Technoarete®
e-ISSN: 2583-3480
Technoarete Transactions on
Recent Research in Applied Microbiology and Biotechnology
Volume 4, Issue 2, September 2026

Biosolutions for Green Agriculture: Using DNA Sequence Chromatograms Produced by an Applied Biosystems to Identify the Various Rhizobacteria Components that Encourage Plant Growth

S. Aravind[1*], V. Rajeswari[2], N. Meenakshi[3]

[1, 2, 3]Assistant Professor, Department of Microbiology, PKN Arts and Science College, Madurai, Tamil Nadu, India
[*]Corresponding Author Email: aravaravindsethu@gmail.com

Abstract

Conventional agriculture's widespread use of chemical fertilisers and pesticides has led to serious health and environmental problems, including the rise of infections and pests that are resistant to these treatments. With their dual advantages as biofertilizers and biocontrol agents, Plant Growth Promoting Rhizobacteria (PGPR) provides a sustainable substitute. This study explores how PGPR promotes plant development through pathogen control, phytohormone synthesis, and nutrient solubilisation. The economic feasibility and use of PGPR, especially under abiotic stress situations, are also investigated. By increasing nutrient absorption and generating growth-promoting compounds, PGPR enhances plant development directly. It also inhibits phytopathogens indirectly through processes like siderophore synthesis and lytic enzyme release. Despite its promise, strain specificity, formulation stability, and regulatory obstacles are obstacles to PGPR's commercialization. The study emphasizes the necessity of continuing studies to improve our comprehension of plant-microbe interactions and create more reliable PGPR formulations. Reducing reliance on synthetic agrochemicals and incorporating PGPR into conventional agriculture techniques would require addressing these issues. Adopting PGPR successfully may result in more sustainable farming methods that support healthier crops and ecosystems. Additionally, our current work, which is based on this evaluation of the literature, clarifies important technical issues that may arise both before and during the interpretation of DNA chromatograms in Sanger sequencing and provides solutions. This study emphasises how important it is to recognise and address these technological constraints throughout the chromatogram analysis. Acknowledging these issues can help enhance the quality of downstream studies for Sanger sequencing data, which provide significant improvements in accuracy, dependability, and capacity to supply critical genetic information in clinical analysis.

Keywords

Chromatogram, Electropherograms, Phytopathogens, Plant Growth Promoting Rhizobacteria (PGPR), Sanger sequencing, Siderophore synthesis and Lytic enzyme.

A BRIEF OVERVIEW

To boost productivity and prolong the shelf life of agricultural produce, chemical fertilizers and pesticides are crucial. There is rising worry about the detrimental effects of chemical pesticides on human health, despite their indisputable benefits in managing pests and plant diseases.

Our immediate surroundings, including terrestrial and marine environments, have been negatively impacted by chemical pesticides, resulting in long-term damage in beneficial creatures. A wide range of microorganisms, including helpful ones, are impacted by chemical pesticides' persistence in the soil microenvironment [1]. Plant fungal disease resistance to azole, a broad-spectrum strobilurin, is the finest example of how the extensive usage of these compounds over time has resulted in the emergence of resistant pests and diseases [1]. Higher doses of chemical fungicides are now required due to the development of pesticide resistance, which raises the hazards these chemicals pose to human health and the environment [2]. The hunt for substitute chemical pesticides are more effective and have different deadly modes of action while being less harmful to human and environmental health has been spurred by this circumstance. The development of new chemical pesticides has become difficult due to the stricter environmental impact assessment studies that must be completed before a new chemical pesticide is approved for commercialization.

Additionally, between 1993 and 2018, about three-quarters of the legally accessible pesticides were removed due to their detrimental impacts on human health and the environment, making it more difficult than ever to bring a new chemical into the market. Agronomic methods have changed as a result of the detrimental effects of chemical pesticides, and biological control agents (BCAs) are increasingly being used to manage diseases. BCAs are becoming more and more well-liked as an important part of integrated pest management (IPM) techniques throughout the world due to their enormous potential as a substitute for or addition to chemical control [3].

The IPM system's employment of biological control agents encourages the production of wholesome crops with negligible effects on the agroecosystem and human health. In order to manage pests and diseases, biological control makes use of naturally existing organisms or their products.

Despite its growing popularity, biological control is not without its difficulties, chief among them being the dearth of large-scale, commercially accessible, effective biocontrol agents [4]. Bacteria, protozoans, yeast, fungus, and viruses are among the many biocontrol agents found in nature. The primary separation of BCAs from the soil rhizosphere, which is rich in microorganisms because of its nutritional content, is where the name "rhizobacteria" first appeared [5]. Beneficial rhizobacteria, neutral rhizobacteria, and detrimental rhizobacteria are the three main categories into which these rhizobacteria are divided. Plant growth-promoting rhizobacteria (PGPR) are the beneficial groupings [5].

Because PGPR plays a part in sustainable agriculture methods, it is of great agricultural significance. In addition to enhancing vital nutrient availability in plants, PGPR is crucial for controlling and suppressing phytodiseases [6]. PGPR enhances plant health and growth in a number of ways. The direct approach is either facilitating plants' absorption of the nutrients already present in the soil or supplying additional nutrients through microbes. By eradicating or lowering the microbial burden of phytopathogens in the rhizosphere, PGPR can also have an indirect effect [7].

