Environmental Uses and Pathogenic Potential of Paenibacillus lautus

by Kathryn O'Donnell

Paenibacillus lautus has a wide variety of environmental and clinical sources and contains many survival mechanisms that may contribute to pathogenicity. *P. lautus* demonstrates proficiency in the degradation of cellulose and diesel, resistance to high temperatures and heavy metal exposure, biofilm and endospore formation, and persistence in various digestive systems. It has also been shown to utilize swarming motility and reversible phenotypic switching, both of which may be factors in increasing pathogenicity and human infection prevalence due to the extensive bacterial communication necessary to carry out these processes. It is important to be aware of these factors as they relate to the broader *Paenibacillus* species, particularly by comparing the phenotypic switching and complex swarming motility patterns of *P. lautus* to those of *P. dendritiformis* and *P. vortex*. Targeting this communication may be the key to developing effective antibiotics in the case of a multi-drug resistant strain of *P. lautus*. By knowing the pathogenic implications of the survival strategies utilized by this species and weighing the benefits of its cellulolytic and pollutant-degrading potential against its ability to cause opportunistic infection through tick vectors, we may be more equipped to handle an increase of human infections caused by this bacterium.

Introduction

Paenibacillus lautus is a gram-positive, spore forming species of bacteria cultured from a wide variety of environmental and human sources (Saez-Nieto et al. 2017). Originally labeled *Bacillus lautus,* it was reclassified as part of the *Paenibacillus* genus in 1996 due to the extensive genetic similarities it shares with other *Paenibacillus* species (Heyndrickx et al. 1996). *P. lautus* is typically rod shaped; however there is evidence of phenotypic switching that may contribute to motility and population control (Mangwani et al. 2014). This phenotypic switching is reversible and involves the bacteria changing shape from rods to cocci in response to environmental stress.

Paenibacillus species have been found in varied environments, including human digestive tracts, cow feces, landfills, hot springs, ticks, human saliva, and soil (Yadav and Dubey 2018; Mead et al. 2012; Loong et al. 2018; Tetz et al. 2016; Saez-Nieto et al. 2017). The diversity in natural habitats may relate to the ability to form a biofilm on objects, the ability to form endospores, and the ability to participate in phenotypic switching, which gives *P. lautus* a variety of tools to persist and replicate in stressful environments (Celandroni et al. 2016).

P. lautus can break down cellulose and diesel, which may make it an important biological source of environmental cleanup in landfills or diesel polluted land (Yadav and Dubey 2018; Mauricio-Gutierrex et al. 2020). However, we also must be prepared for an increase in opportunistic, or possibly pathogenic, human infections caused by *P. lautus*. These infections seem to be connected to an overflow of waste, which attracts rodents and therefore ticks, which have been shown to harbor the bacterium and may spread it to humans (Loong et al. 2018; Canpolat and Biterge-Sut 2019).

Environmental Sources and Uses

An important aspect of bacterial studies is determining which bacteria contain enzymes that can assist humanity in breaking down waste products in landfills and similar environments. These enzymes are called cellulases and are used often in the food and paper industries. *P. lautus* has been shown to have cellulolytic properties, which could make it a candidate in studies regarding the breakdown of cellulose as well as diesel pollutants in soil-rich environments (Yadav and Dubey 2018; Mauricio-Gutierrez et al. 2020).

Cellulose and Diesel Degradation

P. lautus was isolated from Indian soil, evaluated for its cellulolytic ability using a cellulase enzyme assay, and was found to be efficient at biodegradation. The *P. lautus* strain BHU_3 has the ability to efficiently produce cellulases at a rate similar to fungi, which can be utilized at a large scale to break down cellulose (Yadav and Dubey 2018). *P. lautus* contains 28 genes that contribute to fermentation, which makes it a valuable tool in breaking down food products, as well as over 1,000 genes that code for the ability to transport and metabolize carbohydrates (Mead et al. 2012; Yadav and Dubey 2018). The ability to utilize carbohydrates as an energy source is useful for a potential biodegrading organism, as they often come across a wide variety of food sources.

