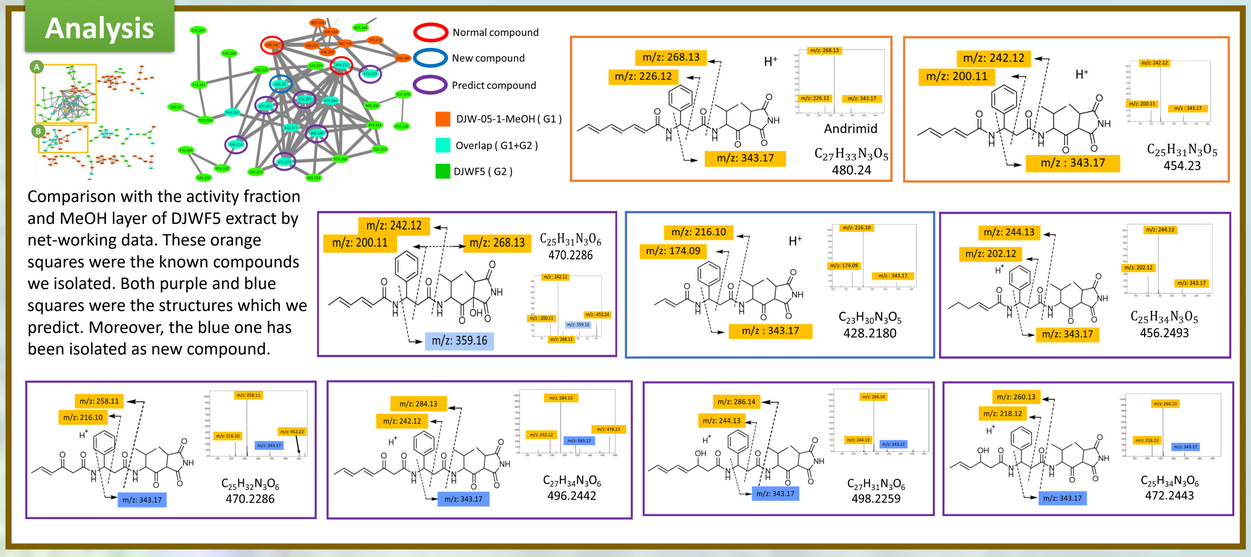


Specific medium

Active bacteria

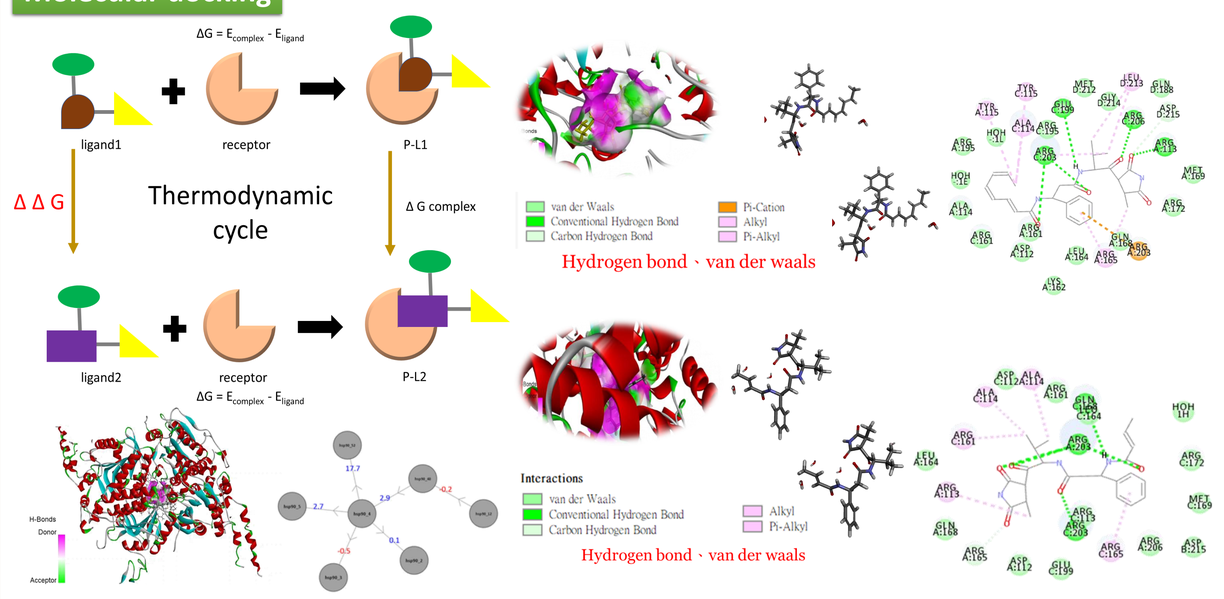
Indicator bacterium	DJW-05-1	TS1-5	E5-1
Staphylococcus aureus	V	V	V
Staphylococcus epidermidis	V		
Acinetobacter baumannii	V		
Candida albicans		V	V
Escherichia coli	V		

Net-work Analysis



Comparison with the activity fraction and MeOH layer of DJWF5 extract by net-working data. These orange squares were the known compounds we isolated. Both purple and blue squares were the structures which we predict. Moreover, the blue one has been isolated as new compound.

Molecular docking



Free Energy Perturbation (FEP) is focus on the thermodynamic cycle. The molecule in the natural would change the pose all the time. A lot of reason can influence the pose such as temperature, pH value, solvent system… etc. Each pose would have an energy, and the higher energy of the compounds were more unstable. Instead that the lower energy, it suggested that the compound would be more stable. On the other hand, the complex also had an energy when the compound docking with the input receptor. Thus, we can calculate the value of subtraction of the ligand and complex's energy. ($\Delta G = E_{complex} - E_{ligand}$) However, it would be a huge mission when we compared different analogues if we still followed this method. For solving the time, we can directly calculate the value of subtraction between different analogues and complexes.

Conclusion

New drug development is cost- and time-consuming. Thinking a effective method to isolate a bioactive microbial strains is important so that we modify the process of the active bacteria isolation and purification, active compounds analyzation, and structure-active relationship (SAR) elucidation by molecular net-working and molecular modeling (docking) technology.

In this thesis, we plan to combine the modern technologies, such as molecular network and molecular modeling, to build a rapid way to search the active compounds from bioactive microbial strains from marine microbial resources. Then, we will further study the mechanism of antibacterial action of those bioactive compounds we found for new drugs discovery.