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Chromium Reduction and Plant Growth Promotion by *Staphylococcus arlettae* in Wheat

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

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Dedicated to ALLAH Almighty, Hazrat Muhammad (PBUH) and my brother Mohsin Raza who has been a constant source of motivation and encouragement during the challenges and supporting me spiritually throughout my life.

CERTIFICATE OF APPROVAL

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To start with the greatest name of **Allah Almighty**. Most gracious and merciful, whose bounties are unbounded, whose benevolence is eternal, whose blessings are uncountable, whose being everlasting, whose mercy is limitless, whose provisions are un-ending, and whose Love is our life, whose worship is our faith. Words seem too little to express my gratitude to **Allah Almighty** who has bestowed me with more than I deserve.

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Abstract

Wheat is a crop extensively cultivated for its seed, a cereal grain which is a worldwide staple food. It is the most tremendous health-building food. Microbial habitations are severely damaged by the indiscriminate use of organic fertilizers to meet the increasing demand of food supply. For supportable food production development bio fertilizers are used as a hopeful tool in different subdivisions comprising farming, ecosystem and remediation. A substantial environmental concern is the high concentration of heavy metal pollutants in soil and water bodies resultant from industrial development remains. *S. arlettae* strains were isolated from textile and tannery industries with azo dye degradation potential and plant growth promoting property from sewages. Essential bacterial strain of *Staphylococcus arlettae* was used to check its influence on growth of wheat plant (*Triticum aestivum .L*). The impact of plant growth promoting bacteria on numerous parameters on crop growth and development was examined. *Staphylococcus arlettae* has the ability of *Staphylococcus arlettae* to simultaneously produce PGPP and reduce Cr cytotoxicity seed showed increased proportion of germination, length of radicle, coleoptile length of coleoptile, wet and dry weight. It is also exposed that in absence as well as presence of Cr *S. arlettae* treated seeds showed increased percentage of germination. *S. arlettae* treatment resulted in an increase in shoot length over control untreated seeds in the absence of Cr and also showed healthier rooting response. Hence the progression of the bio fertilizers and the promotion of bacterial inoculation of *S. arlettae* in the field is an ecologically approachable way to meet the worldwide necessity to uplift crops harvests. Additionally, developments in new technologies leading to the enhancement of biofertilizer shelf-lives, facilitation of distribution and application in the fields, are essential for their use to be extended in the future.

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Abbreviations

AM:	*Arbuscular mycorrhizae*
ANN:	Artificial Neural Networks
ATP:	Adenosine Triphosphate
BNF:	Biological Nitrogen Fixation
CoNS:	Coagulase-negative *Staphylococci*
Cr:	Chromium
ECM:	*Ectomycorrhizae*
ET:	Evapotranspiration
FT-IR:	Fourier Transform Infrared Spectroscopy
HMW:	High Molecular Weight
IAA:	Indole Acetic Acid
$K_2Cr_2O_7$:	Potassium Dichromate
LB:	Luria Broth
N/ha:	Nitrogen Per Hectares
PGPB:	Plant Growth Promoting Bacteria
RSM:	Response Surface Methodology
TOC:	Total Organic Carbon
UV-vis:	Ultraviolet–visible Spectroscopy

Chapter 1

Introduction

1.1 Background

Wheat is a crop widely cultivated for its seed, a cereal grain which is a globally staple food [1, 2]. The genus *Triticum* is comprised of many species of wheat among them the most widely grown is common wheat (*T. aestivum*) [3]. For more than one third of the world population wheat is considered the most important stable food crop and contributes to the world diet more calories and proteins than any other cereal crops [4, 5, and 6]. It can be processed into various types of food and it is nutritious, easy to store and transportable. Protein, minerals, B-group vitamins and dietary fiber are the major nutrients from wheat [7, 8]. Nutritional composition of wheat grains with its essential coating of bran, vitamins and minerals can be affected by the environmental conditions. It is the most excellent health-building food. Bread, produce biscuits, confectionary products, noodles and vital wheat gluten are prepared from wheat flour. Animals use wheat as their feed and it is used in many processes such as for ethanol production, brewing of wheat beer, raw material for cosmetics made from wheat, wheat protein in meat substitutes and also in making of wheat straw composites. A good source of dietary fiber is provided by wheat germ and wheat bran and it can be helping in the prevention and treatment of some digestive disorders [8]. Soluble fibers have

health benefits which are not shared by insoluble fiber and these are reduced by the phenolic acid cross-linking [9]. However, insoluble fiber may also have benefits in delivering phenolic antioxidants into the colon and colon-rectal cancer is reduced [10, 11].

Microbial habitats are severely damaged by the indiscriminate use of chemical fertilizers to meet the growing demand of food supply that has undoubtedly directed to contamination and insects. Excess chemical inputs has made the crops more prone to diseases and reduced soil fertility [12, 13]. For sustainable agriculture development biofertilizer are used as a promising tool in different sectors including agriculture, ecology and remediation. To flourish agricultural productivity and to feed the growing population with the deficit amount of available nutrients, the world certainly needs a sustainable and ecofriendly way. Hence, the use of chemical fertilizers, pesticides, herbicides, fungicides, and insecticides it is unavoidably required to reconsider many of the existing agricultural approaches [14]. The use of synthetic chemical fungicides and chemical fertilizers help to minimize mycofungicides and fungal biofertilizers.

This is beneficial as synthetic chemical compounds likely to have detrimental effects on humans and the environment [15, 16]. For crop growth nitrogen is one of the major important nutrients that are very essential. 80 percent of nitrogen volume in the atmosphere is in Free State.

To determine nitrogen balance in soil ecosystem the elemental nitrogen that finds its way into the soil is entirely due to its fixation by certain specialized group of microorganisms by the process known as Biological Nitrogen Fixation (BNF) is considered to be an important process. Sustainable agricultural production is supported through BNF. By the application of biofertilizers the value of nitrogen fixing legumes in improving and higher yield of legumes and other crops can be achieved [17].

Bacteria that are free-living, and form specific symbiotic relationship with plants are known as plant growth-promoting bacterium (PGPB) such as bacterial endophytes that can colonize at some portions of plant tissue, and *Cyanobacteria*

[18]. Common plant growth promoting bacteria include *Rhizobium, Bradyrhizobium, Sinorhizobium, Azospirillum, Nostoc, Anabaena, Acetobacter, Bacillus megaterium, Azolla, Bacillus polymyxa,* etc. which are very that help in enhancing crop yield and overall plant growth significantly.

A significant environmental concern is the high concentration of heavy metal contaminants in soil and water bodies resulting from industrialization remains. Chromium (Cr) is one of the most prominent heavy metal contaminants which are a product of tannery, chrome plating, metal cleaning and wood processing industries. Due to high water solubility, permeability through cell membranes and interaction with DNA and cell proteins it is highly toxic [19].

For remediation of affected environments an ecofriendly process is bioreduction of Cr into less toxic and soluble trivalent chromium. The role of different microorganisms in chromium reduction has shown by many investigations [20]. Adverse effects on the growth parameters of plants are induced by chromium phytotoxicity such as stoppage of early seedling development, decrease in biomass, reduction in root growth and chlorosis [21, 22].

Staphylococcus arlettae is one of the coagulase-negative *staphylococci* (CoNS) species it was first isolated from the skin and nares of poultry and goats [23]. Subsequently, S. arlettae strains were isolated from textile and tannery industries with azo dye degradation potential and plant growth promoting property from effluents [24, 25]. It consists of clustered cocci. It is resistant to novobiocin. *Staphylococcus arlettae* has the ability concurrently produce PGPP and reduce Cr toxicity seed showed increased percentage germination, radicle length, coleoptile length, wet and dry weight is shown in the *S. arlettae* treated seeds in the absence as well as presence of Cr [26, 27]. Genome sequencing is performed in the laboratory to achieve whole genome sequencing of *Staphylococcus arlettae* [28, 29]. Mag Attract High Molecular Weight (HMW) DNA Kit, GridION X5 system and FASTQ processing program are the analysis used to sequence the whole genome of *Staphylococcus arlettae* species [30, 31].

1.2 Aims of Research Study

The objective of this study was the evaluation of chromium reducing bacteria *Staphylococcus arlettae*, for plant growth promotion property. It is potential in enhancing *Triticum aestivum* seedgermination and plant growth was evaluated.

1.3 Objectives of Research Study

- To utilize essential bacterial strain of *staphylococcus arlettae* to check its impact on growth of wheat plant (*Triticum aestivum .L*).
- To formulate different concentrations of heavy metal chromium to check metal uptake and its growth and developmental effects on wheat crop.
- To check the cytotoxicity of chromium on the growth of plants.
- To analyze and study the impact of biofertilizers on various parameters of crop growth and development.

Chapter 2

Literature Review

2.1 Wheat Plant

The most extensive cereal crop which grows worldwide is wheat (*Triticum aestivum linn*) bearing stalks with bristly spikes is the portion known as head with attached awns having high nutritious value and grains being used as staple food, annual covering area is about 237 million hectares (Figure 2.1) [32, 33]. Genus Triticum comprises of different species of wheat, *T. aestivum* is widely grown among them. World trading of wheat is greater than other crops [33]. Gluten proteins present in grains have adhesive and viscous properties which increase global demand for wheat which facilitate the production of processed foods [34]. Wheat is the major source of carbohydrates and vegetable proteins in human food, it provides multiple nutrients and dietary fiber when eaten as the whole grain [35].

2.1.1 Nutritional Value

Essential nutrients are provided by wheat such as proteins, dietary fiber, multiple vitamins (thiamine and vitamin-B) and significant minerals are the major contents with 13% of water, 71% are carbohydrates 2.1% minerals and fat contents are 1.5% [36, 37]. The protein part is gluten these proteins have essential amino

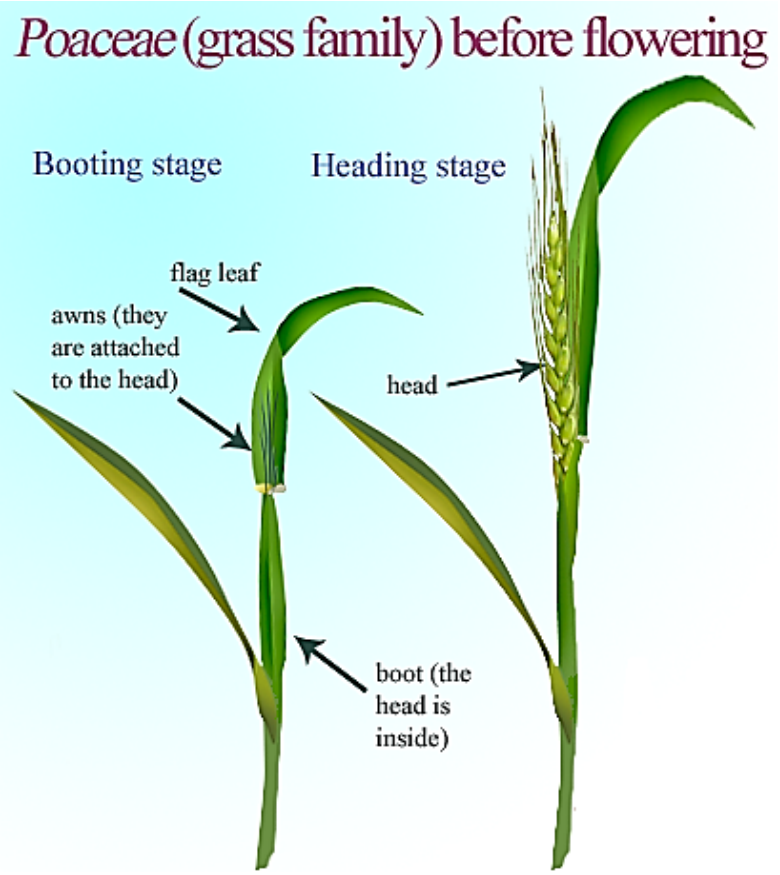

FIGURE 2.1: *Triticum aestivum* (Wheat) [32].

acids for humans, lysine is although in a less quantity, now a days lysine rich crops are developing by plant breeding efforts [38]. Trace minerals like selenium and magnesium provided by the wheat are the nutrients essential to good health. Caryopsis that is wheat grain, consists of the pericarp or fruit and the true seed. 72% of the protein is stored in the endosperm of the seed, which comprises 8-15% of total protein per grain weight. Pantothenic acid, riboflavin and some minerals, sugars etc. are also rich in wheat grains [39, 40].

