



Review Paper

Pseudomonas Syringae: An Overview and its future as a “Rain Making Bacteria”

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Available online at: www.isca.in, www.isca.me

Received 24th October 2014, revised 27th December 2014, accepted 3rd February 2015

Abstract

Bioprecipitation is a process of precipitating water by precipitation causing microorganisms by its ice nucleating properties. The concept of rain-making bacteria is known since 1980's but lack of research data makes it unrevised. *Pseudomonas syringae* is a Gram-negative bacterium mostly known to have ice-nucleating properties causing plant diseases. Their huge numbers of pathovars were identified in different hosts each having different modes of action. As always known for its pathogenesis in plant species with its ice-nucleating gene (*ina*), a concept of ice minus bacteria was created in 1970's which is against wild type *P.syringae*. So the bacterium lacking ice nucleating gene (*ina*) competed with wild type strain and succeeded. But findings say that a bacterium (wild type *Pseudomonas syringae*) was found on rain drops of different parts of the world and that bacterium is literally raining. More studies in this bacterium as a rain-making element may give as a better chance to know more about its role in life cycle.

Keywords: Bioprecipitation, pathovars, pathogenesis, bacterial nucleation, rain-making bacteria.

Introduction

Looking into the changes or effects of global warming in the environment, it gives one observed and most concerned problem, that is rainfall. Two thirds of the global food production depends largely on rainfall. Any decrease in rainfall will directly affect the agriculture. Precipitation is the main component of the water cycle. Condensed atmospheric water vapour that falls under gravity is precipitation. The different forms of precipitation include drizzle, rain, sleet, snow, graupel and hail. Water condenses and “precipitates” when a local portion of the atmosphere becomes saturated with water vapour. When water vapour does not condense sufficiently that's when fog and mist occurs so they are not precipitates but only suspensions. Precipitation is responsible for depositing most of the fresh water on the planet.

Microbes can also cause precipitation called as bioprecipitation. The concept of rain making bacteria was first proposed by David Sands in 1970's. Depending on the nucleating material a bacterium can cause ice formation even at -1°C but normally the pure water freezes approximately at -36°C¹. Bacteria could therefore be considered as climate altering factors in which clouds play a vital role in driving the climate system. However in 2010, C. Hoose *et al.* estimated that aerosol bacteria have, at most, a 0.6% influence on a global scale². Several different organisms are said to be involved, particularly those that are easily suspended in the air column. Organisms potentially involved in bioprecipitation include: *Exserohilum turcicum*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, *Pantoea agglomerans*,

and *Xanthomonas campestris*. David Sands explains that the same bacterium that was causing the yield of crops (frost damage to the plants) was found in samples of the clouds and made him to believe that the bacteria were literally raining down over the plants³. Ice-nucleation is a process by which bioprecipitation occurs⁴. The most well described organism that demonstrates ice nucleation is *Pseudomonas syringae*, which was determined to specifically supply a source of ice nucleators by Leroy Maki in the 1970's. Most of the ice nucleating bacteria is also plant pathogens.

This review mainly focuses on a particular bacterium, *Pseudomonas syringae*. It includes from identification to possible future of *Pseudomonas syringae* in causing rainfall.

Pseudomonas syringae: *Pseudomonas syringae* which was first characterized and named by C.J.J.van Hall in 1904 but before that in 1899 it was initially isolated by M.W.Beijerinck from the diseased lilac called *Syringe vulgaris* L⁵ and proposed by Migula in 1894. So the species was linked directly to its host from which it was initially isolated. *P.syringae* is a Gram negative, rod shaped bacterium with polar flagella causing disease in most of the plant species. Recently using the comparative analysis of 16S rRNA, the fluorescent, poly-beta-hydroxybutyrate negative pseudomonads associated with the type species, *P.aeruginosa*, and including *P.syringae* and related species, are now included in δ -proteobacteria⁶. It gives negative result for oxidase and arginine dihydrolase which makes it different from other fluorescent pseudomonads. It also forms polymer levan on sucrose nutrient agar. Secretion of the lipodepsinonapeptide plant toxin syringomycin and it owes its

