Bioprospecting Potential of *Pseudomonas putida* for Access and Benefit Sharing

Reviewed by Rahel Tilahun

Genetic Resources Access and Benefit Sharing Directorate

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1. Introduction

Ethiopia is a country endowed with biodiversity such as microorganisms, plants, and animals which helps in identifying their use and shares the benefit. The sustainable use of its components and the fair and equitable sharing of the benefits arising out of the use of genetic resources are the two goals of convention on biological diversity (CBD) in which Ethiopia is applying it according to Proclamation No 482/2006 and Regulation 169/2009 (Access to Genetic Resources and Community Knowledge and Community Rights). An Access and Benefit Sharing Directorate is working on introducing applicable genetic resources for bioprospecting and Access and Benefit Sharing (ABS) agreement.

Consequently, the aim of this review is to promote the use of *Pseudomonas putida* for Agriculture, Biocontrol, and Bioremediation application for any company involved in bioprospecting or a person interested to work on it.

2. Description and Taxonomy of the bacteria

Pseudomonas putida is a commonly studied saprophytic soil bacterium strain in laboratories. It is grouped under rod-shaped, gram negative bacteria. Requiring oxygen for growth and survival, this aerobic bacterium inhabits soil, water, and other habitats, where there is availability of abundant oxygen and organic nutrients. It is motile and uses polar flagella for movement. In scientific history, the first living organism to obtain patent was *P. putida*. In the taxonomical classification, *P. putida* is grouped under the Class *Gamma proteobacteria*, Family *Pseudomonadaceae* and Order is *Pseudomonales*.

3. Cell structure and metabolism

Pseudomonas putida is rod-shaped, non-spore forming, gram-negative bacteria that utilizes aerobic metabolism. This bacterium also has multiple polar flagella for motility. The flagella have a waveform that of usually 2 to 3 wavelengths long. *Pseudomonas putida* is sensitive to the environment and suppresses the changes in the direction of flagella rotation upon sensing chemo attractants. This is very helpful in guiding the *Pseudomonas putida* to propel towards the seeds of the plants which provides nutrients to the bacterial cells (Harwood *et al*., 1989). *Pseudomonas putida* is able to tolerate environmental stresses due to its diverse control of proteins including protein and peptide secretion and trafficking, protein modification and repair, protein folding and stabilization, and degradation of proteins, peptides, and glycopeptides

(https://www.researchgate.net/publication/221847539_Industrial_biotechnology_of_Pseudomonas_putida_and_related_species. Some important proteins include the global regulatory proteins which link the pathway genes to the cell status. *Pseudomonas putida* exercises a very complex metabolism: the proteins control a particular pathway that depends on not only the signal received, but also the specific promoters and regulators in the pathway. In turn, once the signals are received, the protein informs the cell of the oxygen and nutrient availability. The Crc protein is another important protein which is part of the signal transduction pathway moderating the carbon metabolism. It also functions in biofilm production (Ruiz *et al*., 2005).

4. Ecology

Pseudomonas putida are significant to the environment due to its complex metabolism and ability to control pollution. There is a high versatility of bacterial communities towards contaminations which is further increased by certain catabolic sequences on the Toluene and m- & P-xylenes (TOL) plasmids in the cell. (Reanney *et al*., 1983)

Even the plasmids are important in sensing the environmental stress. Some of the environmental stresses are caused by benzene, xylene, and toluene, the main components of gasoline and are major sources of water contamination. *Pseudomonas putida* can degrade the hydrocarbons of these organic solvents through oxidative reactions therefore placing *Pseudomonas putida* as one of the most important microbes in bioremediation (Otenio *et al*., 2000).

Pseudomonas putida also interacts with other organisms in the soil. Its interaction with *Saccharomyces cerevisiae* in the rhizosphere led to beneficial effects on the state of the *Pseudomonas putida*. Fungi *Saccharomyces cerevisiae* produced the necessary glucose and also maintained the pH which was both favorable to the bacteria *Pseudomonas putida* (Romano and Kolter, 2005). The complex interaction of *Pseudomonas putida* and *Saccaromyces cerevisiae* together regulate plant health. Moreover, the bacteria itself is a great maintainer of abundant plant life. The production of the siderophores, such as pyoverdine and pyochelin, protect the plants from fungal pathogens. The mutual relationship benefits both partners. While *Pseudomonas putida* is able to reside in the plant seed and rhizosphere, the plant is, in turn, protected from plant pathogens and able to obtain vital nutrients from the bacteria (Espinosa *et al*., 2000).

5. Application of *Pseudomonas putida* in different sectors

5.1. Use in Agriculture

A beneficial property of all pseudomonas bacteria is that, it helps the plants to absorb nutrients from the soil medium. It also aids in counteracting pathogenic fungi, like Fusarium and Pythium. Fusarium causes wilt disease in plants; while Pythium inhabits the rhizosphere, and causes damping off disease in newly sprouted seedlings and young plants. Since from then, scientists have completed the sequencing of several strains of *P. putida*. Results showed that this simple bacterium is made up of about 6.2 million base pairs. Huge interest is shown in sequencing the genome of other strains for use in pollution control measures. Practical use of this bacterium has also been put forth for degrading oils and other organic pollutants. The plus point of employing this bacterium strain over other species of pseudomonas is its non-pathogenic nature.