By secreting different growth inhibitors, including lytic enzymes, bacteriocins, siderophores, and antibiotics, or by stimulating the host plant's innate resistance, they can also demonstrate indirect biocontrol effect on pathogens [8] [9]. The method of action of PGPR, how it promotes plant development under abiotic stress, and its commercialization constraints as biofertilizers and biocontrol agents are the main topics of this study.

More on Synopsis of History

The use of microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), to stimulate plant development has a long history that dates back to the late 1800s. Soil microorganisms and their positive effects on plants were studied by early researchers including Sergei Winogradsky and Martinus Beijerinck [10]. However, Kloepper did not officially coin the term "plant growthpromoting rhizobacteria" until 1978. He described PGPR as bacteria that colonise plant roots and promote plant growth through a variety of processes [11]. Considerable progress was made in comprehending how PGPR stimulates plant development during the 1980s and 1990s. Nitrogen fixation, phosphate solubilisation, phytohormone synthesis, and plant pathogen biocontrol are some of these processes. Many PGPR strains, including those from the genera Azospirillum, Bacillus, and Pseudomonas, were identified and described at this time [12].

The late 1990s and early 2000s saw a surge in the commercial use of PGPR, with products like Serenade and Rhizo Plus hitting the market. These compounds were created to protect plants from disease and increase agricultural production [10]. PGPR research has increased dramatically in the twenty-first century because to the demand for sustainable farming methods. A better knowledge of the genetic and molecular connections between PGPR and plants has been made possible by developments in molecular biology and genomics. As a result, more potent PGPR strains and formulations have been created. The function of PGPR in reducing abiotic stressors such salt, drought, and heavy metals has been emphasized in recent research.

For instance, studies have shown that by generating exopolysaccharides that boost soil structure and water retention, PGPR can increase plant resistance to drought [13]. All things considered, PGPR's historical evolution shows a trajectory of growing sophistication and applicability, in line with more general trends in biotechnological innovation and agricultural sustainability.

PGPR'S MODE OF ACTION

To maximise PGPR's potential to increase plant production, a thorough knowledge of its method of action is necessary. The two modes of action of PGPR are shown in Figure 1. PGPR has always used both direct and indirect methods of operation. PGPR effects that take place outside of the plant system are often referred to be indirect. On the other hand, activities that directly affect a plant's metabolism are referred to as direct [14]. The direct method of action involves processes that use growth regulators to alter the equilibrium of plant development.

The PGPR produce these regulators, which the plant system then integrates. Sometimes, the PGPR serves as a good source of phytohormones, as highlighted in Table 1, thus inducing plant growth and resistance to abiotic stress. The indirect mode of action leverages the plant’s natural defense mechanism through the microbial signaling process. Furthermore, through indirect mechanisms, PGPR protects plants against environmental stress [15] [16].

ACTION'S DIRECT MODE

Direct PGPR activity is the improvement of plant development in the absence of pathogens. This includes promoting the absorption of nutrients and the synthesis of plant phytohormones, which have an effect on the phyllosphere and rhizosphere.

Inorganic Phosphate Solubilisation

Second only to nitrogen, phosphorus is an important macronutrient for plants. Despite being widely available in nature, phosphorus is frequently insoluble and cannot be absorbed by plants. Phosphorus can only be used by plants as mono- and dibasic phosphate. Aluminium phosphate, tricalcium phosphate, rock phosphate, and other complex insoluble organic phosphates are broken down into simpler soluble inorganic forms in the soil by PGPR. Plants can more easily get phosphate as a result [17]. Bacterial strains that produce phosphatase use a number of methods to solubilise insoluble phosphate, mostly by releasing organic acids when carbohydrates are broken down. Acetic, lactic, malic, gluconic, tartaric, butanoic, and succinic acids are a few examples of these organic acids [17] [18]. These organic

acids release phosphate into its soluble phosphatic form by effectively chelating Ca^{2+}. These acids have been found using a variety of instruments, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) [18].

Table 1. PGPR's mechanism of action to promote plant development

PGPR forms	Definition	Mode of action	Ref
Phytostimulator	These rhizobacteria have the unusual capacity to create phytohormones, such as gibberellin, cytokinin, and indole acetic acid (IAA).	Phytohormone production	[19]
Biofertilizer	When applied to a plant, these living creatures or products derived from living organisms, particularly bacteria, colonise the root microenvironment and stimulate greater plant development by increasing the host plant's access to vital nutrients.	Inorganic phosphate solubilisation Fixation of nitrogen	[20]
Biopesticide	These microorganisms aid in the development of plants by opposing dangerous phytopathogens.	Systemic resistance, both acquired and induced Production of lytic enzymes Siderophore, antibiotic, and hydrogen cyanide (HCN) production	[21]

About 20–40% of the soil microbial population is made up of phosphate-solubilizing bacteria, most of which are found in the rhizosphere [18].

According to studies, PGPR that use phosphate have higher metabolic activity than those that are separated from different sources. Phosphate reacts with cationic ions like iron, aluminium, and calcium-forming salts during precipitation, making it difficult for plants to absorb. Iron and aluminium salts make up inorganic phosphates in acidic soil, while calcium phosphate predominates in calcareous soil [12]. In soils with a high organic matter concentration, the majority of the soluble phosphate is organic phosphate.