yellow fluorescent appearance when cultured *in vitro* on King's B medium to production of the siderophore pyoverdinin is the property of many but not all strains of *P.syringae*. *P.syringae* does not rot potato which distinguishes it from *P.viridiflava*⁷. The infrasubspecific epithet pathovar is used to distinguish among bacteria within the species that exhibit different pathogenic abilities⁸. Then it was evolved that *P.syringae* is a complex representing a single species with distinct populations capable of infecting limited ranges of host⁹. Colonization marks the most predominant way by which *P.syringae* infects the plant species. So the microscopic view of it gives us a clear evidence of bacterial population in a particular leaf surface. The development of new approaches that combine molecular biological tools with light microscopy techniques has enabled to extend our scale of investigation of epiphytic communities to small scales and has revealed unanticipated features of leaf surface microbial communities^{10,11}.

Pathovars: The first record of 40 pathovars along with *P.mori* was published in 1978¹². Pathovars of *P.syringae* has involved in lots of debate about which belong as its pathovar and which are not and which should be considered as a new species table-

1. Most of the biochemical and nucleic acid based tests (e.g., DNA hybridization, restriction fragment length polymorphism, and repetitive DNA PCR-based genetic fingerprinting) have also been found useful in determining pathovars of *P.syringae*¹³⁻¹⁷.

Based on various tests groupings are made on the basis of host range. Strains within most of the pathovars exhibit rather narrow host ranges. The exception may be pathovar *syringae*, which includes the strain originally isolated from lilac (i.e., the type strain for the species). From published data, it is not clear whether pv. *syringae* is a repository for strains that may in actuality have quite limited host ranges^{18,19,20}. Although the symptoms of the diseases are more or less similar for the species, strains within the pathovars are clearly different with respect to a number of phenotypes, including nutritional, biochemical, and serological parameters, phage sensitivity, DNA based characteristics, and others^{21,22}. When ribotypical analysis was introduced, incorporation of several pathovars of *Pseudomonas syringae* into other species was proposed (eg. *P.amygdale*, *P.tomato*, *P.coronafaciens*).

