Myxobacteria and the Exploration into Their Relationship as Keystone Species

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Keystone species are an integral part of biodiversity and ecosystems. Without keystone species, the biodiversity in an ecosystem would diminish because of the overproduction of species lower in food pyramids. Keystone species help ensure the diversity of species and communities. While manymacroscopickeystonespecieshavebeenstudied,littleattentionhasbeengiventopotential microbial keystone species. Myxobacteria are gram-negative bacteria common and abundant in almost all soil and are bacterial predators. Although myxobacteria have been suggested to be keystone species, this has yet to be shown, and this research project sought to test thishypothesis. Bacterial prey species were cultured from a soil sample collected at Bagley Nature Center and allowed to grow with or without myxobacteria present as a predator. If myxobacteria were a keystone species, the diversity of prey bacteria was expected to be increased. However, it wasobservedthatmyxobacteriahadaslightlydeleteriouseffectonoverallmicrobialdiversityin our soil samples. It was observed that myxobacteria appeared to preferentially prey upon a gram-positive species that were observed to have multicellular-motility properties that maybe unique among prokaryotes.This new bacterial species was named after its colony morphology ("starburst"), and its strange motility opens up a potentially new area of study in bacteria.

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1966, Robert T. Paine conducted a groundbreaking experiment on the concept of keystone species. A keystone species is a species that fills a specific ecological niche and has a disproportionatelylargeeffectonitsenvironmentcomparedtoitsabundance.Akeystonespecies is essentially the glue that holds the environment together and without it, the ecosystem collapses. Dr.Paine's research was conducted with the use of a starfish species and a mussel species.Heselectivelyremovedeitherthestarfishorthemusselinnaturallyoccurringtidepools. He found that the starfish acted as a predator towards the mussels and the removal of the starfish caused the mussels to take over areas where other species once were. When the mussels were removed, a less drastic effect took place and only a small amount of biodiversity decreased. Dr.Paine'sresearch demonstrated that even though starfish may not seem to have a profound effect on the ecosystem they inhabit, they actually do (Paine, 1966).

After this pioneering study, it was discovered that many more ecosystems can contain keystone species, and without these keystone species biodiversity can be quite low (Kaimer et al., 2020). One ecosystem that has been underrepresented in the study of keystone species is the soil-basedecosystemsofsoilthatcontainawidevarietyofinteractionsbetweenmicrobesacting as producers, nutrient fixers, and predators. One well-characterized soil-base predator is the bacterial taxa myxobacteria which use their gliding motility, chemotaxis, and aggregation feeding to devour other bacteria. *Myxococcus xanthus* is an especially well-studied predatory myxobacteria that utilizes a feeding strategy called 'wolfpackfeeding' that involves coordinated and social behavior. *M. xanthus* aggregate to form these wolfpacks and then continue to disperse and search for more food. They are good predators because they use chemotaxis, which are chemical signals that allow the species to sense prey. The use of both the feeding strategy and chemotaxis alone does not make them good predators, but their gliding motility allows them to rapidly glide across smooth surfaces toward prey and outcompete other predators (Kaimer and Thiery, 2020). Utilizing these abilities allows myxobacteria to dominate and predate in soil, and these abilities are due to genes. There are special secretion systems and outer membrane exchange complexes that allow them to dominate. After cellular recognition, certain effectors are delivered to the target cells. This causes cell death, which allows the bacteria to have easier access to nutrients (Kaimer et al., 2020).

Myxobacteria are gram-negative unicellular rod-shaped bacteria (Munoz-Dorado et al., 2016). Myxobacteria are typically present in most soil sources. Myxobacteria are typically considered predators since they prey on other organisms within the soil. Predators play a key role within an ecosystem, and when predators are removed, it disrupts the food chain. Since myxobacteria are predators and their removal disrupts the ecosystem, it could be hypothesized that myxobacteria are keystone species within a soil microbiome (Petters et al., 2021).

Myxobacteria have unique characteristics which include their predator-prey interactions.These interactions shape microbial communities (Phillipsetal.,2022). An experiment was conducted to compare the biodiversity of an ecosystem with or without myxobacteria acting as a possible keystone species.