About 80% of this organic phosphate is made up of phytate, yet certain microorganisms may hydrolyse this complicated phosphate by producing phytases. Nevertheless, this enzyme frequently combines with metals including calcium, iron, and aluminium to create complexes [20]. Bacillus polymyxa, B. coagulans, and B. megaterium are the most well-known rhizobacteria that solubilise phosphate [22]. According to research, Pseudomonas fluorescence is a potent gluconic acid generator that helps dissolve mineral phosphate [23]. According to published research, Bacillus amyloliquefaciens and Bacillus licheniformis are known to produce a combination of isobutyric, isovaleric, and acetic acids, while Bacillus firmus, Rhizobium leguminosarum, and Ensifer meliloti are excellent producers of organic acid 2-ketogluconic acid, which effectively solubilises mineral phosphate [19] [20].

Nitrogen Fixation

There are two types of Rhizobacteria strains that can fix atmospheric nitrogen: free-living nitrogen fixers and symbiotic bacteria. Leguminous plant roots are naturally home to symbiotic bacteria, which pierce the root to produce nodules [24]. Strains from the genera Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium, and Azorhizobium are among the examples [23].

The other category, free-living nitrogen fixers, helps plants fix nitrogen by forming intimate relationships with crop roots. Unlike symbiotic associations, this kind of relationship is often regarded as loose and nonspecific [10]. Due to their capacity to fix nitrogen in both the environment and the lab, several PGPR strains of Azospirillum and Azotobacter have gained popularity. These strains have been in use since the turn of the 20th century and are still in use today [25]. These organisms are skilled at making phytohormones such auxins, cytokinins, and gibberellic acid in addition to fixing nitrogen [26]. Using Azospirillum and Azotobacter as agricultural inoculants has reportedly boosted cereal crop productivity. There have recently been reports of novel PGPR strains in the genus Bacillus [14] [17].

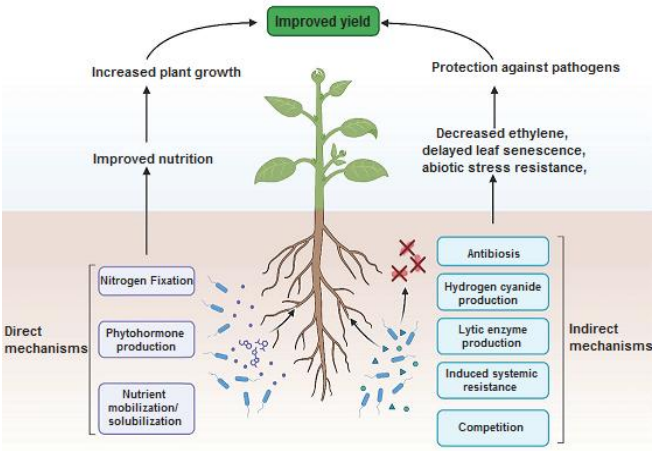


Figure 1. Diagrammatic representation of PGPR's mode of action

It has also been demonstrated that several Methylobacterium species engage in nitrogen fixation while interacting with plants. In particular, nitrogen-fixing bacteria have been identified as M. nodulans, M. symbioticum, and M. radiotolerans [14] [17] [27].

Production of Siderophore

As a coenzyme in vital physiological functions including respiration, photosynthesis, and nitrogen fixation, iron is an important macronutrient for plants. The plant will suffer from a lack of this important macronutrient. Despite the soil's high iron content, soil microbes and plants may not always have easy access to it [27]. The most common type of iron is oxidised, which easily produces insoluble hydroxides and oxides. Microbes and plants cannot absorb these insoluble oxides. Nonetheless, plants have developed two equally effective strategies for absorbing iron. In the first way, the iron is chelated and made soluble by the organic acid secreted by the plant. After diffusing in the direction of the plant root, this acid is reduced and absorbed there. The second is more straightforward: Iron may be absorbed by some plant species in its complex oxide and hydroxide forms and then reduced inside the plant system. Siderophore, an excellent chelating agent found in the soil rhizosphere, is produced by several PGPRs. These siderophores cause soil iron to migrate in the direction of better plant iron absorption [28].

Siderophores' low molecular weight (less than 1 kDa) and the chemical characteristics of their functional group allow them to bind reversibly with iron. The three most prevalent classes of siderophores are hydroxamates, carboxylates, and catechols. For effective binding to iron, the structures provide the perfect spacing between molecules. In soil, siderophore concentrations are frequently rather low (10–30 mM). Pseudomonas aeruginosa and Pseudomonas fluorescence are two siderophores that PGPR strains of Pseudomonas are particularly good at producing [29].

Because these PGPR strains have strong antibiotic qualities, they release their siderophores (pyoverdine and pyochelin) to outcompete other rhizosphere-dwelling microorganisms [7]. The function of siderophores in promoting plant development has been shown in a number of investigations [30] [31]. By increasing iron absorption, siderophore-producing PGPR strains promote plant development while preventing the growth of harmful fungi through antibiosis. Furthermore, because fungal phytopathogens are unable to exploit the iron-siderophore complex, siderophore might restrict iron delivery and result in iron shortage.