Dietary source for fiber, potassium, phosphorus, magnesium, calcium, and niacin in small quantities are provided by the barn consisting of pericarp testa and aleurone. Kernel weighs about 83% of the endosperm provides the source of white flour [41, 42]. The greatest share of the protein in the whole kernel, carbohydrates, iron, many B-complex vitamins (riboflavin, niacin, and thiamine) are contained in the endosperm. Kernel weighs about 14.5% of the Bran [43, 44]. Dietary flour that is indigestible cellulose material, a small amount of protein, larger quantities of the

B-complex vitamins listed above and trace minerals are contained in whole wheat, the bran [49]. The germ or embryo of the wheat kernel is comparatively rich in protein, fat and several of the B-vitamins [45, 46]. A higher concentration of protein, vitamins and phytic acid is contained in the outer layers of the endosperm and the aleurone than the inner endosperm. Most of the starch and protein is contained in the grain. Wheat is being milled for flour, starch and proteins are separated [47, 48].

2.1.2 Crop Development

For the development of crop with all the developmental stages such as sowing, harvesting requires 110 to 130 days normally depending upon different factors, climate, seed type, soil conditions. At some stages fertilizers, herbicides and effective growth regulators are required to apply for optimum growth management. Climate risks are also very important to be identified with the knowledge of each stage. Flag leaf appears during grain filling period which shows 75% of photosynthesis, at this stage crop is preserved from disease for ensuring good yield [50].

2.1.3 Conditions for Growth

2.1.3.1 Ecology and Climatic Requirements

Temperature that is best suited for optimum growth and development is about 18 to 25°C, high intensive temperature could cause hastening growth and shortening developmental stages [51, 51]. Wheat is highly adaptive crop although temperature and humidity are the major limiting factors in yielding as humidity enhances the chances of diseases to occur. Site selection for the cultivation is also dependent upon temperature factor although fertile well drained sand and clay foam with low night temperature area plays significant role [53]. Climate, as the regular weather state of a habitation for at least thirty years regulates, not only what the agriculturalist can plant, but main traditional practices and occurrence of pests

and infections. The main crop regulating aspects of weather in the tropics are high temperature and moisture. Information of phases is significant to classify phases of higher risk, in terms of weather.

2.1.3.2 Sowing Date

Crop growth and development is significantly influenced with sowing date [54, 55]. Temperate conditions are required for wheat to grow. Crop is exposed to aphid and stem borer attacks after delay in seed sowing [56]. Wheat is best suited to be usually sown between November/December and best to be harvested during March/April [57]. Mid-November is the optimum time for seed sowing, earlier or later sowing than mid-November will not yield optimum crop [58].

2.1.3.3 Seed Treatment

Seed treatment is a vital process in achieving an adequate crop stand as it is prerequisite for a successful wheat crop. High percentage germination of a crop will be ensured by seed treatment. Better seed germination by preventing/ reducing the incidence of pests is achieved by seed treatment [59].

2.1.3.4 Land Preparation

The cropping history of the land is important for land preparation requirement. Land preparation starts early November after the harvest of the last rain-fed crops as a dry season crop. Before the end of October it is common to have land preparation done. When to begin the process of land preparation will be determined by the current crop under cultivation prior to incorporating the wheat crop into the cropping system.

Ploughing and harrowing should be done to a good tilt for better site. Gravitational pull required to be achieved a level of slope with water movement. Free water movement will be ensured by leveling the field to a slope of 0.25 – 0.30% to

a drain located at the end of the field. Free water movement is allowed through sunken beds [60].

In rain fed zones, the field preparation must be finished with maintenance as preservation of humidity is dependent on it. Planking must be completed initial in the morning. A buffer region of 10 to 30 m breadth should be reserved to evade pollution from the inorganic field.

2.1.4 Crop Water Requirement (CWR)

Water is lost through evapotranspiration so a particular amount of water is required to meet this loss. Simply it is the required quantity of water for the optimum growth of any crop.

Optimal conditions under which a plant is grown, crop that is uniformly grown with no developed diseases, also covers the complete field and encourages soil fertility must need adequate water amount. The factors necessary to meet the required needs are:

2.1.4.1 Climate

Per day more water is required in dry and hot climate areas as compared to cool and cloudy climatic areas.

2.1.4.2 Crop Type

Many crops such as sugarcane and maize essentially require high amount of water as compared to sorghum and millet crops.

2.1.4.3 Crop Growth Stages

Plants which are newly grown should have less watering as compared to properly grown plants [61].

2.2 Bio Fertilizers

Substances contain microbes which are active they improve the fertility of soil and growth of plant is improved when soil id added with these bio fertilizers. Rhizosphere or plant interior is colonized when the seeds of plant or soil containing crops are applied with bio fertilizers which as a result promotes plant growth and nutrients are efficiently provided to the host plant which is primarily required by them by increasing the supply to the plant [62].

For the low-income farmers expensive chemical fertilizers and pesticides are a limiting factor and increase the cost of crop production. Bio fertilizers have been proved eco-friendly effective as compared to the chemical fertilizers so proved to be economical and sustainable alternative [63, 64]. As compared to synthetic fertilizers bio fertilizers are cost effective as they diminish environmental pollution and agricultural production is enhanced and they stimulate plant growth, and restore soil fertility [65]. Bio fertilizers are economical, ecological in nature, and their prolonged use improves the fertility of the soil significantly [66, 67].

Among the vast benefits of bio fertilizers following are the main advantages: The nutrients are economically delivered, organic matter is also supplied efficiently, growth hormones are secreted enormously, micronutrients and chemicals are provided, which as a result counteract the adverse effects of chemically synthesized fertilizers [68]. Soil is composed of vital microorganisms which perform multiple biological activities in soil medium which results in the mobilization of nutrients and make soil fertile and dynamic for crop development and complete nutrients are delivered to the plants [69]. Common examples which are being used as bio fertilizers include bacteria which fix nitrogen effeciently such as Nostoc, Aulosira, Scytonema, Tolypothrix, Anabaena and Plectonema etc. [70, 71]. Strain PCC7120 of Anabaena sp. contains a promoter that induce light which drives HetR gene expression this gene expresses the protein formation and nitrogenase activity is highly expressed as compared to the wild type of strain. Paddy shows improved growth development when this strain was utilized to check its impact [72].

2.2.1 Fungal Bio Fertilizers

Assemblages of living microorganisms and microbial inocula comprised by bio fertilizers utilize multiple mechanisms which show benefits directly or indirectly and result in improved growth of crop [73]. Atmospheric nitrogen is fixed and phosphorus is solubilized by these microorganisms they also perform decomposition of organic matter and soil elements such as sulfur is oxidized [74]. That nutrient supply is beneficial to agricultural production [75].

2.2.1.1 Types of Fungal Bio Fertilizers

2.2.1.1.1 Arbuscular Mycorrhizal Fungi

This type of fugal bio fertilizer is known among the abundant agricultural soil fungi. By hormone action or antibiosis the production of crop is improved as a result of high concentration of available nutrients and their uptake by stimulating the growth of crop, stimulates growth of a plant by decomposition of organic residues [76].

2.2.1.1.2 Mycorrhizal Fungi

In land plants most of them such as 80% make symbiotic mutualism with mycorrhizae these include trees of forests having healthy growth outcomes [77, 78]. Mycorrhiza are of seven types such as ectomycorrhiza, endomycorrhiza or arbuscular mycorrhiza, ect- endomycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, and orchidoid mycorrhiza [79, 80].

2.2.1.1.3 Ectomycorrhizae (ECM) and Arbuscular Mycorrhizae (AM)

These improve the uptake of nutrients and water amount and pathogens are defensed. Only a few plants make mutualistic association with both of these mycorrhizae [80]. Mycorrhizal associations are universal in terrestrial ecosystems, and it is likely that these associations assisted to facilitate land colonization by plants.

Ectomycorrhizas are transitional in their capability to take up nutrients, being more competent than arbuscular mycorrhizas and fewer so than ericoid mycorrhizas, creating them valuable in a transitional nutrient condition.

2.2.2 Classification of Bio Fertilizers

In the production of bio fertilizers microbes make beneficial association with crops are exploited. On the basis of nature and function they are classified into different categories.

2.2.2.1 Rhizobium

Rhizobium is the most effective biofertilizer because of the magnitude of of nitrogen fixation performed by them [81]. They are grouped into seven genera which are performing nodulation in legumes and are very specific [82, 83].

2.2.2.2 Azotobacter

In culture media chroococcum is the dominant in habitant in soils having enough air and it fixes nitrogen in enormous amount. Slime production by the bacterium helps in soil aggregation.

Because organic substances are less and antagonistic microbes are in high concentration chroococcum rarely exceed 105/g soil. For the enhancement of the tomato growth, primary substances are required for optimum growth. Azotobacter species are effective in producing these growth materials including cytokinins, auxins and G-A like substances [84].

2.2.2.3 Azospirillum

Primary inhabitants of soil are *Azospirillum lipoferum* and *Azospirillum brasilense* (Figure 2.2) which are the rhizosphere and root cortex with its intercellular spaces

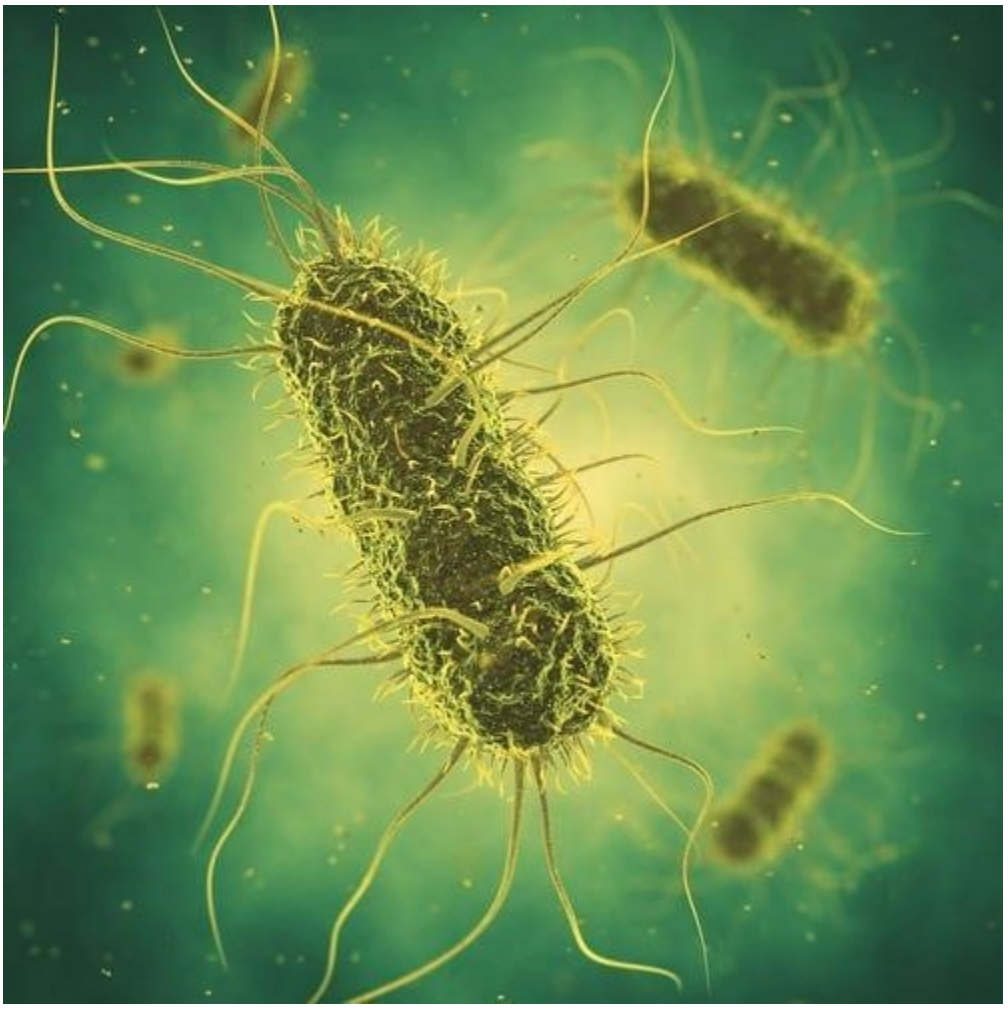

FIGURE 2.2: Azotobacter.

of graminaceous plants are the primary inhabitants of soil. Associative symbiotic relationship is developed by them with graminaceous plants. Additional benefits of Azospirillum have benefits such as the production of substances which promote growth, also resist diseases and perform nitrogen fixation and drought conditions are tolerated well by them [85].