LiteratureReview

Myxobacteria's Unique Characteristics

Myxobacteria display a particular social behavior that is only seen in predators. They separate and hunt to find their prey, which is typically referred to as "wolf pack feeding behavior" (Kaimer & Theiry, 2020). Wolf pack feeding behavior is sped up by the use of their gliding motility (Dawid, 2000). This behavior is thought to reveal that myxobacteria arekeystone species, based on their behaviors. Myxobacteria have adapted well to unfavorable conditions such as starvation.During starvation, the bacteria aggregate into a large mound,called a fruiting body (Shimkets,1999). The fruiting body allows the cells to grow in a swarm rather than as single cells, which increases the likelihood of survival. This unique feature allows myxobacteria to fill an ecological niche that many other bacteria cannot since it can grow in conditions that are typically too hostile for other organisms (Shimkets et al., 1970).

Are Myxobacteria Keystone Species?

A recent study from 2021 (Petters et al.) asked the question if myxobacteria are keystone species. Testing different European soil samples allowed the researchers from the 2021 study to gather data from 28 metatranscriptomes. Different predatory species within the soil samples were analyzed and taken from the whole data set when compared with predatory versus non-predatory species. After SSU rRNA transcripts were completed, myxobacteria covered a substantial portion of the predators. This substantial portion of the myxobacteria indicates that they are important for the soil microbial community.

Myxobacteria and Their Effects on Ecological Systems

Predatory myxobacteria have an essential role in nutrient cycling since they can break down organic matter into important nutrients such as nitrogen or phosphorus, which go back into the ecosystem. They also produce antibiotics that help control the spread of harmful bacteria, which allows for a healthy and balanced soil microbiome (Rainer et al., 2019). Myxobacteriaalso play an important role in the ecological balance and stability of their habitats. Keystone species help researchers to understand how important an organism is to that particular ecosystem. Keystone species are broken down into three separate groups, which include predators, ecosystem engineers, and mutualists. Myxobacteria fall under the predator category since they control the prey species. This affects the number of other species or organisms within the food web and ecosystem. Many have recently hypothesized that most predatory species are typically keystone species since they have the greatest impact on each trophic level and ecosystem.

Myxobacteria Ecological Stability

Myxobacteria, through their unique hunting and multicellular formation abilities, are key players in promoting ecological stability. These bacteria play a critical role in breaking down organic matter, making nutrients available to plants, and promoting soil health. By doing so, myxobacteria contribute to the overall health and stability of the ecosystem. Some researchers have suggested that the presence of myxobacteria can even help to predict the stability of an ecosystem (Keller & Krug, 2004). They play a major role in controlling plant pathogens, which can be attributed to producing a special linear peptide antibiotic. Their contribution to nutrient cycling is by breaking down nitrogenous waste or fixing nitrogen, which can be used by other organisms. This creates a symbiotic relationship that can prompt ecological stability (Kjelleberg & Matz, 2005). Myxobacteria play an essential part in maintaining the delicate balance of the environment. This complexity is what makes myxobacteria such fascinating organisms to study, and their potential applications in promoting ecological stability are vast. The contribution of myxobacteria to ecological stability cannot be understated. These bacteria's unique abilities to break down organic matter, promote soil health, and cycle nutrients make myxobacteria essential players in maintaining the balance of the environment. As researchers continue to explore the potential applications of myxobacteria, they may uncover new ways in which these fascinating organisms can help promote ecological stability and contribute to a healthier planet.

Myxobacteria and Their Effect on Agriculture & Pollution

Myxobacteria can help fix pollution by using their many unique characteristics.These bacteria can consume many pollutants, such as heavy metals and organic compounds. Myxobacteria can break down pollutants at a faster rate than many other types of bacteria (Chen Shouwen et al., 2019). The main focus was on many bacteria that can degrade hydrocarbons since hydrocarbons are a large source of pollution. This makes myxobacteria a potential tool in the cleanup of contaminated soils and waterways. Farmers utilize myxobacteria in their soil instead of fertilizer which can cause runoff and continually pollute the waterways. In addition to theirpotential to decrease pollutants, myxobacteria can be used in agriculture to promote healthy soil and plant growth. By breaking down organic matter, myxobacteria can make nutrients more readily available to plants, which can help to increase crop yields. This increase in crop yields can decrease the price, which can in turn feed more people. This can be particularly important in areas where the soil is depleted of nutrients due to intensive farming practices or areas of higher poverty. Myxobacteria can help to reduce the use of harmful chemicals in agriculture. By promoting soil health, these bacteria can help to reduce the need for fertilizers and pesticides, which can be harmful to both the environment and human health.