***Phytohormones* Production**

Auxin, cytokinin, gibberellins, ethylene, abscisic acid, brassinosteroids, and jasmonates are examples of phytohormones, commonly referred to as plant hormones. These hormones may be produced by microorganisms in bulk soil, but rhizobacteria are more effective at it [17]. Phytohormones generated by microbes are easily absorbed by the plant. Leguminous and nonleguminous root growth, cell division, and other plant system functions are all regulated and modulated by these hormones [32].

(1) Cytokinin One of the main classes of phytohormones produced by PGPR is cytokinin. The functions of auxins, gibberellin, abscisic acid, and cytokinin in boosting plant growth and yield have been thoroughly covered in the literature [33]. Auxin and cytokinin have comparable effects on plants when administered exogenously. It enhances the development of root hair, cell division, and root growth. Among other physiological reactions, it aids in the initiation of shoot development and prevents root extension [33]. The microbial synthesis of cytokinin by PGPR has been thoroughly investigated by researchers, leading to the isolation, identification, and characterisation of several strains. These strains have been identified from a wide range of plants, including wheat, canola, beans, and barley, and from several taxa, including Bacillus, Azospirillum, and Pseudomonas [12] [34].

It has been observed that B. megaterium, a PGPR strain, improves tea growth by producing more cytokinin. According to reports in the literature [35], several PGPR strains from the genera Xanthomonas, Escherichia, Klebsiella, and Proteus can biosynthesise cytokinin.

The two main biochemical forms of cytokinin are kinetin and zeatin. The N6 of the adenine backbone is swapped out for an isoprenoid in both situations. The adenosine monophosphate, or tRNA, pathways are used in PGPR to synthesise zeatin. It has been observed that the culture fluid of Bradyrhizobium japonicum and Bradyrhizobium leguminosarum contains a cytokinin-like substance [36]. Zeatin was subsequently identified as this cytokinin-like material. Zeatin and isopentenyl adenine were shown to be produced in significant quantities by two PGPR isolates of Rhizobium spp. IC3342 and ANU240 [37].

(2) Auxin Indole-3-acetic acid (IAA), a phytohormone essential to several plant metabolic processes, including apical dominance, cell division, cell elongation, and tissue differentiation, is frequently produced by PGPR. The production and mode of action of this important phytohormone have been extensively studied by researchers. Coinoculating plants with auxin-producing PGPR has been shown to enhance plant growth, yield, and metabolic performance [38]. Auxin causes both fast (short-term) and nonrapid (long-term) reactions in plants, according to a related study. Nonrapid reactions include cell division and differentiation, whereas rapid responses usually include cell elongation. The authors' research in [38] indicates that plants grown under long-term auxin treatment improve their root systems, which improves their capacity to absorb nutrients and their general development.

Auxin can be produced by more than 80% of PGPR. The hormone initiates a combinatory action that stimulates plant growth when it mixes with the native plant auxin. Auxin helps every component of the plant, but it particularly strengthens the root system. The soil contact surface area of these roots increases as they get bigger, heavier, and more

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Technoarete Transactions on
Recent Research in Applied Microbiology and Biotechnology
Volume 4, Issue 2, September 2026

branched. The plant's growth potential and nutrient pool are enhanced by the improved root characteristics, which also boost the root system's effectiveness in searching for nutrients. The redifferentiation of roots from stem tissue has been effectively shown by auxin [39].

These biosynthesis pathways' precise biochemistry is still mostly unclear [10] [39]. The indole-3-pyruvic acid (IPyA), indole-3-acetamide (IAM), and tryptamine pathways are the three L-trypto-phan-dependent processes [40].

(3) Gibberellin Gibberellins, a large group of phytohormones, consist of 130 structurally diverse molecules. This hormone affects many plant development processes, such as stem elongation, flowering, fruit set, and seed germination.

Only four gibberellins (GA 20, GA 4, GA 3, and GA 1) have been identified from seven bacteria strains. In addition, researchers have identified and characterized 28 gibberellins from 7 fungi species and 128 plant species [41].

Strains from the genus Bacillus are generally nonproducers of gibberellins, except for some known strains, namely, Bacillus licheniformis and Bacillus pumilus [39]. This group of phytohormones can translocate from the root to the plant's aerial region, positively affecting plant growth. Microbes can produce gibberellins, which consist of a 19–20 carbon atom backbone [39] [41]. GAs is more efficient for aerial plant growth, especially if the PGPR also produces auxin. This enhances the root system, thereby facilitating growth in the aerial part and improving the nutrient supply.

Researchers first documented GAs produced by PGPR through characterization using some physiochemical methods. They carefully examined Ensifer melilotigrown on gnotobiotic culture and found 4 GAs: GA 20, GA 9, GA 4, and GA 1. Many strains of bacteria have been reported to produce GAs, although the methods used for many characterizations, such as in vitro bioassay and thin-layer chromatography, are unreliable [39].

Gas-chro-matography-mass spectrometry (GCMS), a more reliable physiochemical method, confirmed the production of Gas in Herbaspirillum seropedicae and some Bacillus spp. [42]. The biosynthesis pathways of gibberellins in fungi are similar to those of higher plants; however, the required enzymes differ. Ongoing exploration into the mode of action of gibberellins produced by PGPR emphasizes their ability to enhance seed germination. While PGPR capable of producing gibberellins are termed beneficial microbes, fungi that can produce the substance are considered phytopathogens [24].