P. lautus and other gram-positive organisms seem to have the ability to break down organic pollutants. One frequently problematic soil pollutant is diesel spills, which can occasionally be relieved using the addition of biodegrading bacterial species. Instead of degrading cellulose, *P. lautus* would be given the task of breaking down diesel into digestible forms. Luckily, *P. lautus* has protective mechanisms that allow membrane-bound oxygenases to utilize hydrocarbons to alter the hydrophobicity of its plasma membrane, thus protecting the organism from being broken down by toxic pollutants like diesel (Mauricio-Gutierrez et al. 2020). Many indigenous bacterial species in diesel-polluted soil have hindered growth when exposed to high levels of pollutant, but there is evidence suggesting that some *P. lautus* strains are able to resist this decrease in growth, possibly due to adjusting their hydrophobicity with hydrocarbons (Mauricio-Gutierrez et al. 2020).

The ability of *P. lautus* to degrade diesel varies greatly depending on the specific strain used. Paenibacillus lautus M1CH27 was able to break down a significant amount of diesel compared to other bacterial species but was not able to continue growth as well as *Paenibacillus lautus* M1CH19, which could proliferate when exposed to high levels of diesel in soil but could not break down the diesel efficiently (Mauricio-Gutierrez et al. 2020). This may suggest that there is also strain-dependent variation in cellulase production, which could be a topic of future study (Mead et al. 2012; Yadav and Dubey 2018). There is great variety in environmental capabilities within organisms classified as *P. lautus*, which could make this species an important contributor in the search for biological solutions to polluted soil.

Temperature Resistance

P. lautus has been found in extreme environments, including Obsidian Hot Spring in Yellowstone National Park. This indicates that *P. lautus* has adaptations that allow it to persist despite harsh environmental pressures, including high temperatures (42-90°F) and acidic pH (6.37). The optimum temperature for *P. lautus* is about 37°F (Mead et al. 2012). An important factor that could explain this ability is that *P. lautus* is a known spore former, and endospores are extremely resistant to threatening environments due to their ability to create a tough, outer cortex around the cytoplasm of the original bacterial cell. Another explanation may be the biofilm formation of *P. lautus* (Celandroni et al. 2016; Mangwani et al. 2014). Biofilm formation allows bacteria to survive in hostile environments by decreasing susceptibility to antimicrobials and macrophages. It is unknown whether biofilm formation would be a factor in surviving high temperature environments like a hot spring.

P. lautus Prevalence in Ticks

Another environmental source of *P. lautus* is within ticks (Loong et al. 2018; Canpolat and Biterge-Sut 2019). *P. lautus* was found in the intestines of Ioxides granulatus Supino, a tick typically found in Asia (Loong et al. 2018). These ticks were collected from rodents and sanitized with ethanol before being homogenized and spread onto a sheep's blood agar plate to harvest cultivable bacteria. The strain of *P. lautus* identified from these plates had identical antibacterial susceptibility to clinical *P. lautus* strains, which creates a concern that these ticks could spread these bacteria to humans or animals through the process of blood feeding. However, there are virulence genes typically present in clinical strains that were not present in the strain isolated from ticks, which could indicate a difference in pathogenicity (Loong et al. 2018).

Ecologists and microbiologists have also studied the effects of heavy metals on *P. lautus*, and it seems to have a high tolerance for heavy metal contamination compared to other related species. This finding makes the possibility of *P. lautus* being spread by ticks more concerning, as antibiotic resistant bacteria are often also resistant to heavy metals in their environment (Canpolat and Biterge-Sut 2019).

Clinical Sources and Uses

While the environmental uses and sources of *P. lautus* are many, there are also clinical sources of *P. lautus* found in hospital environments and biological fluids. The risk of pathogenicity rises as more patients are infected, and the fear in these environments is often the development of antibiotic resistance. To know how antibiotic resistance develops, it is important to know which antibiotics *P. lautus* is susceptible and resistant to.

Human Samples

P. lautus is increasingly being found in clinical environments, including biological fluids such as blood, abscesses, and wounds, as well as hospital environment sources like gloves (Saez-Nieto et al. 2017; Tetz et al. 2016). *P. lautus* has also been isolated from sterile body sites, meaning that it is most likely not a contaminant. These human samples are indicative of an increasing prevalence of human infections, which may support the idea that different strains of *P. lautus* are adopting human habitats.