2.2.2.4 Cyanobacteria

Cyanobacteria are also known as blue-green algae which are prokaryotic form which performs aerobic photosynthesis and it is known to be the oldest form of life in planet earth. They have 50 genera and more than 2,000 species including unicellular, branched filaments and colonial species having five sub-sections

FIGURE 2.3: Azospirillum [85].

i.e. Oscillatoriales, Chroococcales, Pleurocapsales, Nostocales and Stigonematales [86]. In paddy soils they are abundantly found and make soil fertile and texture better without any cost [87].

2.2.2.5 Azolla

FIGURE 2.4: Azolla (water fern) [89].

Azolla is a fern of water and floats freely and nitrogen fixation is performed by it (Figure 2.3). Azolla behaves as a substitute of nitrogen source or as an enhancement to sustainable nitrogen fertilizers. It contributes 40 to 60 kg N per ha for every rice crop and used as bio fertilizer for wetland rice [88]. Azolla is a heterosporous pteridophyte, with seven species. In Asian countries some experiments are carried out which can be predominantly compared to the bibliographical available data for the development of environment [89].

2.2.2.6 Phosphate Solubilizing Microorganisms (PSM)

Pseudomonas, Bacillus, Penicillium, Aspergillus have species which are effective in lowering the soil pH and produce organic acid in their vicnity and perform dissolution of phosphate element in the soil. Culture inoculation of Bacillus polymyxa and Pseudomonas striata improves the growth of wheat crops and potato [90].

2.3 Rhizobacteria (Plant Growth Promoting)

Bacterial group such as rhizobacteria stimulate growth of plants with multiple beneficial effects are known as plant growth promoting bacteria PGPB. These are categorized in numerous genera such as: *Agrobacterium, Bradyrhizobium, Alcaligenes, Arthrobacter, Actinoplanes, Azotobacter, Bacillus, Erwinia, Enterobacter, Pseudomonas sp., Rhizobium, Amorpho sporangium, Flavobacterium, Cellulomonas, Streptomyces* and *Xanthomonas* [92]. PGPR is increasing day by day due to frequent studies done on different species of plants and some improvements made in the taxonomy of bacterial species and also development of understanding the mode of action and mechanism of PGPB.

Plants are interacted with microorganisms and it is significant to have competency to colonize the habitats effectively. A single strain can adhere to the plant cell surface and divides to make dense aggregates generally called as biofilm formation. There are steps of macro colony formation include attraction towards plants, to

recognize them, adhere there and invade. Colonization is performed through signaling as the roots of plants produce signals and microorganisms recognize them and colonize there known as crosstalk [93]. Chemotactic signals are used by PGPR to actively move and to reach surfaces of roots with the help of flagella. There are a few factors upon which competency of PGPR depend such as to obtain the environmental advantages and show adaptation to the changed condition of soil and plant species.

Plant roots initiate crosstalk with soil microbes by creating signals that are recognized by the microbes, which in turn produce signals that recruit colonization. PGPR reach root surfaces by active motility enabled by flagella and are guided by chemotactic responses. This implies that PGPR competence extremely depends either on their abilities to take advantage of a specific environment or on their capabilities to adapt to changing conditions or plant species [94].

2.3.1 Mechanisms of Plant Growth-Promoting Rhizobacteria

Rhizobacteria adapt various mechanisms (Figure 2.4) which can promote plant growth which can be classified according to their mode of action in:

1. The production of substances that can be assimilated directly by plants [95].
2. The nutrient mobilization [96].
3. Plant stress resistance is induced.
4. Plant diseases are prevented [97].

PGPB encourage the growth of plants by diverse mechanisms but these are categorized into two mechanisms such as direct mechanism and indirect mechanism [99]. Direct mechanism is responsible for fixation of nitrogen, production of phytohormones, solubilization of phosphate and growing iron accessibility and indirect

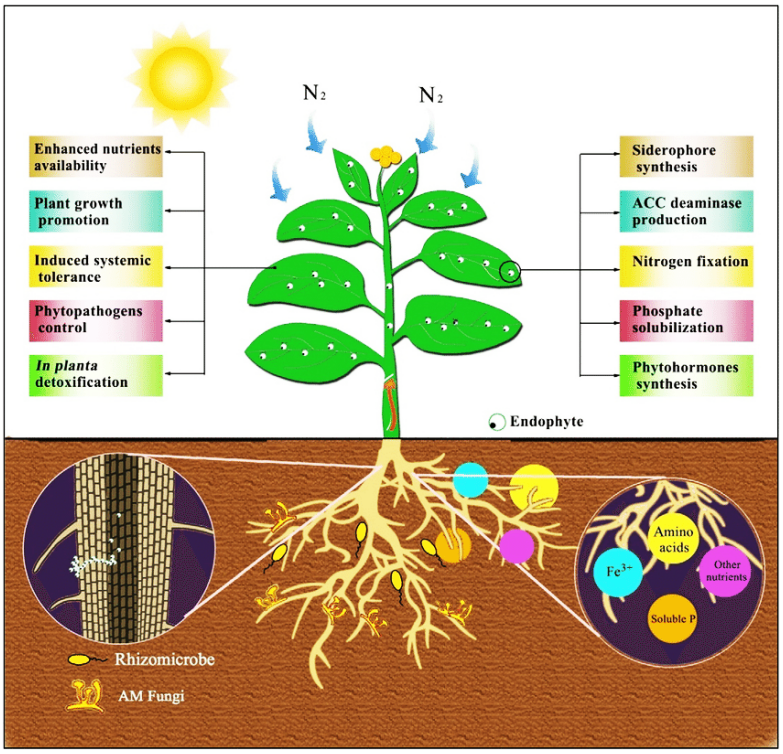

FIGURE 2.5: Mechanism of plant growth promoting rhizobacteria [98].

mechanism results in the production of lytic enzymes and volatile and non-volatile antiobiotics (Figure 2.5).

2.4 Nitrogen Fixation

Among the most important nutrients nitrogen is required for the essential plant growth and survival of bacteria. Nitrogen fertilizers are being used to meet the deficiency to achieve plant requirements for maximum plant yield in most soils. Nitrogen is present in gaseous form that is not consumed by majority of microbes; it is first converted to ammonia suitable for plant assimilation. Significant amount of energy is required for this conversion to ammonia for the stability of the triple bond in nitrogen [99, 100].

Nodule formation is shown by a great number of microorganisms performing nitrogen fixation bya adapting symbiosis, a broad range of nitrogen fixing bacteria have been identified. Rhizobium, Sinorhizobium, Allorhizobium, Mesorhizobium,

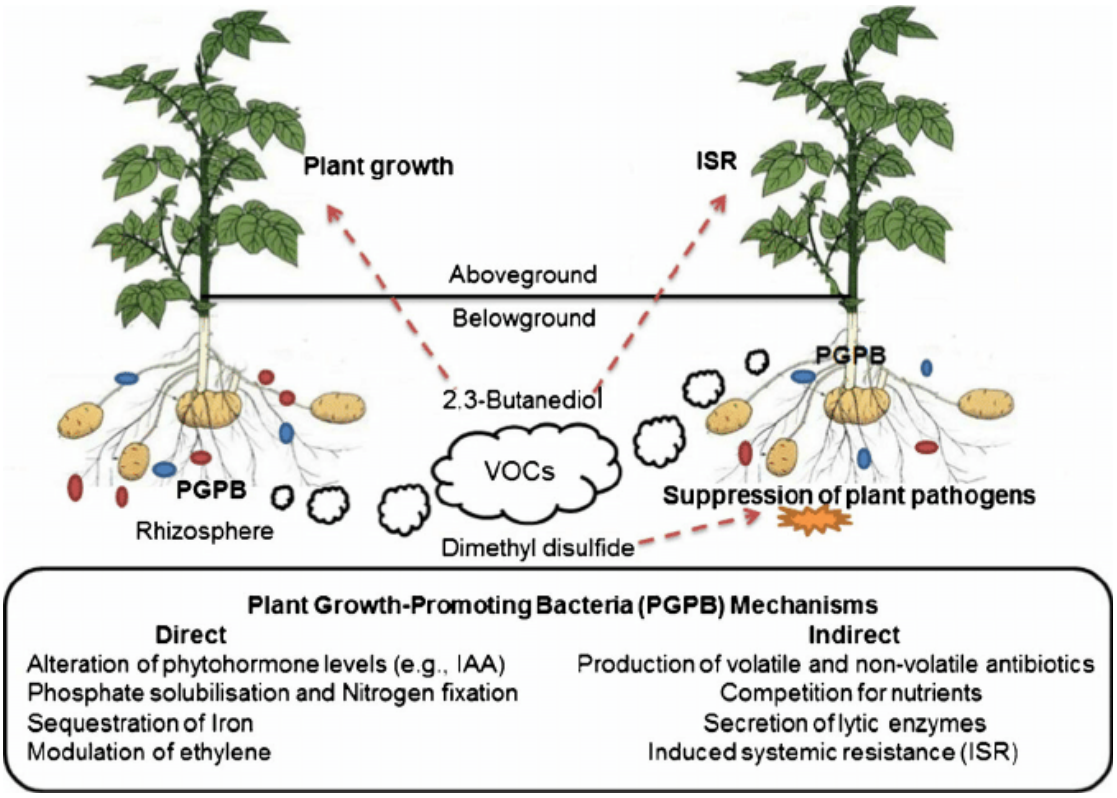

FIGURE 2.6: Mechanism of plant growth promoting rhizobacteria [99].

Bradyrhizobium, Azorhizobium, Frankia, Azoarcus, Achromobacter, Burkholderia, and Herbaspirillum are the examples of symbiotic nitrogen fixers [101].

Symbiotic relationships majorly with legumes are formed by Rhizobia which are gram-negative bacteria, interactions among rhizobium and plants is very specific. Some studies show that rhizobia also colonize cereal crops. Rhizobium resides in the cells of rots of a plant results in the complex kind of changes in development like nodulation. In root nodule, bacteria is present in the form of bacteroid having no cell wall and utilize the nitrogenase enzyme to perform nitrogen fixation (Figure 2.6) and produces ammonia [102].