(4) Other Phytohormones In addition to auxin, gibberellin, and cytokinin, PGPR can produce several other phytohormones that play significant roles in plant growth, development, and stress responses. Some strains of PGPR have been reported to produce abscisic acid (ABA), a phytohormone involved in various physiological processes such as seed dormancy, stomatal regulation, and responses to abiotic stresses. ABA production by PGPR can contribute to enhanced stress tolerance in host plants by regulating water balance as depicted in Figure 2. Certain PGPR strains can also produce ethylene, a gaseous phytohormone that regulates diverse aspects of plant growth and development, including fruit ripening, leaf abscission, and responses to biotic and abiotic stress.

PGPR-produced ethylene modulates plant responses to acute environmental change and promotes adaptation to changing conditions [43] [44].

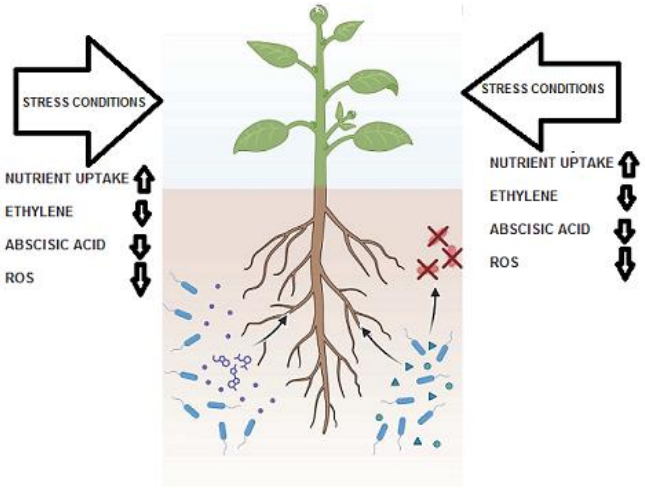


Figure 2. Process by which crop resistance to abiotic stress is enhanced. Activation impact is shown by the upper arrow, while decrease of effects is indicated by the downward arrow

Furthermore, Some PGPR can also synthesize polyamines such as spermidine and spermine, which are involved in various physiological processes including cell division, differentiation, and stress response. These additional phytohormones further highlight the diverse array of signaling molecules produced by PGPR, underscoring their importance in mediating plant-microbe interactions and promoting plant growth.

INDIRECT MECHANISM OF ACTION BY PGPR

Rhizobacteria's function as biocontrol agents is the main indirect way in which they encourage plant development (Figure 3).

As shown in dtas, PGPR exhibits a variety of biocontrol actions, including as competition for resources, niche exclusion, induced systemic resistance, and the synthesis of antifungal and antibacterial metabolites.

Competition

For a PGPR strain to be an efficient biocontrol agent (BCA), it must persevere in the soil and colonize the plant root even under the harsh abiotic conditions of the field. Therefore, the BCA must successfully colonize the plant root and compete with other rootdwelling microbes for nutrients and niches. Competition is a critical trait of a good BCA. It is well-documented that PGPR, with their rapid colonization of the root, efficiently protect the plant from pathogenic microbes. They do this by preventing pathogens from attaching and limiting nutrient availability to the pathogen

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[45]. Furthermore, the BCA generates secondary metabolites that are detrimental to the pathogens [46]. It's interesting to note that some root exudates have secondary metabolites that are harmful to the majority of bacteria. Nonetheless, PGPR can acquire a competitive edge and fill the ecological niche with the required detoxification equipment. In conclusion, PGPR competency is based on their capacity to maximize and adjust to potentially challenging micro environmental settings.

Hydrogen Cyanide (HCN) Production

HCN is known to be produced by PGPR via de novo synthesis from smaller precursor molecules. Because of its antibacterial qualities, this HCN can be used to suppress illness [47].

Certain PGPR strains have a special capacity to produce HCN, which makes them effective biocontrol agents against phytopathogens. HCN damages cytochrome oxidase, an essential enzyme of metabolic respiration, which distorts the electron transport chain and ultimately causes cell death [48]. Notably, a large number of Bacillus and Pseudomonas species are excellent HCN makers [48]. Furthermore, PGPR strains of other genera, including as Burkholderia, Rhizobia, and Chromobacterium, have been shown to exhibit bacterial cyanogenesis [49], underscoring the variety and potential of these biocontrol agents. The 45% reduction of Fusarium wilt in a tomato plant inoculated with Brevundimonas oleic Prd2 [50] provides proof that Pseudomonas, especially the Juorescent strain, are effective HCN makers as part of their secondary metabolism. Additionally, it has been observed that Pseudomonas fluorescence LRB3W1 and Pseudomonas sp. RhB-12 are very effective against Fusarium oxysporum f. sp. lycopersici.

Hydrolytic Enzymes by PGPR Production

By generating enzymes that break down the pathogen's cell wall, PGPR can demonstrate biocontrol action.