Virulence markers have been found in *P. lautus* in recent studies, lending an explanation to this trend (Saez-Nieto et al. 2017; Celandroni et al. 2016). There are also many virulence markers that have been unveiled in a related species of Paenibacillus, specifically *Paenibacillus* sp. strain VT-400 (Tetz et al. 2016). This species has been discovered in saliva samples of pediatric patients who suffer from lymphoblastic leukemia. *Paenibacillus* sp. strain VT-400 is related to *P. lautus* in multiple ways, one of which is the fact that they both seem to cause mainly opportunistic infections. However, both species seem to have the genetic capability to be pathogenic instead of an opportunistic contaminate (Tetz et al. 2016; Saez-Nieto et al. 2017). One of the most concerning aspects of this possibility is the fact that both *Paenibacillus* sp. strain VT-400 and P. lautus are spore-forming, and spore forming bacterial infections are particularly difficult to treat using antibiotics due to the inherent toughness of endospores.

Antibiotic Resistance

The susceptibility of *P. lautus* to antibiotics is well documented. *P. lautus* is susceptible to ciprofloxacin (Canpolat and Biterge-Sut 2019; Loong et al. 2018; Celandroni et al. 2016), imipenem (Canpolat and Biterge-Sut 2019; Loong et al. 2018), and tetracycline (Canpolat and Biterge-Sut 2019; Celandroni et al. 2016). Vancomycin is also typically included in this list, though there has been some evidence of resistance to this antibiotic in *P. lautus* (Canpolat and Biterge-Sut 2019; Saez-Nieto et al. 2017; Celandroni et al. 2016).

P. lautus seems to be consistently resistant to ampicillin (Canpolat and Biterge-Sut 2019; Loong et al. 2018; Saez-Nieto et al. 2017), penicillin (Canpolat and Biterge-Sut 2019; Loong et al. 2018; Celandroni et al. 2016), and rifampicin, although this antibiotic also contains some inconsistencies in resistance (Loong et al. 2018; Saez-Nieto et al. 2017). One chemical that seems to be ambiguous in terms of susceptibility and resistance is chloramphenicol, which *P. lautus* was listed as susceptible to in one study and resistant to in another (Canpolat and Biterge-Sut 2019; Loong et al. 2018).

The ability to fight a *P. lautus* infection with antibiotics is especially important to watch as human cases continue to rise, and the possibility of a fully antibiotic resistant strain is particularly concerning. In general, if a person were infected with *P. lautus,* according to current studies, they could fight that infection with doses of ciprofloxacin, imipenem, or tetracycline, while doses of ampicillin or penicillin would be ineffective.

Phenotypic Switching and Swarming Motility Patterns

There are a variety of tactics that bacteria such as *P. lautus* use to remain viable in stressful environmental conditions, whether that environment is inside a living body or deep in the dirt. Some of those tactics, described by Be'er et al. (2011), Mangwani et al. (2014), and Celandroni et al. (2016) include forming biofilms with swarming motility patterns and developing reversible phenotypic switching mechanisms to encourage colony survival. Both strategies contribute to the bacteria's ability to act as a pathogen or proliferate in an opportunistic infection scenario. *P. lautus* is now grouped with other *Paenibacillus* species based on the many genetic similarities to species like *P. vortex* and *P. dendritiformis* (Heyndrickx et al. 1996). It would not be a far stretch in logic to expect consistencies in motility and defense across these species.

Swarming Motility in P. vortex vs. P. lautus

Paenibacillus vortex cells can form biofilms, which include vast amounts of bacteria that create vortex-like swarming patterns (Ingham and Jacob 2008). The *P. vortex* cells are physically curved with multiple peritrichous flagella, meaning the flagella are uniformly distributed around the cell body. These cells swim together in a clockwise pattern in optimal agar concentrations, which is hypothesized to be instrumental in infection, as it requires a large amount of communication between bacteria (Ingham and Jacob 2008). This communication may be similar to quorum sensing, which is a process in which many bacteria sense the size of the colony around them and subsequently make collective decisions regarding movement or chemical secretions. *P. vortex* cells must communicate with each other about when to start swarming, which direction to swim, and when to send out snakelike projections which often form smaller vortices of their own. These offshoots occasionally loop over each other as they travel, but they largely avoid pathways where other projections have already been (Ingham and Jacob 2008).

There is not a consensus on whether these swarming patterns are replicated in *P. lautus.* One study suggests that swarming behavior is seen in P. lautus biofilms, and that there is a specific window of time in which *P. lautus* will begin to form vortex-like patterns (Celandroni et al. 2016). However, another study suggested that there is no complex cooperative behavior within the specific strain of *P. lautus* (Mead et al. 2012). Future studies should focus on determining whether *P. lautus* directly models the swarming capabilities of *P. vortex,* or if it demonstrates novel cooperative behavior between cells within a biofilm.