Nitrogen fixation requires multiple factors to be handled with the help of nitrogenase "nif" genes. To active the Fe protein for the cofactor biosynthesis such as iron molybdenum, donation of electrons, these functions are handled with the help of structural genes. There are as many as 20 different proteins which are encoded by 7 operons present in the form of 20-24kb size having nif genes in nitrogen fixing bacteria [103]. It is possible to improve nitrogen fixation by genetic engineering

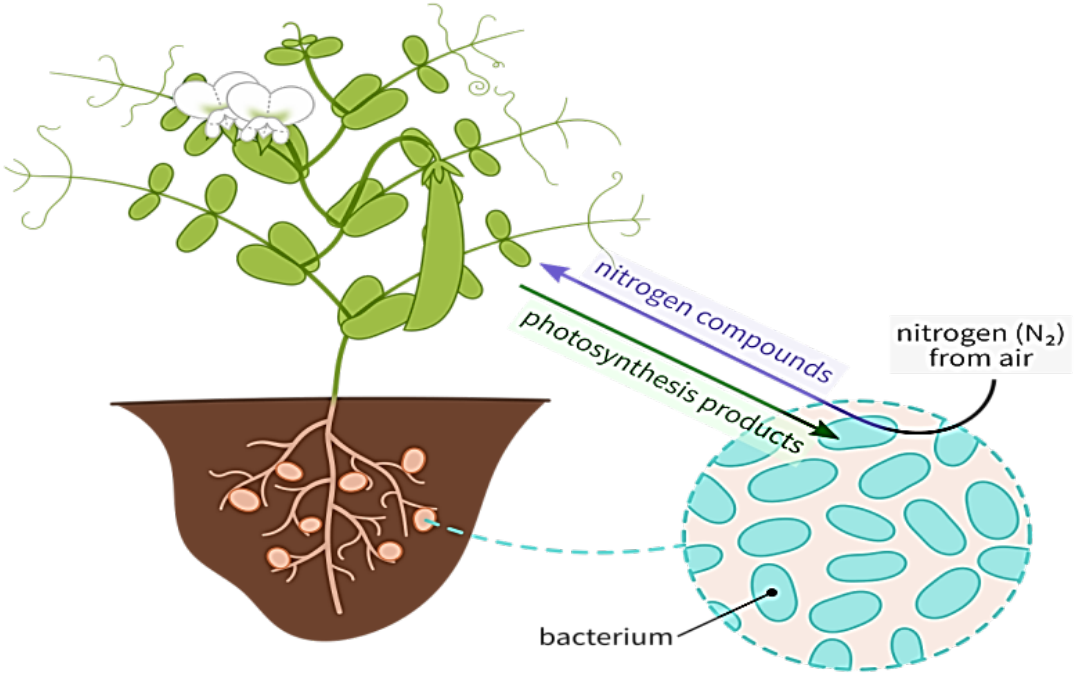

FIGURE 2.7: Root nodule formation [102].

according to some scientists, they believe that if once nif genes are isolated and characterized, some explained the possibility to genetically engineer plants which would in return able to fix nitrogen. When Rhizobium tropici is induced with a deleted gene which is responsible for the production of glycogen synthase and plants of beans are treated with genetically modified bacterial strain results in nodule formation and increased biomass as compared to the untreated strain of wild-type. Unfortunately, it is not flourished in the environment of soil. Genetically engineered strain successfully improved nodulation and dry weight of plant in the field [103]. Inhibition of the enzyme nitrogenase and negative regulation of nif gene expression is dependent upon oxygen although, it is essential for the respiration of species of Rhizobia (Figure 2.7).

2.5 Phosphate Solubilization

It is the fact that phosphorus is not soluble so does not promotes the growth of a plant although phosphorous is present in a large amount in the soil environment much high such as 400 to 1,200 mg/kg of soil.

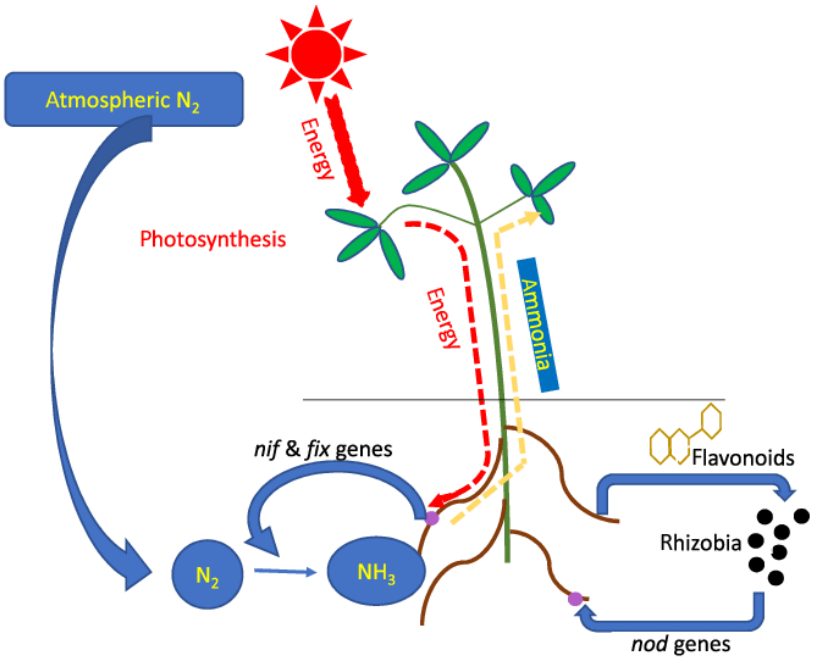

FIGURE 2.8: nif gene expression in nitrogen fixing *Rhizobia* [103].

It exists in the form of inorganic matter and organic matter as well which include phosphomonesters, inositol phosphate, and phosphotriesters [104]. Plant growth is adversely effected when sufficient amount of phosphorous is not able to reach there this is because it is combined with soil and bioavailability is limited that ultimately effects growth of plant as this is very essential nutrient.

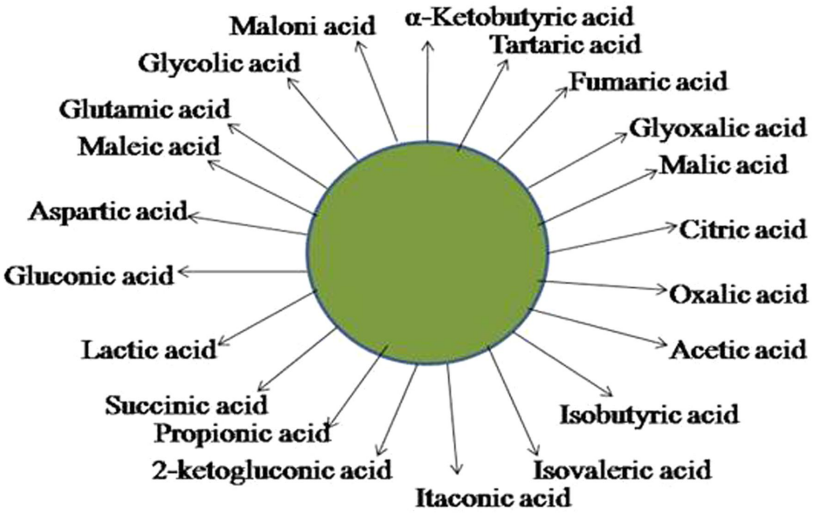

FIGURE 2.9: Organic acids produced by PSM [106].

Inability to obtain sufficient phosphorus often limits plant growth due to limited bioavailability of phosphorus from the soil combined as this element is essential

for plant growth. Compounds which dissolve reserves such as different organic acids which include (malonic acid, gluconic acid, tartaric acid, isobutyric acid etc.), siderophores production, protons and hydroxyl ions and CO_2 as well are produced by phostate-solubilizing bacteria as shown in (Figure 2.8), organic acids are responsible to lower the pH which results in the discharge of Phosphorous. Phosphate-solubilizing bacteria thus, solubilize and mineralize phosphorus as it is known capacity of PGPB in promoting plant growth as well as in fungi like *mychorrizae* [105, 106].

2.6 *Staphylococcus arlettae*

- *Staphylococcus arlettae* is among gram +ive bacterium. Taxonomic classification is given in Table 2.1.
- It consists of clusters of cocci (Figure 2.10)

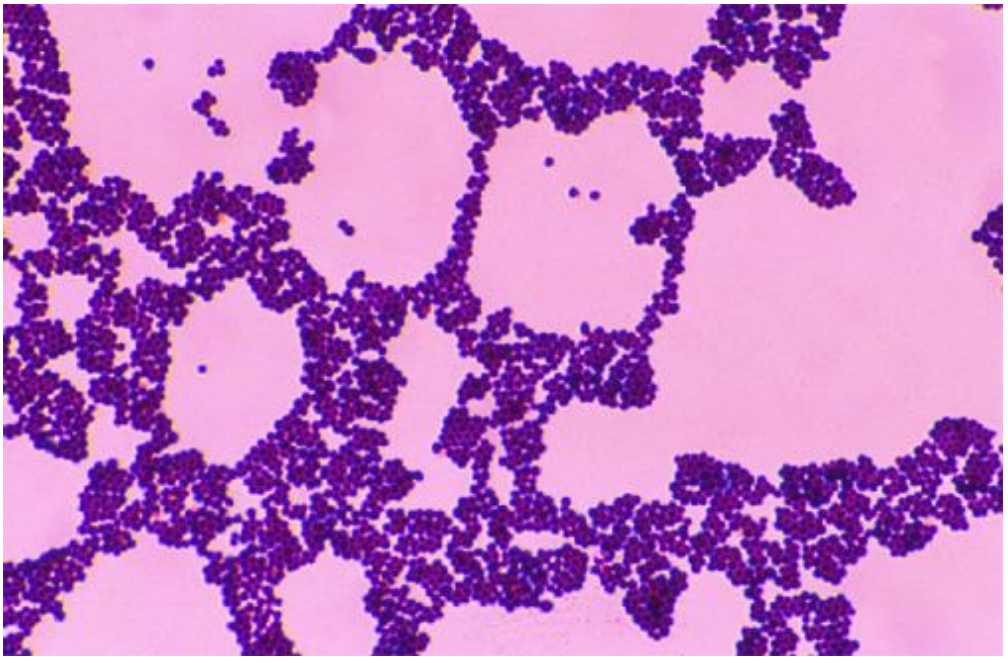

FIGURE 2.10: Clustered cocci [108].

- It is the member of genus Staphylococcus which is coagulase-negative.
- It is being isolated from the skin of mammals and birds.

TABLE 2.1: Scientific Classification of *Staphylococcus arlettae* [108].

Kingdom	**Bacteria**
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	*Staphylococcus*
Species	*S. arlettae*
Binomial name	*Staphylococcus arlettae*

- It is resistant to novobiocin.
- It degrades azo dyes (this bacterial strain is obtained from textile factories and sewages) [108, 109].
- The chemical conformation of the cell walls of this new species is comparable to that of S. xylosus and S. saprophyticus.

2.6.1 Origin

Staphylococcus arlettae is isolated from skin of mammals and nares of goats and poultry. Veterinary and clinical samples are also examined and proved to be the source of this strain [108].

Subsequently, *S. arlettae* has potential to degrade azo dyes to promote growth of plants being separated from textile industries and effluents from tannery industries, respectively [110, 111].

Some sites such as soil, surfaces of things used frequently by humans like mobile phones are found to be the source as well. Biological cabinets used in laboratory which are disused, also existed with this strain [112, 113].

Genomic DNA from *S. arlettae* strain CVD059 was sequestered by means of DNeasy miniprep kit, and genome sequencing was accomplished by Ion Torrent Personal Genome machine. A total of 2,997,560 reads with a normal read length of 245 bp were achieved, which yielded 734,402,200 sequenced bases.

2.6.2 Previous Studies

2.6.2.1 Arsenic Hyper Tolerance

Based on analysis an arsenic hyper tolerant bacterium was isolated from arsenic polluted site a bacterium was separated on the basis of 16S rRNA analysis named as *S. arlettae* strain NBRIEAG-6 that is arsenic hyperactive tolerant. *S. arlettae* possessed arsC gene which had capability to erase arsenic when applied to liquid media, having arsenate reductase activity. It was also able to produce acetic acid, carboxylic acids and siderophores when biochemical profiling was performed. The bacterial inoculation on mustard plant *Brassica juncea L.* significantly improved the plant growth by the increased development of dry weight, protein content, carotenoids and

2.6.2.2 Decolorizing Azo Dyes

From activated sludge of textile industry a facultative *Staphylococcus arlettae* bacterium was isolated successfully who decolorized multiple azo dyes in microaerophilic environment and the decolorizing percentage was $> 97\%$. Fourier Transform Infrared Spectroscopy and Ultraviolet–visible spectroscopy techniques were applied to characterize degradation products and their toxicity was aso measured with respect to *Daphnia magna*.

The concentration of amines and total organic carbon (TOC) levels were observed during biodegradation. In microaerophilic state and the aerobic environment, aromatic amines were formed which shown that the azo reductase activity and oxidative biodegradation as well. Aromatic amines were formed by reducing the breakdown of azo bonds under aerobic conditions and oxidized into non-toxic metabolism in a bioreactor when *Staphylococcus arlettae* strain was used to form aromatic amines. Anaerobic and microaerophilic microorganisms diminish azo bonds non-specifically in anaerobic situations leading to dye decolorization. Methyl red being the unassuming azo dye was used in this study to recognize the bacterial decolorization of azo dyes.