By breaking down the fungal phytopathogen's cell wall, enzymes such glucanase, protease, chitinase, and cellulase produced by PGPR strains act as biocontrol agents [51]. The lytic enzyme chitinase breaks down the components of chitin (1-4-N-acetyl-glucosamine).

A crucial part of the fungal cell wall is chitin. According to reports, @ 1-4 glucanase generated by PGPR strains of Streptomyces and Paenibacillus may break down the fungal cell wall in Fusarium oxysporum [51] [52]. Similar to this, it has been discovered that a variety of bacteria, such as Serratia marcescens, Pseudomonas fluorescence, Pseudomonas aeruginosa, Enterobacter agglomerans, Bacillus thuringiensis, Bacillus subtilis, Bacillus cereus, and Bacillus circulans, have strong chitinolytic activity [52]. This group of lytic enzymes demonstrates their harmful potential by rupturing the structural integrity of their target pathogen.

Research has demonstrated that Serratia marcescens may manufacture chitinase with strong antifungal, chitinolytic, and biocontrol properties against recognised fungal phytopathogens such Rhizoctonia solani and Fusarium oxysporum [52]. Under a microscope, the mycelia of these fungal pathogens showed remarkable abnormalities, such as hyphae bursting, curling, and swelling, after inoculation with PGPR, offering concrete proof of the biocontrol mechanism in operation.

Antibiotic Production by PGPR

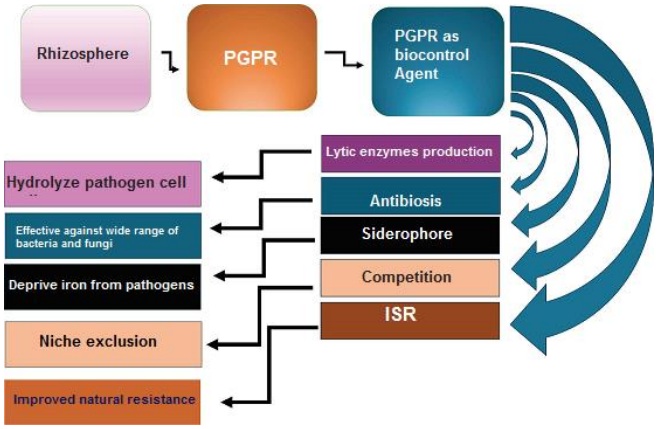


Figure 3. PGPR's biocontrol agent method of action

For economically significant crops, using natural enemies of phytopathogens has proven to be a viable substitute for chemical pesticides. The capacity of some PGPR strains of Pseudomonas and Bacillus to inhibit pathogens by secreting antibiotics has been identified. Even at low concentrations, these PGPR strains may effectively fight plant pathogens by producing secondary extracellular chemicals. The genus Bacillus, for example, produces an extracellular compound with well-defined antibacterial and antifungal properties. The potential of these antibiotic compounds, including sublancin, subtilosin A, TasA, and subtilin, as well as others from the families of iturin, fengycin, and surfactin, is enormous [53]. Extracellular substances with strong antibacterial qualities are reported to be produced by strains of Pseudomonas aeruginosa and P. fluorescence [54]. These substances have several uses in addition to being effective biocontrol agents against bacteria. Their antiviral, anticancer, antihelminthic, antioxidant, phytotoxic, and cytotoxic properties make them an intriguing field of study [53]. Cepaciamide, phenazine-1-carboxamide (PCN), 2-4-diacetyl phloroglucinol (DAPG), pyoluteorin, oomycinA, pyrrolnitrin, butyrolactone, ecomycins, and cepafungins are a few examples of these extracellular metabolites [54].

RESULTS AND DISCUSSION

Common Software Used to Read DNA Chromatograms

The ability to read DNA chromatograms is a crucial skill in the field of molecular biology and genetics research. Both the forward and reverse chromatograms provide valuable information on the nucleotide sequence included in the examined DNA fragment. The process of interpreting chromatograms from Sanger dideoxy sequencing is essential to the accuracy and reliability of sequencing results. During sequencing efforts, instances of misinterpretations,

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Technoarete Transactions on
Recent Research in Applied Microbiology and Biotechnology
Volume 4, Issue 2, September 2026

base-calling mistakes, and artifacts may occur, and identifying them can be particularly challenging if one only looks at the forward or reverse chromatogram. Sanger sequencing chromatograms can be examined more easily with the use of a variety of software tools. For example, Gene Codes Corporation's Sequencher software allows both forward and reverse chromatograms to be visualised and analysed simultaneously. Accordingly, Chromas, created by Technelysium Pty Ltd, enables users to see and work with chromatograms, identifying discrepancies and errors. There are several specialised tools available to help read DNA chromatograms, such as 4Peaks (available only on Mac), SnapGene Viewer, FinchTV, Sequence Scanner (compatible with PC and MAC), Chromas (available only on PC), and SnackVar [55].