Table-1
Pathovars of *P.syringae*

S.No.	Pathovar	Host
1.	<i>Pseudomonas syringae</i> pv. <i>syringae</i>	<i>Syringa</i> , <i>Prunus</i> and <i>Phaseolus</i> species.
2.	<i>Pseudomonas syringae</i> pv. <i>japonica</i>	Barley (<i>Hordeum vulgare</i>)
3.	<i>Pseudomonas syringae</i> pv. <i>aptata</i>	Beets (<i>Beta vulgaris</i>)
4.	<i>Pseudomonas syringae</i> pv. <i>atrofaciens</i>	Wheat (<i>Triticum aestivum</i>)
5.	<i>Pseudomonas syringae</i> pv. <i>lapsa</i>	Wheat (<i>Triticum aestivum</i>)
6.	<i>Pseudomonas syringae</i> pv. <i>pisi</i>	Peas (<i>Pisum sativum</i>)
7.	<i>Pseudomonas syringae</i> pv. <i>aceris</i>	Maple Acer species
8.	<i>Pseudomonas syringae</i> pv. <i>actinidiae</i>	Kiwifruit (<i>Actinidia deliciosa</i>)
9.	<i>Pseudomonas syringae</i> pv. <i>aesculi</i>	Horse chestnut (<i>Aesculus hippocastanum</i>)
10.	<i>Pseudomonas syringae</i> pv. <i>dysoxylis</i>	The kohekohe tree (<i>Dysoxylum spectabile</i>)
11.	<i>Pseudomonas syringae</i> pv. <i>panici</i>	Panicum grass species
12.	<i>Pseudomonas syringae</i> pv. <i>papulans</i>	Crabapple (<i>Malus sylvestris</i>) species
13.	<i>Pseudomonas syringae</i> pv. <i>tomato</i>	Tomato, <i>Arabidopsis</i>
14.	<i>Pseudomonas syringae</i> pv. <i>oryzae</i>	Rice
15.	<i>Pseudomonas syringae</i> pv. <i>glycinea</i>	Soybean
16.	<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	Tobacco
17.	<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	Bean
18.	<i>Pseudomonas syringae</i> pv. <i>savastanoi</i>	Olive
19.	<i>Pseudomonas syringae</i> pv. <i>morsprunorum</i>	Stone fruit, Cherry trees
20.	<i>Pseudomonas syringae</i> pv. <i>maculicola</i>	Cruciferous plants
21.	<i>Pseudomonas syringae</i> pv. <i>cannabina</i>	Cabbage
22.	<i>Pseudomonas syringae</i> pv. <i>cerasicola</i>	Cherry trees
23.	<i>Pseudomonas syringae</i> pv. <i>coryli</i>	Hazelnut Orchards
24.	<i>Pseudomonas syringae</i> pv. <i>corona faciens</i>	Oats
25.	<i>Pseudomonas syringae</i> pv. <i>delphinii</i>	Delphinium species
26.	<i>Pseudomonas syringae</i> pv. <i>erobotryae</i>	Loquat trees (<i>Eriobotrya japonica</i>)
27.	<i>Pseudomonas syringae</i> pv. <i>helianthi</i>	Sunflower
28.	<i>Pseudomonas syringae</i> pv. <i>mori</i>	Mulberry
29.	<i>Pseudomonas syringae</i> pv. <i>mellea</i>	Tobacco plants
30.	<i>Pseudomonas syringae</i> pv. <i>lachrymnas</i>	Cucumber, zucchini squash, honey dew melon

Identification: After the development of genomic level identification tests in late 1980's it is possible to identify a pathogen based on its gene level but the basic species differentiation tests still be used for identification of plant pathogenic species based on simple phenotypic tests. For identification of pathovars of *P.syringae* the use of determinative tests was shown to be of limited value²⁴ with primary reliance for classification being increasingly oriented towards poly-phasic and molecular methods. Successful identification system of isolates is possible as the host is known and only need to differentiate a few pathogenic species or pathovars²⁵. PCR primers can offer a reliable method for the confirmation of identification of pathovars. Palacio-Bielsa *et al.*, recorded a total of 246 papers describing primers for plant pathogenic bacteria²⁶. Out of which 30 describe primers for 19 members of the *P.syringae* complex, it includes the species: *P. avellanae*, *P. cannabina* and *P. fuscovaginae*, and the pathovars *actinidiae*, *alisalense*, *atropurpurea*, *coryli*, *glycinea*, *maculicola*, *morsprunorum*, *papulans*, *phaseolicola*, *pisi*, *savastanoi*, *sesami*, *syringae*, *tagetis*, *theae*, and *tomato*. There is always a need for comprehensive studies to confirm specificity if false positive and false negative results are to be avoided. However now it's possible to identify and characterize the particular strains of *P.syringae* based on phenotypic properties and whole cell protein patterns^{27,28} and repetitive PCR and multilocus sequence typing (MLST) are also done²⁹. More molecular level identification and comparison tools were used to distinguish each pathovar of this group^{30,31}. Lots of whole genome sequence analysis studies were conducted on different strains³² and complete genome (PPI) availability of most of the strains makes it easy for its identification and Bioinformatics genome database makes it much easier for comparison as well.

As a plant pathogen

As a pathogen, *P.syringae* affects large group of plant species and mostly symptoms of the diseases are similar. There is a great deal of specialization, within the species, with respect to plants with which individual strains are likely to interact. Colony formation on the host is the simple and most causative way through which bacteria causes diseases so if the plant is a host for particular pathovar it forms better colonies³². Some but not many pathovars of *P.syringae* can cause disease in various plants. Until 1970's, all the strains of *P.syringae* was considered as a pathogen and scientists also looked for pathogens in all diseased tissue. Is it really necessary that all strains of *P.syringae* should be pathogenic? Answer comes in early 70's, when frost injury to plants and ice nucleating bacterial association was discovered. After this discovery searches began for bacteria that were active as ice nuclei³³. Then, an ice nucleation active bacterium becomes a most predominant source of research and the findings were placed in the strain of *P.syringae*.