2.6.2.3 Lipase Activity

Genetic analysis algorithm was generated through Rock salt mine was explored to get bacterial strain *Staphylococcus arlettae* JPBW-1 showed lipase production and higher lipase activity. This strain shows great stability for solvents including xylene, benzene, n-hexane, ethanol, methanol, and toluene has been shown by this strain. Metal ions such as K^+, Co^{2+} and Fe^{2+} inhibit the activity of lipase and metals such as Mn^{2+}, Ca^{2+} and Hg^{2+} stimulate this activity.The lipase activity has been found to be inhibited by metal ions of K^+, Co^{2+} and Fe^{2+} and stimulated by Mn^{2+}, Ca^{2+} and Hg^{2+}. Thus, considerable potential to tolerate extremophilic conditions of high temperature, pH and salt concentration has been shown by the lipase from *S. arlettae* [115].

2.6.2.4 Extremophilic Lipase Activity

Genetic algorithm was generated through response surface methodology which shows *Staphylococcus arlettae* to be solvent tolerant and alkaline lipase producer. Using binary coded genetic algorithm an optimum lipase yield has been obtained. Extremophile lipase shows tolerance under halophilic conditions and for solvents could contribute to introducing this to sector of manufacturing biotechnology and also proved possible choice pharmaceuticals, chemical production and food items [116, 117].

2.6.2.5 Degradation of Phenolic and Non-Phenolic Compounds

Ability to degrade variety of phenolic and non-phenolic compounds is shown by oxidoreductase enzymes such as lactase. Versatile oxidoreductase enzyme having a capability of degrading variety of phenolic and non-phenolic compounds is Lactase.

Staphylococcus arlettae S1-20 obtained from waste of tea produced lactase (S1-20LAC) extracellularly and it was stable under thermo-alkali conditions. Numerous progressive arithmetical software tools such as response surface methodology

and artificial neural networks were employed to increase the enzyme yield by 16 folds.

The overall quality of lactase is measured by the comprehensive dynamic and thermodynamic studies which exposed this is spontaneous process at high temperature. A large number of substances are degraded and synthesized by the focus of this study which proves to be great product and notably shows property which proves it to be green biocatalyst for coming future viewpoints [118].

2.6.2.6 Triglyceride Removing Ability

Soil fabric has the ability to remove triglycerides are shown by the lipases which are the enzymes for laundry detergent industries which limit chemical cleansers which are composed of phosphate for the formation of detergents. Salt mine was assessed and separated *Staphylococcus arlettae* JPBW-1 for partially purified bacterial lipase and its triglyceride removing ability. Lipase shows great stability for surfactants, oxidizing agents and chemical, lipase has shown removing ability.

When non-ionic detergent was used in 0.5% with lipase it enhanced the washing efficiency of detergents. By the usage of alkaline lipase of bacteria, chemical free detergent was formulated by enzyme-based detergent has been proved to be shown by this study [119, 120].

2.6.3 Genome Sequencing

Genome sequencing is performed in the laboratory to achieve whole genome sequencing of *Staphylococcus arlettae*. A sterilized moistened swab is used to swab the floor of lab to pick up the microbes. Luria Broth plate is streaked by swabbing. After incubation for overnight at 37°C the petri plates showed one colony. Further this colony is refreshed by streaking onto another fresh plate of LB. This experimental procedure is performed three times [121].

The resulted isolate which is named P2 analyzed for 16SrRNA gene that shows complete identity such as 99.95 with 16SrRNA arrangement of *S. arlettae* strain CVD059, motivated scientists to define the first ever closed genome sequence of *Staphylococcus arlettae* strain. P2 strain is inoculated in the luria broth until early stationary phase, after those cells are cultured at 37℃ [122, 123].

Mag Attract high molecular weight (HMW) DNA Kit is used to prepare genomic DNA of high molecular weight from selected cultures microbial strain, this DNA is further subjected to long and short read sequencing. GridION X5 system is used to perform long read sequence. Ligation sequencing Kit (ONT) is used to construct library of 1.0 μg unfragmented genomic DNA. For long read sequence generated 52502 reads having average length of 12,711bp for 10hrs running time [124]. For short read MiSeq instrument is used to prepare DNA library. FASTQ processing program is used for raw sequencing data, yielded 1.05m bp short reads with average base pairs having length of 1528bp [125]. Unicycler is used for the complete DE novo genome assembly [126]. Pilonv.123 is used for the final polishing step and generated one singular circular contigs having chromosome of length 2,629,900bp with 33.7% G+C content and plasmid circular contigs with chromosomes of length 22,364bp with 33.7% G+C content. SV-Quest is software program which is used to map the signals for structural gaps which are not detected. When P2 chromosome is compared to CVD059 it has 45kbp shorter than CVD059 shows 99.25 of gap identity and 93.8% symmetrical identity although CVD059 has not plasmid reported [128, 129]. This represents first report on closed genome sequence for *Staphylococcus arlettae* strain and got registered in database publically so that essential comparative analysis can be performed in coming future.

2.7 Chromium Cytotoxicity

Living bodies and human health are on risk from a dangerous heavy metal that is toxic to plant vegetation is chromium, as the environmental pollution is increasing

with the consumption of plant materials effected with Cr, plants being part of food chain and high toxic to vegetation [130].

Chromium is being highly used in the production of chemicals, pulp formation and paper manufacturing, wood is preserved, pigments production as well in metallurgy on large scale majorly spreader Cr pollution in environment which shows serious threat to humans and animals [131]. The mechanisms involved in plant growth metabolism, respiratory and photosynthetic mechanisms and defensive mechanisms as well are harmfully affected by Cr. Many growth parameters are adversely harmed by Cr phytotoxicity such as: early seedling development is stopped, biomass is decreased and root growth is reduced [132].

2.8 Sources

Chromium becomes available naturally and anthropogenic (environmental pollution) by the activities of humans such as: coal and oil burning, application of fertilizers on crops, oxidants usage, metal plating tanneries and drilling of oil well [133]. Factories discharge consisting of toxic metal is transferred from place to another and goes into streams, largely damage aquatic life including fungi, algae, phytoplankton, zooplankton and other microorganisms [134].

2.8.1 Effects of Chromium

A large number of enzymes are affected by this toxic metal including catalases, peroxidases and reductases which results in chlorophyll loss thus plant tissues and organs die with the toxicity [135]. Cellular components when get in contact with toxic metal metabolic responses lead to growth shifts. Normal quantity of crop yield is unable to be achieved due to accumulation in soil, as many nutrients are unable to reach the plant roots which results in poor yield of quantity and plant organs are not developed [136]. In our society and environment the most life challenging and health risk in the ecosystem is due to the bio toxic substances which

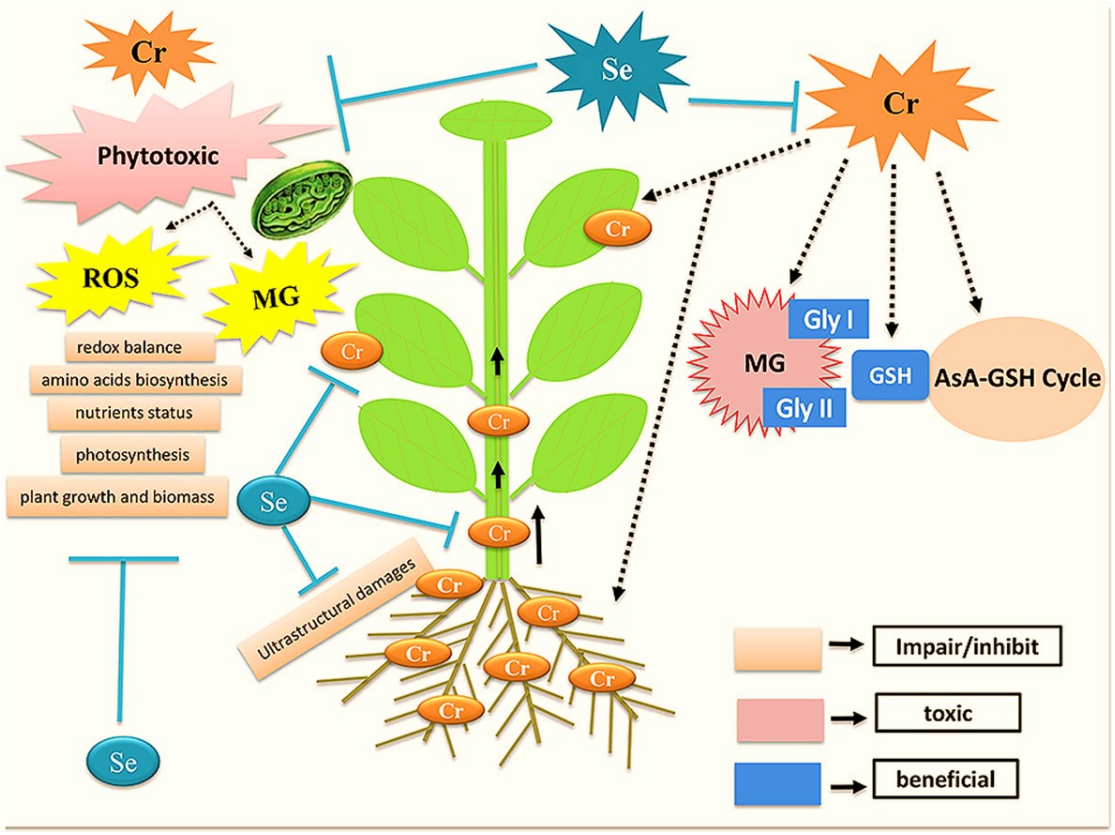

FIGURE 2.11: Toxic effects of chromium [136].

include heavy metals in the discharge of untreated tannery waste water. Damaged oxygen supply, microbes are denatured, reducing bioremediation capacity by chromium and cadmium lethal metals (Fig 2.11) [136,137]. Besides propagation, also root development is regularly affected by heavy metals. Reduced root surface in Cr stressed crops may pay to reduced ability of plants to exploration for water in the mud contributing to water tension.

2.8.2 Mechanism of Toxicity

Enzymatic functions are broken down when they react with thiol and carboxyl groups by competitive and noncompetitive interactions. When Reactive oxygen species (ROS) are produced, it is used as catalyst [138, 139]. Ion regulation is destructed and DNA production is affected. Transcription, translation and replication are all affected by intracellular cationic CIII complexes which interact with DNA constituents like phosphate group electrostatically and cause mutagenesis

[140]. Severe injury can be caused to cytoplasmic molecules, DNA, lipids by metals like Copper I and Copper II which catalyze the ROS production acting as soluble electron carriers [141]. Ion balance is disturbed when these adhere to surfaces of cells and enters from channels for ion movement (Fig 2.11) [142].

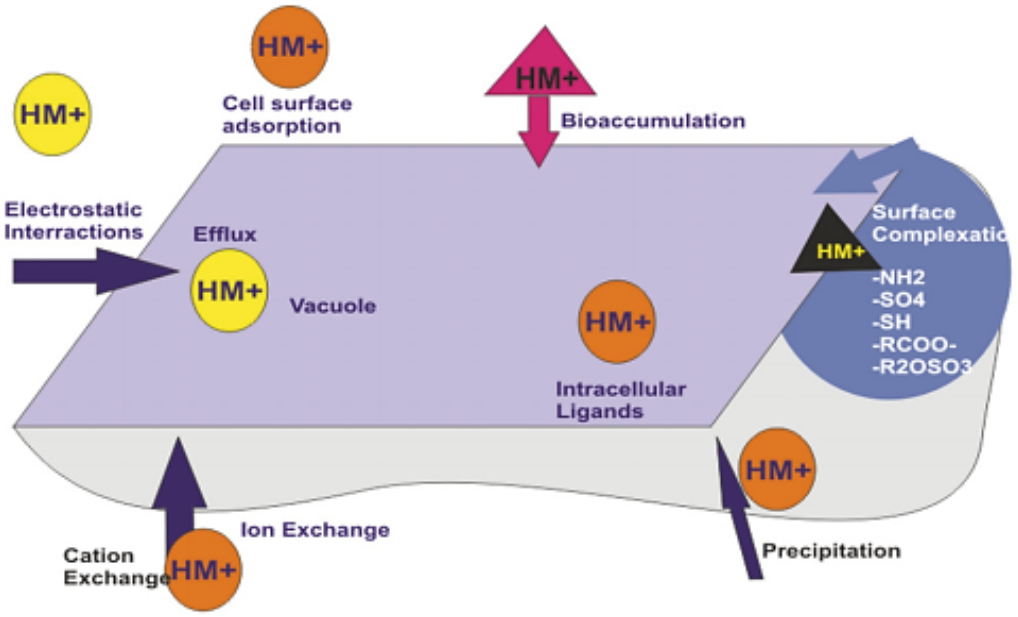

FIGURE 2.12: Mechanism of heavy metal uptake by microorganism [143, 144].