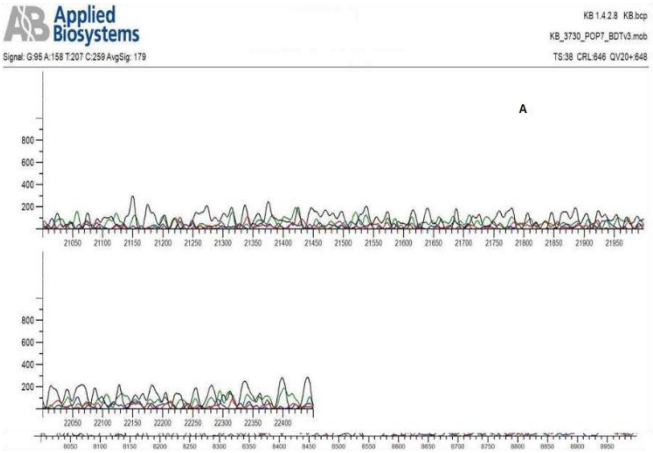


Figure 4A. Main issues encountered in the reading of DNA chromatograms of PCR products based on the Sanger sequencing method

However, precise nucleotide calls are not always made by computer programs. There may be situations where a human observer might identify a different nucleotide in the same chromatogram data while the computer incorrectly detects a nucleotide. Sometimes a call that a human observer would confidently choose a more precise base call will be marked with a "C" by the machine. Even seemingly error-free areas of the gel may exhibit such errors. Quickly scanning the gel for tiny peaks, C calls, and any unevenly spaced peaks or nucleotides is crucial. Understanding DNA chromatograms requires close attention to detail and a thorough understanding of the sequencing process, regardless of the tools used for interpretation. Once again, the presence of human intervention is strongly recommended to rectify all potential errors, with a particular focus on pivotal sites of nucleotide polymorphisms.

Technical Issues in Reading DNA Chromatograms

The accuracy and integrity of the sequencing data taken from DNA chromatograms can be affected by a number of technological issues.

An existence of unwanted noise in the data is a significant technical challenge when interpreting DNA chromatograms from Sanger sequencing. This noise can cause base calling mistakes and make it difficult to distinguish between real signals and background disturbance.

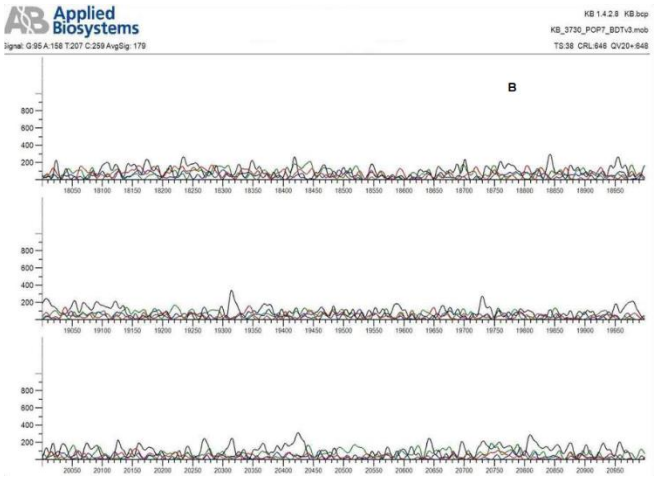


Figure 4B. Main issues encountered in the reading of DNA chromatograms of PCR products based on the Sanger sequencing method

Optimum and Noisy Peaks

Peaks in an electropherogram represent the amount of a particular chemical in the sample. The height, breadth, and shape of each peak provide important information about the sample, including its purity, concentration, and size distribution. Electropherograms' optimal and noisy peaks are important markers of the accuracy and dependability of data obtained from gel electrophoresis.

Optimal peaks provide accurate and reliable information on the number and size of molecules under analysis. On the other hand, in order to improve data quality, less ideal peaks could require more refining or troubleshooting [56].

Sequences Artifacts

Sequence artifacts are mostly caused by non-biological variations in the sequence data that arise from the sequencing procedure rather than from the sample itself. Inaccuracies in PCR amplification, the presence of contaminants or inhibitors, and DNA deterioration during handling or storage are some of the causes of these artifacts [57]. Their presence can cause errors in sequence analysis and make it difficult to distinguish them from real sequence data. Additionally, sequence artifacts might affect the accuracy of subsequent studies including phylogenetic reconstruction, haplotype inference, and variant naming. For example, PCR mistakes may result in false-positive variations or incorrect allele frequencies, which might affect analyses such as clinical genetics or association studies [56] [57]. Similarly, misidentification of microbial taxa or false beliefs about microbial diversity can result from contamination [58].

Technoarete®
e-ISSN: 2583-3480
Technoarete Transactions on
Recent Research in Applied Microbiology and Biotechnology
Volume 4, Issue 2, September 2026

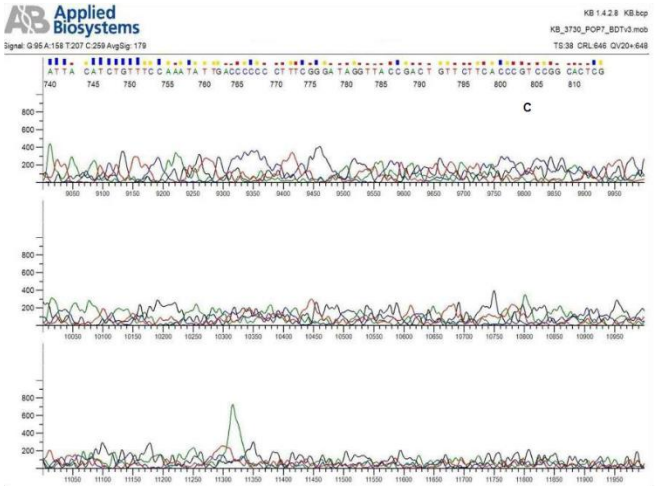


Figure 4C. Main issues encountered in the reading of DNA chromatograms of PCR products based on the Sanger sequencing method