A diversity of bacterial species is known to colonize the

phyllosphere³². The specific types and their relative abundances vary with a number of factors related to the plant such as plant species, phenology, and age, and the environment in which the plants are grown^{33,34,35} (e.g., geographic area and weather conditions within a geographic area). *P. syringae*, more than any mineral or other organism, is responsible for the surface frost damage in plant exposed to the environment. The freezing causes injuries in the epithelia and makes the nutrients in the underlying plant tissues available to the bacteria. For plants without antifreeze proteins, frost damage usually occurs between -4°C and -12°C as the water in plant tissue can remain in a super-cooled liquid state. *P. syringae* can cause water to freeze at temperatures as high as -1.8 °C (28.8 °F), but strains causing ice nucleation at lower temperatures (down to -8°C) are more common. *P.syringae* has an ability to infect wide variety of fruits, vegetables and ornamental plants.

Bacterial nucleation in *Pseudomonas syringae*

Considering two groups G. Vali, R. Schnell, and colleagues at the University of Wyoming and S. E. Lindow, D. C. Arny, and C. D. Upper at the University of Wisconsin- Madison where both worked in two different fields, former with biogenic sources of ice nuclei that play a role in precipitation processes and later with how dried corn leaf powder affected the susceptibility of corn to frost injury, each pursuing completely different lines of investigation in disciplines as widely separated as atmospheric sciences and plant pathology. This research introduced *P.syringae* as their ability to nucleate super-cooled water to form ice³⁶. This discovery opens path for many interesting topics as a winter survival of insects, snow making, etc. Bacterial ice nucleation becomes a very broad field and lots of reviews followed its research^{37,38}.

The ability of bacteria to nucleate super-cooled water to form ice is uniquely limited to *P. syringae*. Strains of *Erwinia herbicola*, *Pseudomonas fluorescens*, *Pseudomonas viridiflava*, and *Xanthomonas campestris* pathovar *translucens* were demonstrated to have the ability to catalyze ice formation in supercooled water. Many studies have demonstrated the potential applications of ice nucleation active bacteria in the food industry including freeze concentration and freeze texturing of food, improving the process of freezing of various foods and preparation of frozen emulsified foods such as ice cream to improve the quality of the product³⁹. Further analysis on this particular species *Pseudomonas syringae*, showed that there was a particular membrane protein which has the ability to act as a nucleation site, called ice-nucleating proteins (INPs). Results of a wide range of experimental and theoretical approaches suggest that ice proteins assemble to form aggregates of various sizes in association with the outer membrane of bacterial cells^{40,41,42}. These nucleation sites allowed water molecules to become particularly aligned in order to promote freezing. Similar to a catalyst, the increase in number of nucleation sites promoted freezing at higher temperatures. As the food crisis increases in world it becomes

the topic of interest. When plants are exposed to below-freezing temperatures ice crystals can form, causing many growth implications and tissue damage. On crops harbouring the epiphyte *Pseudomonas syringae*, temperatures at which freezing occur usually range from 0-5°C³². There are two classes of proteins which can be related to the function of ice: antifreeze proteins (AFPs) and ice-nucleation proteins (INPs). AFPs have particular structures known to inhibit formation of ice crystals by preventing ideal alignment of water molecules for freezing into the crystal structure of ice. INPs do just the opposite, and thus allow freezing to occur at warmer temperatures. Pure water technically can be super-cooled to -40°C in the absence of a heteronucleus, which means that freezing doesn't occur. INPs are able to promote ice formation in raising the nucleation temperature, and *in vitro* this temperature can range from -14 to -2°C depending on the number of proteins that cluster together. To know the structure of INP from *Pseudomonas syringae* an attempt by Graether and Jia based on the comparison with AFP structure which was already determined from insects⁴³. They analyzed the INP sequence of ~60 16-residue repeats similar to a different model organism, and proposed a 16-residue loop for *P. syringae*. Their result suggested that insect AFPs and bacterial INPs may have a similar B-helical structure, even though they have opposite effects on water molecules.