2.8.3 Microbial Detoxification Mechanism by Heavy Metal

Various defensive methods are adopted by microorganisms to survive the presence of metal toxicity such as biotransformation, extrusion, enzyme production, exo polysaccharide production and metallothioneins synthesis [145, 146]. Ingenious mechanisms for metal resistance and detoxification have developed by microorganisms. Along with multiple mechanisms several procedures such as ion exchange, complexion of organic metals, electrostatic interactions, degradation of metal ligands, metal chelator production, precipitation, redox reaction for metal resistance and detoxification [147]. Mechanical means such as metal oxidation, methylation, degradation of metal ligands, pumps for refluxing metals are used to resist heavy metals. Decontamination of metals by microbes can be done by converting valency, volatilization and extracellular precipitation of chemicals. Microbes

involved negatively charged sites such as hydroxyl, amine, phosphoryl, carboxyl, and ester sulfonate and thiol groups for adsorption of metals [148, 149].

2.8.3.1 Bio Sorption

Bio sorption defines as the process that is a physiochemical process that occurs naturally in certain biomasses which permits to submissively concentrate and binding of environment pollutants onto cellular organization. It can be classified into two types:

2.8.3.1.1 Metal Dependent Bio Accumulation

Methods involved in the heavy metal uptake by the cells of microbes occur on exterior of cells.

2.8.3.1.2 Metal Independent Bio Accumulation

Methods involved in the uptake of heavy metals comprised of sequestration, redox reaction and species transformation [150].

2.8.3.1.3 Example

Pseudomonas putida is cadmium tolerant and possesses the capability of intracellular sequestration of heavy metals by the cysteine rich low molecular weight proteins [151].

Chapter 3

Material and Methods

The research was carried out in wet lab of department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology, Islamabad.

3.1 Materials

Materials which were used in the experiment are list below:

3.1.1 List of Equipment

Autoclave, Magnetic stirrer, Measuring balance, Laminar flow, Incubator, Vortex, Refrigerator, Beakers, Spatula, Conical flasks, Eppendrof tubes, Spirit lamp, Inoculation loop, Ethanol, Petri plates, Test tubes, Glass vials, Micropipette, Micropipette tips, Cotton plugs, Cotton swabs, Aluminium foil, Falcon tubes 50ml, Test tube racks, Para film or masking tape, Forceps, Discs etc.

3.1.2 Microorganism and Plant used

Staphylococcus arlettae, *Triticum aestivum* (wheat plant).

3.1.3 List of Chemicals

Nutrient agar, MaCconkey agar, Mannitol Salt agar, Eosin Methylene Blue agar, Luria Broth

3.2 Methodology Flowchart

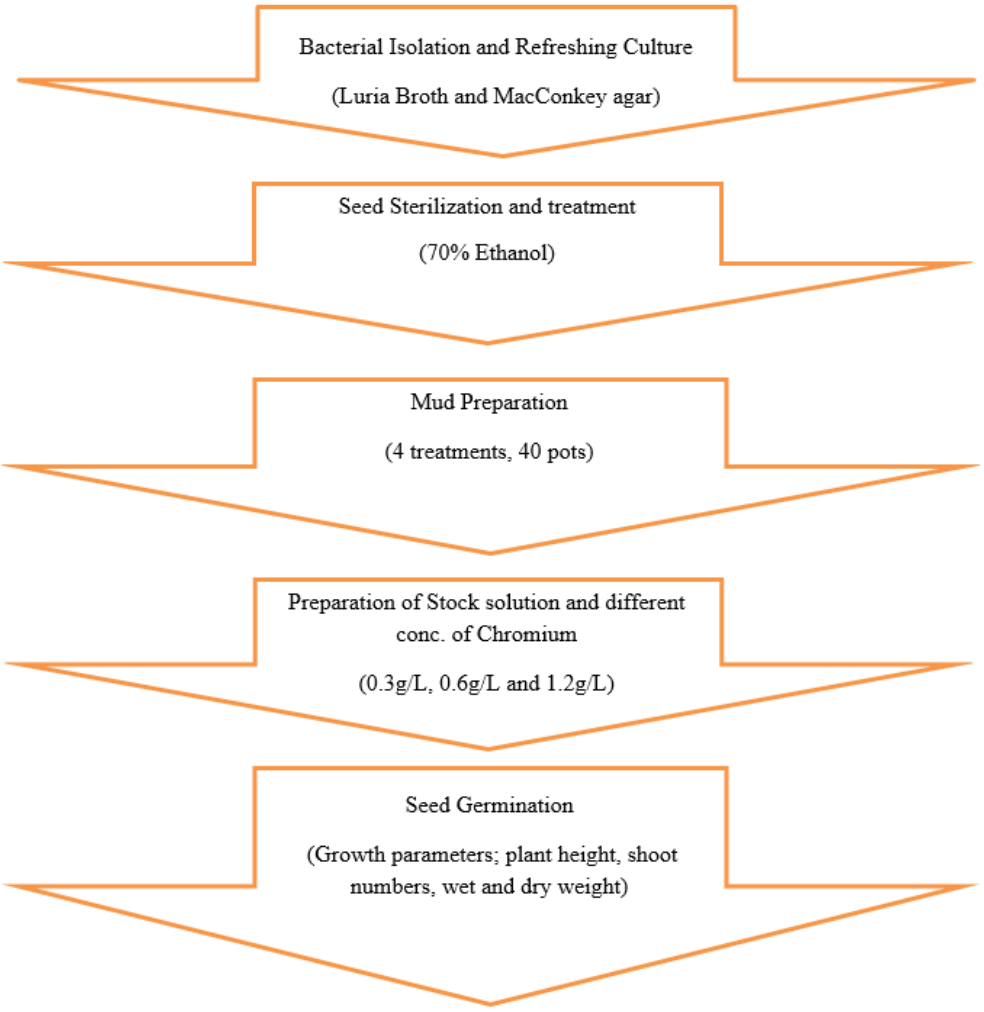

FIGURE 3.1: Methodology

3.3 Bacterial Identification/ Isolation

Different sites of households in Islamabad and Gujjarkhan were designated to gather adult houseflies. From each location 50 flies collected and transported

immediately to the laboratory. Then flies were kept into disinfected eppendroff tubes and autoclaved. Concurrently with the accumulation of one tablet of PBS phosphate buffered solution saline into 100ml of dist. water, PBS was prepared and autoclaved.10ml of this solution was added into eppendroff tubes and vortexed robustly (for 3-5 min). After that these flies were positioned into the refrigerator for further processing [152]. To get the colonies of bacteria associated with the houseflies, bacterial suspension is established on the nutrient agar. By the use of autoclaved tips and micropipettes, 1ml of each suspension contained in the eppendroff tubes having sample were transferred to the petri dishes. With the help of spreader, sample was placed on the plates uniformly. Five plates were labeled with the sample of Islamabad and five were labeled Gujjar Khan and placed in the incubator for 48 hours at optimum temperature 37℃.

3.3.1 Nutrient Agar Treatment

Pathogens association confirmation was done to check the presence on houseflies for that nutrient agar was used to culture these flies. 5.6g of nutrient agar was weighted by measuring balance and out into 200ml of dist. water and placed in autoclave at 121℃ for 2 hrs. Media was then poured into petri plates which were already disinfected. For pouring of sample 10 plates of media were prepared, which were stored in the incubator for further experiment [153]. 5g Luria broth was taken in 100ml of distilled water in 250ml flask and plugged with cotton and sealed then autoclaved after that placed in UV light for 20min. 50μl of bacterial culture was inoculated in LB suspension and placed in the incubator for 24hrs overnight at 37℃. Turbidity appeared showing the growth of *Staphylococcus arlettae* [154].

3.3.2 Streaking of Culture Media on Differential Media

From Nutrient agar bacteria were obtained and streaked on differential media, individual colonies from nutrient agar were streaked. Inoculum streaked was selected on the basis of color, morphology and the shape of specific bacteria. One

specific bacterial colony from nutrient agar was inoculated onto all of the three media (MaCconkey Agar, Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA)) and plates were stored in incubator for 24hrs at 37℃ [155]. After the growth on differential media, colonies were refined further by streaking on these media like MSA was streaked on the MSA, EMB was streaked on EMB, and Macc was streaked on Macc in LFH for 24-48hrs at 37℃. Bacterial pathogens which were obtained from flies among them three most prevalent strains were chosen for further purification for molecular and biochemical characteristics by further streaking onto differential media. For the purpose of purification and duplication total of three plates were streaked of each media. Until pure culture was obtained streaking was performed again and again [156]. To preserve the bacterial strains 100ml of glycerol stock will be prepared, 50ml of glycerol was added into 50ml distilled water in reagent bottle and autoclaved at 121℃ for 2hrs. 40blue tips and 20 eppendroff tubes were autoclaved by packaging them. 2 replicas of each strain was conserved by taking o loop (sterilized by red hot) full of bacterial strains and made suspension within eppendroff tubes and preserved at 4℃ in refrigerator for further processing.

3.4 Seed Sterilization Treatment

Wheat seeds were taken from the market and autoclaved including all the equipment required such as petri plates, flasks and forceps etc. Seeds were surface sterilized prior to germination. Then seeds were washed firstly with distilled water by soaking them for 1min and shifted onto the filter paper with the help of forceps to absorb extra moisture. After that, 70% ethanol was prepared by adding 30ml water in 70ml ethanol. Seeds were soaked in the ethanol for 1min to sterilize the surface and shifted onto the filter paper to absorb extra moisture and another filter paper was placed on above to completely dry the seeds, these were then washed with distilled water to remove ethanol and the process was repeated thrice. After sterilizing process seeds were stored in falcon tubes and stored in refrigerator at 4℃in the dark environment for additional experiment. All this work

was done under laminar flow hood in clean environment and disinfected the hood along with burner turned on to avoid any kind of contamination [156,157].

3.5 Preparing Pots/ Mud Preparation

Soil sample was taken from the soil that is proved to be much fertile every year. Mud was filled in the bigger jars and wrapped in the newspaper and autoclaved to disinfect in case it would be having any toxic microorganisms. This mud was filled in 40 pots (disposable cups already labeled with different treatment setups I am supposed to perform). A small hole was made on at the bottom of each pot so that extra water was drained off and air was reached to the seeds which were sown. Labeling was done accordingly to the setups [158].

FIGURE 3.2: Preparing pots

3.6 Stock Solution

2g/L of stock solution of chromium metal was prepared. Potassium dichromate ($K_2Cr_2O_7$) was purchased from the pharmacy. 0.5g of this was dissolved in 250ml

dist. water (equipment was already autoclaved), the solution was stirred with magnetic stirrer until it was completely dissolved to make Cr ions available and further process was proceeded by making different concentrations.

3.7 Preparation of Chromium Concentrations Dilutions

Seeds were supplemented with three different concentrations of Cr. Following formula was used to calculate different conc. of stock to make dilutions [159,160].

$$C_1V_1 = C_2V_2 \tag{3.1}$$

In the above formula V_1 is the volume of stock, C_1 is the stock conc., C_2 was the required conc. of Cr and V_2 was the required volume of the specific conc. Three conc. were prepared with different dilutions such as 0.3g/L, 0.6g/L and 1.2g/L by using the above formula. For 0.3g/L (by putting the values):

$$0.3g/L \times 50ml = 2g/L \times ? = 7.5ml \tag{3.2}$$

So, 7.5ml from stock solution was added in 42.5ml dist. water in 50ml falcon tubes to get 50ml of 0.3g/L conc. of Cr [175]. For 0.6g/L (by putting the values):

$$0.6g/L \times 50ml = 2g/L \times ? = 15ml \tag{3.3}$$

15ml was added in 35ml of dist. water to make 50ml of 0.6g/L Cr conc. For 1.2g/L (by putting the values):

$$1.2g/L \times 50ml = 2g/L \times ? = 30ml \tag{3.4}$$

30ml of stock sol was added in 20ml of dist. water to make 50ml of 12g/L of Cr conc.