Phenotypic Switching in P. dendritiformis vs. P. lautus

Paenibacillus dendritiformis is a species related to *P. lautus* that uses phenotypic switching, in which the overall shape of the bacterium changes due to environmental pressures. Normally, *P. dendritiformis* is rod shaped and motile. In response to stress, often due to crowded neighboring sister colonies of the same species, *P. dendritiformis* can switch from rods to non-motile cocci. The coccus form of this species can proliferate in the presence of sibling lethal factor (Slf), which is secreted by colonies of *P. dendritiformis* when they near a sister colony (Be'er et al. 2011). Rod shaped bacteria succumb to Slf, forming a barren area between neighboring colonies where all bacteria have been killed, decreasing conflict for resources and subsequent cell death in both colonies. However, if P. dendritiformis is in a coccus shape, it stops moving toward sister colonies, and is immune to Slf (Be'er et al. 2011). These cocci can continue to proliferate in the presence of Slf, and when the danger to the colony is no longer high, those cells can switch back into a rod shape and continue movement in search of resources. This phenotypic switching allows for colony survival security, and therefore extends the life of *P. dendritiformis* colonies.

P. lautus also seems to utilize this survival strategy, demonstrating reversible phenotypic switching in response to environmental stress (Mangwani et al. 2014). Using phase contrast microscopy, confocal scanning laser microscopy, and scanning electron microscopy, *P. lautus* colonies have been seen to react to biofilm crowding by switching the phenotype of cells in the colony from rod to coccus and back again. This is important to note as scientists explore the extent of communication between *P. lautus* cells, since phenotypic switching and swarming motility patterns both require intracellular communication.

Motility's Contribution to Pathogenicity

If, as argued by Mead et al. (2012), *P. lautus* does not participate in swarming, it would be a logical conclusion to assume that *P. lautus* is primarily found in intestinal sources and was found near a hot spring due to nearby animal feces. Bacteria do not need complicated motility patterns when they are adapted to life within a biological system, as there is less of a need for bacteria to provide their own forms of protection and movement, since those functions are already provided by their environment. There are also no antibiotic forming genes, heavy metal resistance genes, or nitrogen fixing genes within *P. lautus* which are normally found in microbes that thrive in hot spring environments. This study argues that *P. lautus* was first isolated from a human child's intestine, which provides an explanation for the source of this species when it is found in extreme environments like a hot spring. *P. lautus* was not found because *P. lautus* thrives in those conditions due to complex motility patterns, but because of animal fecal matter nearby (Mead et al. 2012). This explanation would lean towards *P. lautus* being primarily opportunistic.

However, if Celandroni et al. (2016) has the motility patterns of *P. lautus* correctly pinpointed, the flagellum-driven motility of these bacteria would be an important factor in their pathogenicity. Swarming facilitates the invasion of human and non-human host cellular barriers, as it allows improved communication between cells in a colony (Celandroni et al. 2016). This may be an important idea to study in the future, as developing antibiotics that target cellular communication may be integral in fighting *P. lautus, P. vortex,* or *P. dendritiformis* infections.

Conclusion

P. lautus is found in environmental sources, can break down cellulose and diesel, is particularly resistant to high temperatures, and is possibly being spread to humans through tick bites. It has been identified in various human biological sources, and there are currently a handful of antibiotics to which it is consistently susceptible to in laboratory studies. It may participate in swarming motility and phenotypic switching, like its cousins *P. vortex* and *P. dendritiformis*. Future studies on *P. lautus* could focus on exploring the cellular communication that allows complex motility patterns and/or phenotypic switching, as those mechanisms may be essential in fighting (tick-borne or otherwise) human *P. lautus* infections.

Another research opportunity on this topic would be to determine if the positive influence *P. lautus* could have on landfills as a cellulose degrading organism, or on diesel-polluted grounds due to its ability to break diesel into digestible forms, outweighs the possibility of subsequent opportunistic infections in rodents living near landfills or polluted soil. If *P. lautus* is used as a biological weapon of sorts against these issues, will that usage cause an increase in rodent infections, leading to tick infections and an ultimate transfer to human bodies? By combining the knowledge all the previously cited studies have gathered on *Paenibacillus lautus,* we are aware of the need to fight future infections, animal or otherwise, and we cannot claim ignorance of the possible consequences that may arise from widely utilizing the various degradation tools that this species holds.