Structure of INP: INP is a monomeric protein composed of more than 1,200 amino acid residues with a deduced molecular mass of 118 kDa⁴⁴ and three distinct domains⁴⁵: (i) an N-terminal domain with 175 amino acids, which is hydrophobic and function as the membrane anchor; (ii) a central cylindrical repeating domain (CRD), 48-residue long, which is not essential for membrane anchoring but can be used as a modular spacer to control the length between a heterologous protein and the cell surface and has a catalytic role in the formation of ice crystals, and (iii) a C-terminal domain of 49 amino acids that are hydrophilic and extracellular. The N-terminal domain is anchored to the glycosylphosphatidylinositol (GPI) outer membrane lipid moiety. However, both the C and N-termini of INP are free and exposed on the cell surface, so foreign proteins fused to the C- or the N-terminus of INP can be localized to the cell surface^{46,47}. INP has the ability to maintain its ice nucleation activity after fusion to a foreign protein, which allows the detection of the recombinant proteins on the cell surface by ice nucleation activity assay. The INP protein was used for the first time to display the *Zymomonas mobilis* levansucrase on the *E. coli* surface to produce an immobilized enzyme⁴⁶. This was followed by expression of the *Bacillus subtilis* carboxymethylcellulose (CMCase) on the surface of *E. coli*⁴⁷, the viral protein of the human immunodeficiency virus type 1, and the mutated CMCase gene library generated by gene shuffling⁴⁸. A recombinant oral vaccine was developed to display the hepatitis C core protein on the surface of *S. typhi* Ty21a. Using the N- and C-termini of INP⁴⁹, were able to express salmabin, a thrombin-like enzyme on the surface of *E. coli*. More recently an organophosphorous hydrolase was displayed on the surface of *E. coli* using a truncated INP. The

N-terminal domain of the ice nucleation protein, an outer membrane protein of *Pseudomonas syringae*, was used as an anchor motif for surface display and expression of heterologous antigens of the *Edwardsiella tarda* ghosts and *Ed. tarda* cadaver based combined vaccines⁵⁰. The ice nucleation protein was used as a whole cell biocatalyst to display a heme- and diflavin-containing oxidoreductase⁵¹.

Ice minus bacteria

The discovery of this bacterium came in 1970's when Dr. Lindow found that when a particular bacterium isolated from dried leaf powder of frozen damaged plants was introduced to plants where it is originally absent, the plants became very vulnerable to frost damage. He would go on to identify the bacterium as *P. syringae*, further investigating *P. syringae*'s role in ice nucleation and in 1977, discovered the mutant ice-minus strain. He was later successful at developing the ice-minus strain of *P. Syringae* through recombinant DNA technology as well⁵². At the time of Dr. Lindow's work on ice-minus *P. syringae*, genetic engineering was considered to be very controversial. But now we can consider recombinant *Pseudomonas syringae* (ice minus) as one of the most successful microorganism that was introduced into the environment. Ice minus is a common name give to a strain of *Pseudomonas syringae* which lacks its ability to produce a surface protein called Ina. Mostly wild-type strain of *P. syringae* which is "ice-plus" has the ability to produce Ina proteins found on the outer bacterial cell wall and acts as the nucleating centres for ice crystals. The ice-minus variant of *P. syringae* is a mutant, lacking the gene responsible for ice-nucleating surface protein production. This lack of surface protein provides a less favourable environment for ice formation. Both strains of *P. Syringae* occur naturally. The water is sometimes mixed with *ina* (ice nucleation-active) proteins from the bacterium *Pseudomonas syringae*. These proteins serve as effective nuclei to initiate the formation of ice crystals at relatively high temperatures, so that the droplets will turn into ice before falling to the ground. The bacterium itself uses these *ina* proteins in order to injure plants (Robbins, Jim 2010). The introduction of an ice-minus strain of *P. syringae* to the surface of plants would incur competition between the strains. Will the ice-minus strain succeed? the ice nucleate provided by *P. syringae* would no longer be present, lowering the level of frost development on plant surfaces at normal water freezing temperature - 0 °C (32 °F). Even if the ice-minus strain does not succeed completely, the amount of ice nucleate present from ice-plus *P. syringae* would be reduced due to competition. Decreased levels of frost generation at normal water freezing temperature would translate into a lowered quantity of crops lost due to frost damage, rendering higher crop yields overall. There is also a set of team of agro-scientists working to solve the puzzle of *P. syringae* strain that grows on tomato plants to find out whether its constant reoccurrence, even after potent pesticide applications and the development of GMO tomatoes, shows an incredible ability to adopt, or if it's a completely different