3.8 Setups/ Treatments

Four different setups were taken. First setup is the untreated (control group) had 5 pots and 5 seeds were sown in each pot without treating with bacterial strain and chromium and labeled as C1, C2, C3, C4 and C5.

Second setup was the treatment of seeds with bacteria *Staphylococcus arlettae* strain, had 5 pots with 5 seeds being treated with bacteria labeled as T1a, T1b, T1c, T1d and T1e.

Third setup was the treatment of seeds with *Staphylococcus arlettae* and chromium Cr, 15pots in 3 groups had replica of 5 pots in each group, and labeled with different concentrations of Cr (0.3g, 0.6g and 1.2g) such as pots having 0.3g/L conc. were labeled as T2a1, T2a2, T2a3, T2a4 and T2a5. 0.6g/L conc. were labeled as T2b1, T2b2, T2b3, T2b4 and T2b5. Pots had 1.2g/L Cr conc. were labeled as T2c1, T2c2, T2c3, T2c4 and T2c5.

Forth setup was the treatment with Cr, 15 pots in 3 groups had replica of 5 pots in each group sown with 5 seeds in each pot, labeled with different concentration Such as 0.3g/L were labeled as T3d1, T3d2, T3d3, T3d4 and T3d5. Pots which had 0.6g/L conc. were labeled as T3e1, T3e2, T3e3, T3e4 and T3e5.1.2g/L concentration were labeled as T3f1, T3f2, T3f3, T3f4 and T3f5.

3.9 Seed Germination

Different growth factors like length of shoots (using metric scale), numbers of plants, and number of shoots were recorded after every 7 days of treatment for 3 months.

Fresh and Dry weight was also measured after the plants were removed and separated from the soil to discover out the beneficial properties of *Staphylococcus arlettae* and chromium on wheat plants [161]. Following parameters were observed.

3.9.1 Evaluation of Chromium Cr Reducing Ability of *Staphylococcus arlettae*

Under aerobic conditions with different Cr concentrations (0.3g/L, 0.6g/L and 1.2g/L) Cr pre-exposed log phase *S. arlettae* were grown at optimum temperature 37℃. *S. arlettae* growth and Cr reduction for initial Cr concentrations at different time intervals were observed. Both early and finishing growth conditions were measured in presence and absence of chromium [164].

3.9.2 Heavy Metal Tolerance

The cross reactivity of the *S. arlettae* to toxic metals was established with changing concentrations of chromium in the presence of high concentrations of heavy metals such as (0.3g/L, 0.6g/L and 1.2g/L) and growth competence of bacterial strain was also noticed. The ability of the *S. arlettae* strain to persist in contaminated environments by multiple metal resistances was evaluated [168].

3.9.3 Impact of *Staphylococcus arlettae* on Seed Germination

PGPP production and reduction of Cr toxicity was established by the ability of *S. arlettae* using *T. aestivum* seeds. In the absence as well as presence of Cr *S. arlettae* treated seeds percentage of growth germination was calculated. As compared to control plants in untreated group better-quality germination efficiency was also measured. Wheat seed germination rates as well as length of coleoptile and radicle were evaluated in Cr stress conditions [173].

3.9.4 Impact on Length of Seedlings

Length of shoot was observed by comparing untreated group when treated with various concentrations of Cr. Even at high Cr concentration seedling growth was

detected after treatment of *Staphylococcus arlettae*, compared to control uncoated (without *Staphylococcus arlettae* treatment) Cr treated seeds.

Growth of seedlings was observed with *Staphylococcus arlettae* treatment. In the presence of toxic heavy metal shoot elongation by *Staphylococcus arlettae* was recognized [176].

3.9.5 Impact on Shooting Response

Shooting response of wheat seeds with different treatment groups was studied. Number and length of shoots were observed for *Staphylococcus arlettae* treated seeds as well as untreated seedlings in the presence or absence of Cr [181].

3.9.6 Impact on Rooting Response

Rooting response of different treatment groups (Cr and *Staphylococcus arlettae* treated) was studied. Root length was calculated in the presence and absence of C [181].

3.9.7 Impact on Overall Growth of the Plant

By examining the fresh and dry weight of the seedlings complete impact of *Staphylococcus arlettae* and Cr on the development of plant was observed. As compared to untreated group *Staphylococcus arlettae* treated fresh and dry weight of biomass was calculated. Similarly as compared to control untreated plants Cr treated seedlings fresh and dry weight of biomass was calculated [159].

Chapter 4

Results and Analysis

This chapter covers all the phases which are involved in the reduction of reduction of chromium and growth promotion of plants by utilizing *Staphylococcus arlettae* in wheat with different interpretations and conclusions.

4.1 Bacterial Identification/ Isolation

Houseflies were collected from different surfaces of different substances and pathogens were collected from them and grown again on nutrient agar [162].

4.1.1 Nutrient Agar Growth

The nutrients of agar are peptone, beef extract and agar which are adequate for favorable growth, reproduction of bacteria. Pathogens were grown on nutrient agar and the results indicated the multiple bacterial colonies. Nutrient agar supports the growth of both gram-positive and gram-negative bacteria. It was clearly shown from the results that both bacteria were present. Nutrient agar is used to cultivate microorganisms which support a wide range of non-fastidious organisms, it grows a variety of bacteria and fungi as well because it contains all beneficial nutrients required for the bacterial growth

FIGURE 4.1: Growth of bacteria

4.1.2 MaCconkey Agar Growth

For the detection and separation of all types of dysentery, typhoid and paratyphoid pathogens maCconkey agar is used. This medium support the growth of all salmonella and shigella strains and distinguish between enteric pathogens and coliform group.

MaCconkey agar does not allow the growth of gram-positive bacteria and it differentiates lactose fermenting and non-lactose fermenting bacteria. The bacterial inoculum acquired from nutrient agar were streaked on the MaCconkey agar resulted in different colonies such as neutral red and crystal violet colonies appeared. Gram-negative bacteria are resistant to dyes grown on the MaCconkey agar [163].

4.1.3 Mannitol Salt Agar Growth

Mannitol salt agar is used to separate Staphylococci. It distinguishes bacteria on the basis of fermentation. The results that are obtained after the streaking of

nutrient agar bacterial growth on MSA are significant. Results presented colonies of *Staphylococci* [155].

4.1.4 Eosin Methylene Blue Agar Growth

Eosin methylene agar is a both selective medium for gram-negative bacteria and differential culture medium for coliform and fecal coliforms. EMB media differentiates Escherichia coli, other nonpathogenic lactose fermenting enteric gram-negatives rods, salmonella and shigella genera. These gram-negative bacilli are distinguished on the basis of color of colonies. The bacterial growth streaked on EMB shown significant growth with different colors of colonies. Green sheen appeared which shows the presence of *E. coli* [163]. For the purpose of purification and doubling total of three plates were streaked of each media. Until pure culture was obtained streaking was performed again and again [164].

4.2 Evaluation of Chromium Cr Reducing Ability of *Staphylococcus arlettae*

Under aerobic conditions with different Cr concentrations such as (0.3g/L, 0.6g/L and 1.2g/L) log phase of *Staphylococcus arlettae* that was pre-exposed was grown in autoclaved LB at optimum temperature 37℃. At different time intervals *Staphylococcus arlettae* growth promotion and reduction of chromium for minimum conc. of Cr are shown in Figure 4.1 and 4.2.

For 0.3 and 0.6 g/L reduction of chromium was noticed about 90%. With the initial Cr concentration the early progress of bacteria at the start varies inversely. This may features the cytotoxicity of Cr. Bacterial curve for long term appears to saturate when checked for the same level regardless what was the initial chromium concentration which suggests adaptive mechanism which enables the strain progress resistive tolerance for toxic Cr when it is applied [165].

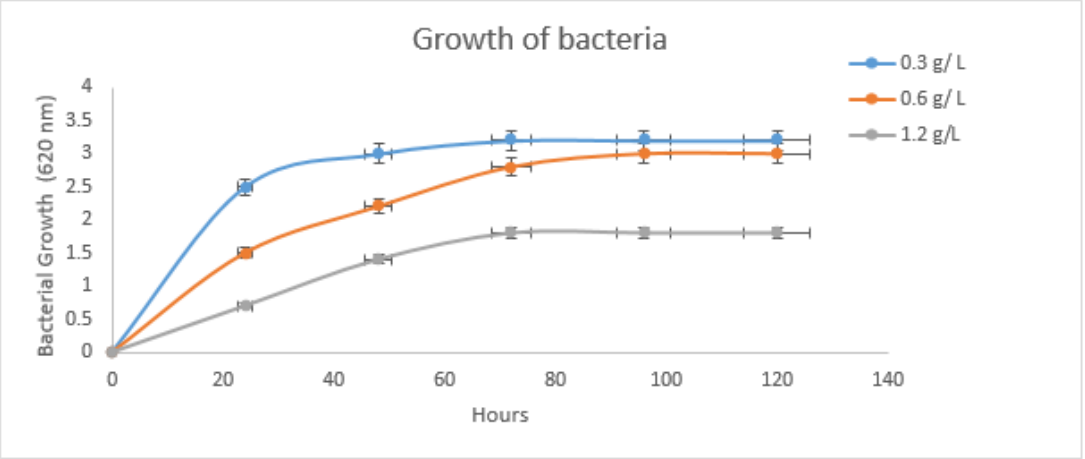

FIGURE 4.2: Bacterial growth curve when different concentrations of chromium applied.

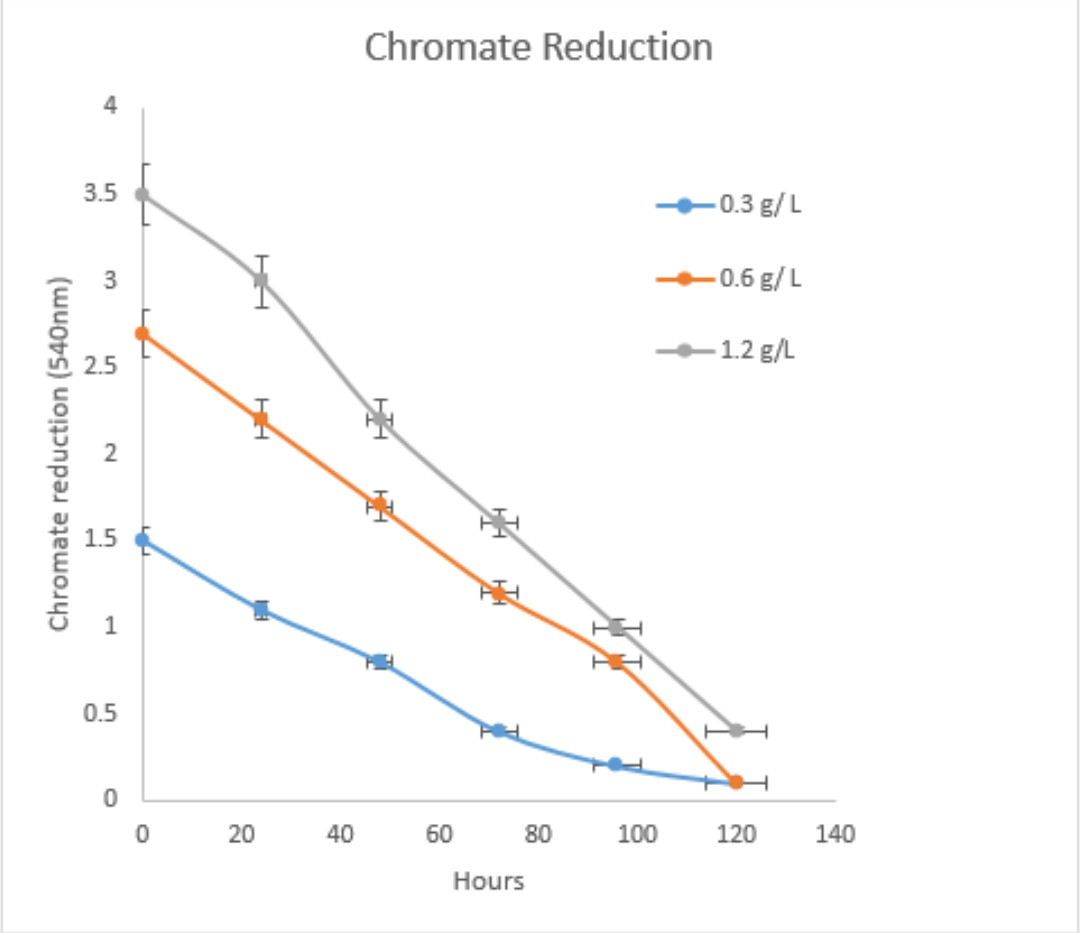

FIGURE 4.3: Chromate reduction shown by bacteria at different time intervals

Reduction of chromium by enzymes, biosorption, metabolic formation and accumulation of Cr etc. may involve in chromium reduction for reducing Cr toxicity

Another study showed Chromium (VI) reducing competence of the bacteria as two initial concentrations by the addition of 10 to 20 mg per liter of chromium and 2% inoculum in M9 minimal salts medium. At 24 and 30 h of incubation bacterial isolates *K. variicola* MKPF8, *K. cowanii* MKPF2, and *Staphylococcus marcescens* MKPF12 reduced the early conc. of chromium entirely although the same conc. was reduced by *A. gerneri* MKPF7 and *K. pneumonia* MKPF5 at 18 and 24 hours, 30 and 36 hours, respectively [166].