References

Be-er A, Fisher CR, Swinney HL, Payne SM. 2011. Surviving Bacterial Sibling Rivalry: Inducible and Reversible Phenotypic Switching in *Paenibacillus dendritiformis*. *mBio. 2*(3).

Canpolat E, Biterge-Sut B. 2019. Determination of Antibiotic and Heavy Metal Resistance in *Paenibacillus lautus* 51ATA. *Turkish Journal of Agriculture- Food Science and Technology. 7*(9): 1465-1468.

Celandroni F, Salvetti S, Gueye SA, Mazzantini D, Lupetti A, Senesi S, Ghelardi E. 2016. Identification and Pathogenic Potential of Clinical Bacillus and Paenibacillus Isolates. *PLOS ONE. 11*(3): e0152831.

Heyndrickx M, Vandemeulebroecke K, Scheldeman P, Kersters K, De Vos P, Logan NA, Aziz AM, Ali A, Berkeley RCW. 1996. A Polyphasic Reassessment of the Genus Paenibacillus, Reclassification of Bacillus lautus (Nakamura 1984) as Paenibacillus lautus comb. nov. and of Bacillus peoriae (Montefusco et al. 1993) as Paenibacillus peoriae comb. nov., and Emended Descriptions of P. lautus and of P. peoriae. *International Journal of Systematic and Evolutionary Microbiology. 46*(4).

Ingham CJ, Jacob EB. 2008. Swarming and complex pattern formation in *Paenibacillus vortex* studied by imaging and tracking cells. BMC *Microbiology. 8*(36).

Loong S-K, Ishak S-N, Lim F-S, Khoo J-J, Tan S-N, Freddy-Jalin E-J, Mohdtaib F-S, Abubakar S. 2018. *Paenibacillus lautus*, an opportunistic bacterial pathogen, isolated from *Ixodes granulatus* Supino (Acari: Ixodidae) collected from a Muller's giant Sunda rat (*Sundamys muelleri. Systematic and Applied Acarology. 23*(4): 597-602

Mangwani N, Kumari S, Shukla SK, Rao TS, Das S. 2014. Phenotypic Switching in Biofilm-Forming Marine Bacterium *Paenibacillus lautus* NE3Bo1. *Current Microbiology. 68*: 648-656.

Mauricio-Gutierrez A, Machorro-Velazquez R, Jimenez-Salgado T, Vazquez-Cruz C, Sanchez-Alonso MP. 2020. Bacillus pumilus and *Paenibacillus lautus* effectivity in the process of biodegradation of diesel isolated from hydrocarbons contaminated agricultural soils. 46(4): 59-69.

Mead DA, Lucas S, Copeland A, Lapidus A, Cheng J-F, Bruce DC, Goodwin LA, Pitluck S, Chertkov O, Zhang X, Detter JC, Han CS, Tapia R, Land M, Hauser LJ, Chang Y-J, Kyrpides NC, Ivanova NN, Ovchinnikova G, Woyke T, Brumm C, Hochstein R, Schoenfeld T, Brumm P. 2012. Complete Genome Sequence of Paenibacillus strain Y4.12MC10, a Novel Paenibacillus lautus strain Isolated from Obsidian Hot Spring in Yellowstone National Park. *Stand Genomic Sci. 6*(3): 381-400.

Saez-Nieto JA, Medina-Pascual MJ, Carrasco G, Garrido N, Fernandez-Torres MA, Villalon P, Valdezate S. 2017. *Paenibacillus* spp. isolated from human and environmental samples in Spain: detection of 11 new species. *New Microbes New Infect. 19*: 19-27.

Tetz G, Tetz V, Vecherkovskaya M. 2016. Genomic characterization and assessment of the virulence and antibiotic resistance of the novel species Paenibacillus sp. strain VT-400, a potentially pathogenic bacterium in the oral cavity of patients with hematological malignancies. *Gut Pathog. 8*: 6.

Yadav S, Dubey SK. 2018. Cellulose degradation potential of Paenibacillus lautus strain BHU3 and its whole genome sequence. *Bioresource Technology. 262*: 124-131.