bacterium that shows up each time. They decided that the bacterium mutates and adapts quickly to get around obstacles placed in its way. These scientists say that new pathogen variants with increased virulence are spreading around the globe unobserved, presenting a potential threat to biosecurity.

Role as a climate changer

As explained earlier there is enough evidence that *P.syringae* is having more ability to cause rain by precipitation (David sands, 1982). Studies by meteorologists and plant pathologists are proving that the bacterium plays a crucial role in the formation of all forms of precipitation like raindrops, hailstones and snow. In 1982, Russell Schnell also discovered that the hail was forming around tiny particles kicked up by tea pickers in the field that carried *P.syringae* while he was attending the University of Colorado where tea plantation in Western Kenya was attacked by hailstorms 132 days of the year. There are more increasing results to support *P.syringae* in rain drops as there was some researchers detected the presence of *P.syringae* in fresh rain, snow and in ice from locations like Louisiana, the French Alps and even in Antarctica. There is another team which found that one-third of the ice crystals in clouds over Wyoming had formed around biological particles. There is no relationship between the strains of *P.syringae* over a plant to the strain which literally rains over it in an area. This evidence was given by Scientists when they discovered that strains of *P.syringae* in rain falling over a soy bean field were different from those on the leaves, which means they probably came from different location. So it's a guess that this bacterium might be creating rain to help them travel long distances. In 2008, Brent Christner and colleagues discovered that every freshly fallen snow sample they collected, even in Antarctica, contained these ice nucleating bacteria and their results showed that these ice nucleating bacteria travelled a long distance and maintained its ice nucleating activity in the atmosphere even in deciduous plants devoid regions^{53,54}. In the same year there was another interesting identification by Christner *et al.*, that ice nucleation by bacteria has been reproduced in the laboratory with samples of rain and snow from around the world (Canada, USA, Pyrenees, Alps and Antarctica), showing that in the samples treated with lysozyme (which hydrolyzes bacterial cell wall) or treated with heat, the ice nucleation (IN) activity was reduced almost 100% at a temperature of -5°C. Therefore, bacteria are responsible of the IN at these relatively high temperatures. Then researchers in the Amazon rainforest (Poschl and colleagues) discovered that primary biological aerosol (PBA) particles, including plant fragments, fungal spores and even bacteria, were a dominant contributor to ice nucleation in clouds above the rainforest. Even though the Earth surface is hot in the Amazon, high enough in the troposphere, it's still below freezing⁵⁵. At Montana State University in May 2012 researchers found high concentrations of bacteria in hailstones that had fallen on campus and they reported that by analysing the hailstones multi-layer structure, finding that while their outer layers had relatively few bacteria, the cores contained high concentrations.