Paenibacillus Genus

The *Paenibacillus* genus is found in soil and is a microbe that is associated with plant roots. It promotes plant growth and is often utilized in agriculture. Paenibacillus produces antimicrobial compounds that are useful in both medicine and agriculture. It produces many enzymes that can be used for bioremediation, which uses microbes to recover an ecosystem. Thegenuscanalsofixnitrogeninthesamewaythatmyxobacteriado,somanyagriculturists inoculate their fields with certain strains to help plant growth and use it as a natural fertilizer.

Unlike myxobacteria, Paenibacillus can create soluble phosphate. Soluble phosphate is extremely rare and only 0.1% exists in a form that can be taken up by plant roots. Synthesized phosphate emits a poisonous hydrogen fluoride gas, so through the use of Paenibacillus production costs and waste expulsion can be decreased. While the Paenibacillus genus can be helpful, it can be detrimental to certain species. *Paenibacillus larvae* can cause American foulbrood, which is a disease that kills many honey bee colonies. This lethal disease can have disastrous effects on wildlife that need pollinators and humans who rely on honeybees(Gradyet al., 2016).

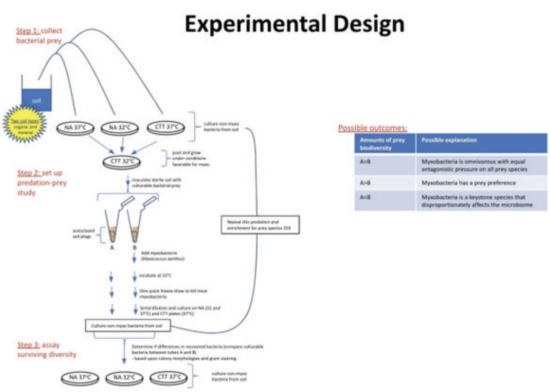
Paenibacillus lautus

Paenibacillus lautus is a gram-positive or gram-variable microorganism that is spore-formingwithmotilerods.Paenibacillus was recognizable through a unique branching chiral vortex morphotype. This impressive movement is done by aggregating dense areas of bacteria that push forward using repulsive chemotactic signals. This formation allows a colony to expand while increasing the amount of surface area the cells can use (Sirota-Madi et al., 2010). In a study done on the biofilm formation of *P.lautus*, it was found that it can actually switch its phenotype when forming a biofilm. It switches between rods and cocci but mainly stays in the rod form when creating a biofilm (Mangwani et al, 2014).

Methodology

The present research with myxobacteria asks a similar question: If myxobacteria are predators, are they keystone species? Using myxobacteria, my research faculty advisor and I divided the organism into organic soils from Bagley Park behind UMD groups and added an antifungal medication, so the fungus would not inhibit the growth of other organisms. The organicsoilwasselectedsinceithasalargerpopulationofmicrobes(Bossangeetal.,2018). The main problem was that since myxobacteria are predators, they are typically in most soil. Since they are in most soil samples, it can be harder to set up a control. My research faculty advisorand I had devised a plan to kill off the myxobacteria and not the organisms through the use of temperature ranges. A control and myxobacteria groups were compared by freezing and incubating the samples. The freezing and incubation period allowed myxobacteria to die and either psychrophiles or thermophiles to continue to live. Using serial dilutions from the soil samples that were collected, we counted the colony-forming units and the biodiversity. This allowed us to see what bacteria were alive after myxobacteria had entered the system and if there was an increase in biodiversity. When myxobacteria would most likely be classified as a keystone species (Dahl and Monjeau, 2023). During our research, a strange phenomenon occurred under the microscope. A gram-positive microbe appeared but only in the mixture that did not have myxobacteria introduced to it. The microbe displayed many similar traits to Myxococcusxanthus,butitsbrightpurplehuecontradictedthatitcouldbeMyxococcusxanthus. This microbe has an interesting rapid gliding movement that was seen under the microscope but without timelapse.