5.2. Bioremediation

The diverse metabolism of wild-type strains of *P. putida* can be exploited for bioremediation; for example, it has been shown in the laboratory to function as a soil inoculant to remedy naphthalene-contaminated soils (Gomes *et al*., 2005).

P. putida is effective for treating polluted soil that contains nicotine, naphthalene, toluene, and oil, as some of the contaminants. These beneficial bacteria also degrade plastic and polystyrene into biodegradable products. Thus, this bacterium is useful for restoring normal conditions in an otherwise polluted soil (Ward *et al*., 2006).

5.3. Application in Biotechnology

Pseudomonas putida has the ability to produce poly-3-hydroxyalkanoates (PHA) from the aromatic hydrocarbon styrene. Styrene, a major environment toxic pollutant, is released in millions of kilograms a year from industrial sites and is known to cause spinal tract irritation, muscle weakness, and narcosis in humans and other mammals. The conversion to PHA allows the cure of styrene pollution.

Because PHA is beneficial to society, it serves in medical applications such as tissue engineering and also used as antibiotics and vitamins. PHA is also very environmental friendly, oil and grease resistant, and has a long shelf-life. Therefore, it is also used in everyday items such as plastic utensils and other disposable items. Unlike styrene, PHA can readily break down in soil

or water. Commonly used styrofoam, aka polystyrene foam, is transformed into biodegradable plastic through the *Pseudomonas putida*'s complex metabolism.

Styrofoam is at first converted to styrene oil where it is introduced to *Pseudomonas putida* to convert to PHA (Ward *et al*., 2005). Within *Pseudomonas putida*, PHA accumulates under unbalanced growth conditions as a means of intracellular storage, storing excess carbon and energy. These PHA polymers are synthesized by enzyme PHA synthase which is bound to the surface of the PHA granules and uses coenzyme A thioesters of hydroxyalkanoic acids as substrates (Ribera *et al*., 2001).

5.4. Biocontrol

Pseudomonas putida has demonstrated potential biocontrol properties, as an effective antagonist of damping off diseases such as *Pythium* and *Fusarium* (Cornelis, 2008). It forms colonies and thrive in the plant roots. They certainly feed on the nutrients found in the rhizosphere. However, these bacteria live in harmonious relationship with the host plants by protecting them from pathogens and increasing the nutrient absorption. Current studies are ongoing for its use in making biopesticides for crops and valuable plants.

5.5. Oligonucleotide usage signatures of the *P. putida* KT2440 genome

Di-to pentanucleotide usage and the list of the most abundant octa-to tetradecanucleotides are useful measures of the bacterial genomic signature. The *P. putida* KT2440 chromosome is characterized by strand symmetry and intrastrand parity of complementary oligonucleotides. Each tetranucleotide occurs with similar frequency on the two strands. Tetranucleotide usage is biased by G+C content and physicochemical constraints such as base stacking energy, dinucleotide propeller twist angle, or trinucleotide bendability. The 105 regions with atypical oligonucleotide composition can be differentiated by their patterns of oligonucleotide usage into categories of horizontally acquired gene islands, multidomain genes or ancient regions such as genes for rizobosomal proteins and RNAs. A species-specific extragenic palindromic sequence is the most common repeat in the genome that can be exploited for the typing of *P. putida* strains. In the coding sequence of *P. putida*, LLL is the most abundant tripeptide (https://www.researchgate.net/publication/221847539_Industrial_biotechnology_of_Pseudomonas_putida_and_related_species).

5.6. Organic synthesis

P. putida's amenability to genetic manipulation has allowed it to be used in the synthesis of numerous organic pharmaceutical and agricultural compounds from various substrates (http://blogs.scientificamerican.com/observations/2011/05/24/newly-discovered-bacteria-lives-on-caffeine).

5.7. CBB5 and caffeine consumption

P. putida CBB5 is a non-engineered, wild-type variety found in soil. They can also live on pure caffeine and have been observed to break caffeine down into carbon dioxide and ammonia (Summers *et al*., 2011).

5.8. Used in research of different species

Pseudomonas putida is very helpful in the research of different species in the genus *Pseudomonas,* especially *Pseudomonas aeruginosa*, a pathogenic bacterium that is one of the leading fatal diseases in humans. Researchers find that *Pseudomonas putida*, although saprophytic, can aid in the research on cystic fibrosis, an inherited disorder caused by a defective CFTR chloride transporter, which leads to recurrent opportunistic infections by *Pseudomonas aeruginosa*. The two bacteria are very closely related and share similar sequenced genomes (approximately 85% are shared), except *Pseudomonas putida* lack the genes that determine virulence. Due to its nonpathogenic nature, many researchers find *Pseudomonas putida* very beneficial to research due to its versatility and ease of handling (Kowalski, 2002).

6. References

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