They have a high concentration of culturable bacteria in the centres, on the order of thousands per millilitre of meltwater. So, in a recent study DeLeon-Rodriguez *et al.*, had shown that the viable bacteria at a 10 km altitude (samples taken above the Caribbean Sea and the Atlantic West) represent 20% of the particles with size between 0.25 and 1 mm, and bacteria are at least 10 times more abundant than fungi, with numbers of 10^5 per m^3 , with a 60% of viable cells by epifluorescence microscopy and quantitative PCR⁵⁶. This suggests that bacteria are an important and underestimated fraction of microparticles of atmospheric aerosols, even at higher concentrations than lower altitudes. Based on this additional evidence gathered, they are now wondering if there might be an entire ecosystem of rain-making bacteria living and reproducing up in the stratosphere. There is more research going around the world about the role of bacteria in causing rain. More research was done by plant biologists; however their results are reviving the interest of atmospheric physicists. Now research teams are speculating about the possibility of directing the fall of precipitation by deliberate production of known biological ice nucleators like *P.syringae*. If the bacterium were grown in dry locations, wind would carry colonies high, where *P. syringae* could act as the coolant around which water vapour condenses into raindrops (or hail). Although rain also forms around dust motes, volcanic ash, and salt particles when it's cold enough, *P. syringae* cools vapour into precipitation at higher temperatures, because of its Ina protein. According to Dr. Snow at the University of Montana, a single bacterium can make enough protein to nucleate 1000 snow crystals and the research continues as the scientists in England are flying into the clouds to take samples of the cloud water and analyzing the DNA of microbes in it. Virginia Tech researchers have sequenced the DNA of 126 strains of the bacterium to create a database that allows scientists to trace the bacteria to their geographic origin⁵⁷.

Conclusion

The future: The occurrence of rain and snow has become more extreme and locations are becoming more and more polarized, although it still rains and snows more or less. There is over heavy rainfall where physical conditions allow it and drought where they don't. This could be partly due to reduced habitat for rain-making bacteria such as *P.syringae*. In the past there is nothing to control the growth and reproduction of *P.syringae* so it grows and reproduce as it like and makes rain in its presence. But now the ability still exists, but probability of it is much lower, as the host plants are disappeared or protected with pesticides. The use of pesticides for industrial agriculture's all over the world with the aim to destroy *P.syringae*, industrial is ranching in different parts of the world which destroyed grasslands even the acres of Amazonian jungle which hosts bacterial colonies. Anyway it's our responsibility to rebalance for what we have done, and to increase the Natures ability to make clouds with bacterium that our farmers despise? So the answer is here, pick a specific location on the windward side of

dry lands to cultivate the bacterium, allow it to multiply on its favourite plants and measure what happens when a good wind kicks up and then look for when and where it rains in nearby mainland. Man would not be at the mercy of the weather, but would be able to predict when and approximately where precipitation would fall. *Pseudomonas syringae*- humankind has marked it as a bad or pests but it essentially needs to find its good side in constructive nature of this rain-making bacterium. As explained earlier about ice minus bacteria which could compete with wild type strain and reduce the possibility of damage to crops but at the same time it's not good when considering its possible cause in affecting rainfall. Still it's possible to protect plants by including antifreeze gene's into its genome so that it produces antifreeze proteins and making themselves less vulnerable against ice plus bacteria and allowing *P. syringae* to continue its role in making rain. So it's always necessary to use the nature's gift to make rain and helping farmers out of it. As India is agriculture based country it is essential to continue more works on this case and increase the possibility of rainfall and water scarcity. Cold weather frosts and bacterial ice action do destroy crops, but crops cannot survive at all without the precipitation generated by ice-nucleating bacteria. Continued experimentation is crucial to increase our understanding of the role *P. syringae* plays within the hydrologic cycle, and to find out how we can enhance, rather than destroy, its ability to create rain where it's needed. So plant biologists, agro-scientists and metrological studies should come together to solve this problem and to find out the missing data. Regardless of their global impact, it seems clear that *P. syringae* has an effect on the local water cycle, which may even play a role in life cycle.

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