Many of the experiments done on myxobacteria have typically been removed before the start of any experiment. This allows for the observation of the entire ecosystem living without myxobacteria and the results would be observed through gram staining and statistical methods.If this removal of myxobacteria leads to a significant change within the ecosystem and the organisms, then it can be classified as a keystone species (Petters et al., 2021). However, many experiments have used gene sequencing in the past, since it allows for far more accurate results. Although some limitations in this experiment included how expensive it is, it will not be used in this research study. Another accurate but expensive method is stable isotope analysis which providesinformationonthetrophiclevels.Thisisdone-byanalyzingtheisotopiccompositionof myxobacteria and other bacteria. This data allows scientists to see if myxobacteria are important in transferring energy and nutrients between trophic levels. Utilizing inexpensive methods allowed us to better understand if myxobacteria are keystone species. Soil samples were collected from Bagley Park behind UMD. Each soil sample was placed onto either a nutrient agar (NA) plate at 32º C or 37º C or placed on CTT-YE agar plates. These were used in conjunction with an incubation temperature of 37º C since that plate and temperature are congruent with the optimal growth/death pattern for *M. xanthus* that grows well on CTT-YE plates not at 37ºC. After all of these plates were incubated at their respective temperatures, they were all pooled together and were allowed the time to grow. They grew at variables favorable to myxobacteria.Two different sets of plates were used. One labeled A,which had no myxobacteria added. One labeled B, which had a concentration of myxobacteria added. After allowing all of the bacteria to grow, the bacteria were pooled and CTT-YE plates were used at a 32ºC temperature. Potting soil from the UMD greenhouse was collected and then autoclaved toremove any bacteria. The greenhouse soil was placed in soil plugs and then a serial dilution was done on the myxobacteria samples. The serial dilution was plated on NA plates and incubated at 32º C and 37ºC. Another was plated on a CTT-YE plate at 37ºC. Our hypothesis was that no myxobacteria would be left on the plates. The samples were placed in half of the soil plugs. The samplewasaddedjustenough-tokeepthesoilmoistwithoutoversaturingit.Aquick-freeze-thaw was done to kill any bacteria after they had enough time to predate other bacteria. Five rounds of serial dilution, plating, and freeze-thaw were done in the hopes of discovering a trend.



Firgure 1.
Experimental Design proposed by Dr. John Dahl Showing the possible outcomes.

Results

A series of experiments were performed through the use of a "freeze-thaw" cycle. This cycle allowed myxobacteria to die and the other prey within the soil microbiome to thrive. The lack of prey preference exhibited by myxobacteria did not carry any significant implications for their role as keystone species within the ecosystem. The proportionate consumption of prey (non-myxobacteria) suggests that myxobacteria may not play a critical role in regulating the population dynamics of this prey species.The interaction between myxobacteria and prey is particularly significant due to the predator's potential role in nutrient cycling, plant-microbe interactions,or other ecosystem functions. The predator-prey relationship between myxobacteria and prey may indirectly affect plant growth, soil health, and overall ecosystem productivity, ultimately influencing the stability and diversity of the ecosystem. Furthermore, the lack of selective predation of myxobacteria on prey may influence competitive interactions among other microbial species. We hypothesized that myxobacteria would have a prey preference but based on cell morphology it was found that most of the myxobacteria are omnivorous with equal antagonistic pressures on all prey species. As well as myxobacteria may have a prey preference. This could have been from not having a proper set up which would cause the myxobacteria to not consume prey properly. Another hypothesis was that there was still too much prey and the myxobacteria were outnumbered by their prey. The most unexpected finding was the other microbe found within our soil samples and was only present when myxobacteria were absent. Using cell morphology, the sample showed that the specimen is gram-positive and spore-forming. After sending the sample to get sequenced, the 16S RNA gene sequence came back with results of Paenibacillus lautus.This organism is consistent with myxobacteria which are both common in soil.