Zygosity Detection or Double Infection Potentials

When trying to ascertain the zygosity (homozygosity/ heterozygosity) potential of a particular peak of interest, reading text files would probably produce inconclusive findings. Usually, electropherograms show a sequence of peaks, each of which represents a distinct nucleotide or fragment in the sample. These peaks indicate fragment sizes in fragment analysis and the nucleotide sequence in the DNA template in DNA sequencing. These peaks' height and arrangement provide important information about the material under analysis.

Stutter Peaks

A frequent problem in Sanger DNA sequencing analysis is stutter peaks. These little peaks, whose height makes up less than one-third of the main peak's height, appear either right before or after the actual signal peak.

Reading Limitations with Larger Size Amplicons

The average signal strength of each nucleotide is represented by the numbers in the sequenced DNA template, which should ideally lie between 200 and 400. Researchers would expect to acquire 500–700 bases of consistently clean DNA sequences outside of this range. DNA sequencers often produce precise data up to 900 or 950 nucleotides with low error rates in situations involving excellent DNA templates. But as time goes on, typical chromatograms gradually become less accurate, as seen by widening and changing peaks, decreasing resolution, and more challenging base calling. The sequencer keeps trying to "read" this data, but mistakes start to happen more often. For instance, the peaks in this chromatogram show widening and reduced resolution when you scroll downstream (to higher-numbered nucleotides, such about 900 nucleotides) (Figure 4B).

Limitations with Smaller Size Amplicons

Due to a number of restrictions, reading DNA chromatograms of smaller-size amplicons might be difficult. Amplicons smaller than 100 base pairs frequently provide weaker signals that are difficult to distinguish from background noise. The precise reading of DNA sequences and the detection of any mutations or variants are hampered by this intricacy. It is challenging to interpret chromatograms toward the end of the trace, especially in tiny amplicons. Low DNA template abundance or technological limitations cause a decrease in signal intensity in this region. This decrease may happen closer to the target sequence in smaller amplicons, making it difficult to determine the last nucleotide precisely.

The low signal intensity at the trace's end might resemble background noise, causing ambiguity in base calling (Figure 4C).

Distorted Peaks

For a variety of reasons, chromatogram files may show distorted peaks. Contamination, column deterioration, injection mistakes, solvent contaminants, and instrument failures are a few frequent offenders. Chromatogram peaks may be distorted by contamination that occurs during sample preparation or chromatography runs, causing peak widening, tailing, or splitting (Figure 1B).

CONCLUSIONS

Through a variety of direct and indirect methods, this investigation of plant growth-promoting rhizobacteria (PGPR) demonstrates their enormous potential to advance sustainable agriculture. These beneficial bacteria create essential phytohormones, boost nutrient availability, improve plant development, and offer strong defence against phytopathogens. Reading DNA sequences from chromatograms produced during sequencing is the process of Sanger sequencing, a popular technique for DNA sequencing. However, a variety of technical nuances that might lead to sequence misunderstanding make it difficult to evaluate DNA chromatograms in many therapeutic applications. In addition to other mistakes, these complications include noise, artifacts, and misunderstanding between heterozygosity and double infection potentials. Therefore, it is essential to identify and address these technological issues in order to guarantee appropriate interpretation of DNA sequencing results. For researchers and analysts looking to improve the accuracy of DNA sequencing data and support downstream studies, this analysis explores these technical facets. By resolving these technical problems, researchers can reduce mistakes and discrepancies in later analyses, maintaining the integrity of the research findings. In-depth chromatogram examination is necessary to troubleshoot failed sequencing reactions or problematic data, hence it is strongly recommended to consult the technical insights supplied if problems occur during data processing. Researchers can proactively detect and address any technical obstacles during the sequencing data processing in clinical applications by taking these technical insights into account before interpreting

chromatograms. This study is a useful manual that helps researchers get reliable and accurate DNA sequencing findings, improving the overall diagnostic quality in technical analysis. Therefore, it is essential to use these technological insights in order to protect the accuracy and reliability of DNA sequencing results.

Funding

This work did not receive funding from any official organization or institution.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Designed the work, collected the data, and wrote the work. BSc., Microbiology students designed the work and helped in the writing of the article. All authors read and approved the final manuscript.

Availability of Data and Materials Collected by the UG Students

All data generated or analyzed during this study are included in this published article by

T.Sriprakash, B.Sc Microbiology, sriprakas756@gmail.com

S.Jagadeesh, B.Sc Microbiology, jegabharathi2022@gmail.com

S. Manikandan, B.Sc Microbiology, manikandan54255425@gmail.com

M.Annamalai, B.Sc Microbiology, annamalaiascii@gmail.com

M.Indhumalini, B.Sc Microbiology, mmindhumalini@gmail.com

D.Angalaparameswari, B.Sc Microbiology, ammuimmu0802@gmail.com

M.Anitha, B.Sc Microbiology, Anitha763988@gmail.com

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