Discussion

The predator's preference for the competitor microbe could reduce the population of myxobacteria indirectly. As the predator focuses on consuming the competitor, myxobacteria could experience reduced competition for resources.However,if the competitor is a food source for myxobacteria, their decline could result in decreased nutrient availability and disrupt the ecosystem's nutrient cycling. The decline of myxobacteria due to reduced competition could trigger cascading effects throughout the ecosystem. Myxobacteria likely have complex interactions with other microbes and organisms in the soil. Their decline could affect the abundance and diversity of other microbial species, potentially impacting plant growth, decomposition rates, and nutrient availability. Based on the results seen, the myxobacteria have preferred prey, which implies its positive effect on its environment when it is present. However, when the myxobacteria are removed, the biodiversity, stability, and recycling of wastes or pollutants significantly decrease. This resulted in the rejection of the hypothesis that myxobacteria may be keystone species and that accepting myxobacteria may simply have a prey preference. Myxobacteria may be an invasive species instead which reduces any form of biodiversity.My research could create important data through the use of comparisons of predator (myxobacteria) and prey, which would allow one bacterial species to bring back all of the soil's diversity. Discovering x myxobacteria can actually enhance soil diversity could be a game changer for how we understand microbial ecology. Comprehending the impact of a single type of bacteria on an entire ecosystem holds significant potential for enhancing nature conservation and promoting sustainable farming practices. This new understanding could lead to innovative strategies for maintaining soil health, benefiting both agricultural productivity and environmental well-being by decreasing water pollution.

The new specimen has been named "starburst" due to its shape and motility on a plate and under the microscope. When looking at the species, it was only present in the absence of myxobacteria.We hypothesize that myxobacteria are either preying on the "starburst", killing a mutualist of the starburst, or creating an antibiotic or toxin against the starburst. After sequencing,the"starburst"has been discovered to be *P.lautus.* The signature vortex is shown in many different species of the genus, Paenibacillus.

Figure 2

Paenibacilluslautus or "starburst" on the right shows its signature vortex. The left side shows Myxococcus xanthus forming a fruiting body.

Conclusion

Myxobacteria may not be keystone species based on the fact that they are predators, have diverse ecological functions, and have unique characteristics that allow myxobacteria to live in an ecological niche and maintain ecological stability. This is based on previous research done recently by Petters et al. in 2021, in which myxobacteria were found at higher concentrations within the soil microbial communities and the soil microbiome. These higher concentrations suggest that mycobacteria are more abundant because they are predatory and can survive at higher rates than other organisms. The fact that myxobacteria have many predatory traits and a certain ecological niche that can not be filled by another species. Many scientists believe predators that have these unique characteristics are usually keystone species, so myxobacteria would have been a likely contender to be classed as a keystone species. Overall, the designation of a species as a keystone species can depend on many factors, including the ecological context and the specific functions and interactions of the species within its ecosystem. While myxobacteria may not have been widely recognized as keystone species in the past, their contributions to ecosystem stability and function are increasingly being appreciated in current research. Myxobacteria have the potential to play a principal role in creating a safer environment by promoting biodiversity, remedying pollution, and promoting healthy soil and plant growth in agriculture (Chen Shouwen et al.,2019). As researchers continue to explore the potential uses of myxobacteria, it is clear that these bacteria have a bright future in helping to create a more sustainable and healthy world.

Although this experimentation could not continue, the experiment would have distinguished between whether an antibiotic or a toxin was being secreted by myxobacteria or whether myxobacteria was acting as a predator. An antibiotic or secretion could be pulled by centrifuging the myxobacteria and utilizing the supernatant to place on a paper disk on a TSA plate. Then by looking for a zone of clearing to show if the starburst died. Or a bacteria growth chart can be used and a supernatant can be added before the log phase and the growth can be tracked.

Future experiments could be done to show whether or not *M.xanthus* produces an antibioticornot. As well as figure out if *P.lautus* has a formation of phenotypic changes when forming a biofilm in its signature vortex. Since the vortex has been studied but not the formation of phenotypic changes during the vortex formation and if that is single cells at once or thousands of cells at the same time.

References

Dawid, W. (2000). Biology and global distribution of Myxobacteria in soils. FEMS Microbiology Reviews, 24(4), 403–427. https://doi.org/10.1111/j.1574-6976.2000.tb00548.x

Ebel, R. (2019). Myxobacteria in Soil: Unexplored Terrestrial Natural Products Chemodiversity. Frontiers in Microbiology. Keystone Species. (n.d.).

Grady, E. N., MacDonald, J., Liu, L., Richman, A., & Yuan, Z.-C. (2016a). Current knowledge and perspectives of paenibacillus: A Review. Microbial Cell Factories, 15(1). https://doi.org/10.1186/s12934-016-0603-7

Kaimer, C., Weltzer, M. L., & Wall, D. (2023). Two reasons to kill: Predation and kin discrimination in myxobacteria. Microbiology, 169(7). https://doi.org/10.1099/mic.0.001372

Krug, J., & Keller, H. (2013). Myxobacteria. In Elsevier eBooks (pp. 119–124). Science Direct. https://doi.org/10.1016/b978-0-12-374984-0.00559-3

Mangwani, N., Kumari, S., Shukla, S. K., Rao, T. S., & Das, S. (2014). Phenotypic switching in biofilm-forming marine bacterium paenibacillus lautus NE3B01. Current Microbiology, 68(5), 648–656. https://doi.org/10.1007/s00284-014-0525-8

Matz, C., & Kjelleberg, S. (2005). Off the hook – how bacteria survive protozoan grazing.

Trends in Microbiology, 13(7), 302–307. https://doi.org/10.1016/j.tim.2005.05.009 Muñoz-Dorado, J., Marcos-Torres, F. J., García-Bravo, E., Moraleda-Muñoz, A., & Pérez, J.

(2016). Myxobacteria: Moving, killing, feeding, and surviving together. Frontiers in Microbiology, 7. https://doi.org/10.3389/fmicb.2016.00781

Petters, S., Groß, V., Söllinger, A., Pichler, M., Reinhard, A., Bengtsson, M. M., & Urich, T. (2021). The soil microbial food web revisited: Predatory myxobacteria as keystone taxa? The ISME Journal, 15(9), 2665–2675. https://doi.org/10.1038/s41396-021-00958-2

Phillips, K. E., Akbar, S., & Stevens, D. A. (2022). Concepts and conjectures concerning predatory performance of myxobacteria. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.1031346

Paine, R. T. (1966). Food web complexity and species diversity. The American Naturalist, 100(910), 65–75. https://doi.org/10.1086/282400

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J. P., & Scow, K. M. (2018). Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLOS ONE, 13(2), e0192953. https://doi.org/10.1371/journal.pone.0192953

Shimkets, L. J. (1999). Intercellular Signaling During Fruiting-Body Development of Myxococcus xanthus. Annual Review of Microbiology, 53(1), 525–549. https://doi.org/10.1146/annurev.micro.53.1.525

Shimkets, L. J., Dworkin, M., & Reichenbach, H. (2006). The Myxobacteria. In Springer eBooks (pp. 31–115). https://doi.org/10.1007/0-387-30747-8_3

Sirota-Madi, A., Olender, T., Helman, Y., Ingham, C., Brainis, I., Roth, D., Hagi, E., Brodsky, L., Leshkowitz, D., Galatenko, V., Nikolaev, V., Mugasimangalam, R. C., Bransburg-Zabary, S., Gutnick, D. L., Lancet, D., & Ben-Jacob, E. (2010). Genome sequence of the pattern-forming paenibacillus vortex bacterium reveals potential for thriving in complex environments. BMC Genomics, 11(1). https://doi.org/10.1186/1471-2164-11-710

Shouwen, C. (2019). Myxobacteria as Natural Efficient Degraders and Potential Bioremediators. Frontiers in Microbiology.

Thiery, S., & Kaimer, C. (2020). The Predation Strategy of Myxococcus xanthus. Frontiers. https://www.frontiersin.org/articles/10.3389/fmicb.2020.00002/full