In a previous study, bacterial strains which can reduce chromate such as *Arthrobacter sp.* SUK, *Arthrobacter sp.* SUK 1205, Pseudomonas putida SKPD 1202, and *Corynebacterium paurometabolum* SKPD 1204 bio transformed highly lethal hexavalent Cr into less toxic trivalent CrIII form during growth, under aerobic conditions [167].

Another group of researchers illustrated that Cr (VI) reduction ability is usually dependent on temperature. *O. intermedium* Rb-2 achieved reduction of chromium quite well (85.8-96.1%) at all the considered temperatures (28, 37, and 42℃), but the optimum temperature was noticed 37℃. When incubation was performed for 72 hrs with initial concentration of chromium 100 μg/ml Rb-2 reduced 96.1% Cr (VI) at 37℃as compared with 88.0% and 92.0% at 28℃and 42℃, respectively [168].

4.3 Heavy Metal Tolerance

With varying concentrations of chromium cross the ability of *Staphylococcus arlettae* to cross react to toxic metal was tested and it showed that when high conc. of toxic metal is applied such as (0.3g/L, 0.6g/L and 1.2g/L) the isolate is capable of growth (Table 4.1). Variety of lethal mutagenic metals and also cations are characterized in the most of industrial wastes by their presence. In the polluted environments strain of *Staphylococcus arlettae* has its ability that would be enhanced by multiple metal resistances. With increase chromate resistance this shows salt tolerance in bacteria [169]. In a study, zinc salts are tolerated at great

level of degree has been reported in Gram-negative bacteria. For instance, *Rhizobium. leguminosarum* exhibited up to a concentration of 92.9 μM. Furthermore, Luria bertani medium was used to grow zinc tolerant strain RL9 for 24hrs and growth of indole acetic acetic was observed when tryptophan was also added in different conc. such as with 20, 60 and 100 μg mL-1 [170].

TABLE 4.1: Tolerance against heavy metal Cr shown by *Staphylococcus arlettae*

S.No	**Concentration of $K_2Cr_2O_7$ (g/L)**	**Tolerance**
1	0.3	+++
2	0.6	+++
3	1.2	+++
4	Untreated(Control)	+++

In another study, the strains which were used such as UFLA 01-659, UFLA 01-663, and UFLA 02-71 of the species *Cupriavidus necator*, as well as strain LMG 19424T of the species *Cupriavidus taiwanensis* as a characteristic strain of the genus.

All metals were tolerated greatly for strain UFLA 01-659 with the following concentrations MIC of 5.00 mmol L-1 Cd, 4.95 mmol L-1 Cu, and 14.66 mmol L-1 Zn. To the respective metals results were as follows for MIC of 5.00 mmol L-1 Cd it was 562 mg L-1, for 4.95 mmol L-1 Cu it was 314 mg L-1 and for 14.66 mmol L-1 Zn it was shown as958 mg L-1. 2.5 times greater MIC of UFLA 01-659 for Cd was shown as compared to the ability of strains to tolerate metals [171].

Another group of researchers illustrated that sample collected at the dumping site four bacterial strains were isolated. It was observed that, Isolate3 revealed higher ability to tolerate under all concentrations of Al2O3 such as 25 ppm, 50ppm, 100ppm, 150ppm although highest was shown in 100ppm concentration of Al2O3. When was used in 50ppm concentration it also shown higher tolerance.

Antibiotic such as Cloxacilin was tolerated and sensitivity to Gentamicin, streptomycin and Rifampicin by the bacteria which is resistant to high aluminum like isolate-3 but more sensitive to Gentamicin [172].

TABLE 4.2: % seed germination of different treatment groups

Treatment	**% seed germination**
Untreated	90
Staphylococcus arlettae treated	95
Cr+*Staphylococcus arlettae* treated	
0.3 g/l	90
0.6 g/l	68
1.2 g/L	50
Cr+ treated	
0.3 g/l	52
0.6 g/l	38

PGPP production and chromium reduction was tested by the ability of *Staphylococcus arlettae* using *T. aestivum* seeds. Before the handling with changed concentrations of Cr (0.3g/L, 0.6g/L and 1.2g/L) surfaces is seeds of *T. aestivum* were sterilized with 70% alcohol and layered with *Staphylococcus arlettae* (Table 4.2). In the nonappearance as well as existence of Cr *Staphylococcus arlettae* treated seeds showed increased percentage of germination (Figure 4.2).

Soil being the objective environment as phytoremediation occurs in soil because it is very complex medium, the ability of *Staphylococcus arlettae* used as bioinoculant for wheat (*T. aestivum*) was established in a setup having pots. Visibly improved germination efficiency was shown by *Staphylococcus arlettae* coated seeds when compared with plants labeled as control untreated group (Figure 4.2). The data was also found statistically significant when T-test was applied as $p < 0.05$.

Staphylococcus arlettae protects plant growth by the reduction of heavy metal and by showing plant growth promoting property despite of the fact of detrimental effects of Cr as it reduces rhizospheric contents completely (Table 4.2). Cytotoxicity can be measured by stopped early growth of seedling indicates the adverse effect on plants was prominent. Seed germination rate was rapidly reduced and the length of coleoptiles and radicles were also reduced this result was shown by the increase

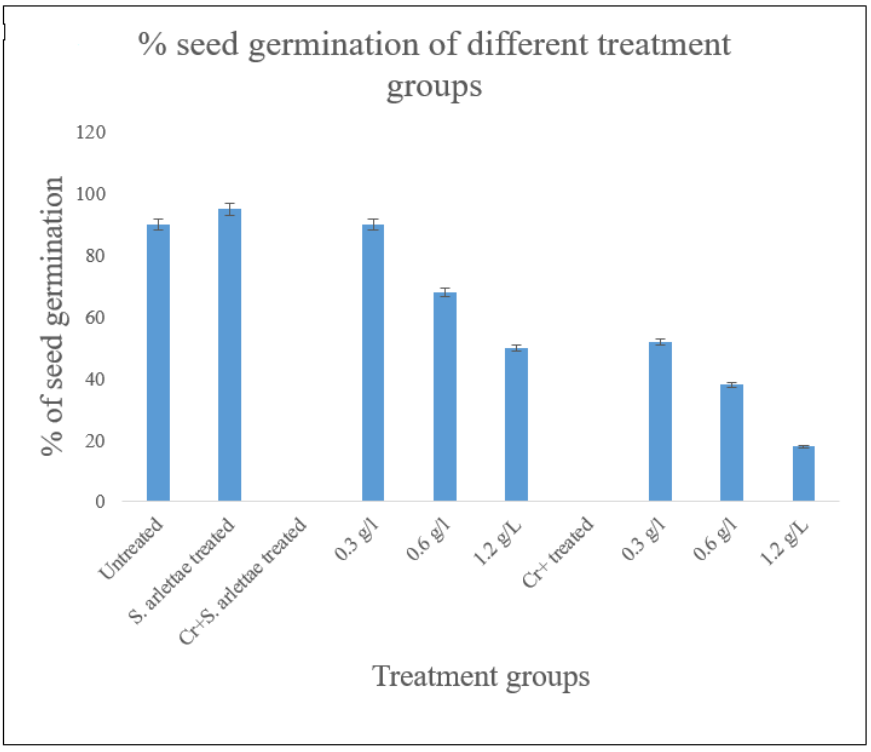

FIGURE 4.4: % seed germination of different treated groups

in Cr stress (Figure 4.2). *Staphylococcus arlettae* treated seeds significantly mitigated this rapid decline in growth and may be recognized as its ability to reduce Cr cytotoxicity (Figure 4.2). Previously, in the existence and absence of increasing concentrations of chromium the ability of the isolate to produce PGPP was examined. The growth was not substantially affected by the increasing concentration of Cr. Bacterial isolate having the ability to form biofilm was not diminished with high Cr concentration suggested by the resulting data. Strain was protected completely from lethal metal and also resulted in improved colonization of rhizosphere and enhanced plant growth promoting property and resistance to metal cytotoxicity was contributed by the strains. By enhancing the heavy metal reducing ability of biofilms, these are helpful in protecting stressful environment. Although results show simultaneous reduction of chromium and plant growth promotion ability of *Staphylococcus arlettae*. For contaminated soil remediation it is thus a potential applicant [173]. It is also reported in a study, that the two crops sorghum and rape having seed germination under stressful cadmium and petroleum with high conditions effectively improved by *Peptococcus activus sp.* SH3- 3-9. It showed the

ability of crude oil is more efficient in improving and less effective in cadmium by *Peptococcus activus sp.* SH3-3-9, which possibly since Peptococcus activus sp.SH3-3-9 is known for its ability to degrade bacteria [174].

In a study, it is showed that at different Cr concentration seed germination of all the varieties (HD2956, HD2932, KO512, and WH775) was reduced as compared to control. Control showed 100% germination however the germination % ranged from 60-95% for HD-2932, 65-80% for HD-2956, 65-100% for KO512, 75-90% for DBW-14, 65-95% for WH 775. Sugars are transported into the embryo axis would be due to the depressive effect of Cr which reduces seed germination when high conc. of chromium is applied [175]. In another study it is stated that, at Greenwich Island from the Antarctic soils twenty-five *Pseudomonas spp.* strains were isolated and tested. Optimum temperature such as 4 to 30 ℃ the isolates developed well and considered as eury-psychrophiles.

When sugar was taken as sole carbon sources in different concentrations, triphosphate was solubilized by the strain at 8 and 16 ℃. The growth of three plant pathogenic fungi was inhibited besides the isolates produced indole-acetic acid, siderophores and hydrogen cyanide, the fungi were (*Fusarium oxysporum, Pythium ultimum* and *Phytophtora infestans*). It was due to the production of metabolites which are soluble and volatile. When seeds of wheat (*T. aestivum*) were treated with bacterial isolates it resulted in significant enhanced root elongation. Wheat (*T. aestivum*) seedlings shown a pronounced improvement in lengths of radicles and shoots, when sterile medium of soil was provided with optimum and controlled temperature such as at 14 ± 1 ℃, as compared to untreated controls which showed less growth [176].

4.4 Impact on Length of Seedlings

Shoot length was observed reduced as compared to untreated group when treated with different concentrations of Cr (Table 4.4 and 4.5). Whereas in the presence of various concentrations of Cr *Staphylococcus arlettae* coated seedlings showed

enhanced growth (Figure 4.4 and 4.5). Treatment with *Staphylococcus arlettae* showed improved shoot length over control untreated seeds in the absence of Cr, (Figure 4.3). Even when chromium conc. was too high the reults were significantly positive and outcome on the growth of seedlings was detected after treatment of *Staphylococcus arlettae*, compared to control uncoated (without *Staphylococcus arlettae* treatment) Cr treated seeds (Table 4.4 and 4.5). Significant improved growth of seedlings was observed with *Staphylococcus arlettae* treatment (Table 4.4) (Figure 4.4 and 4.5). Even in the occurrence of lethal heavy metal *Staphy-*

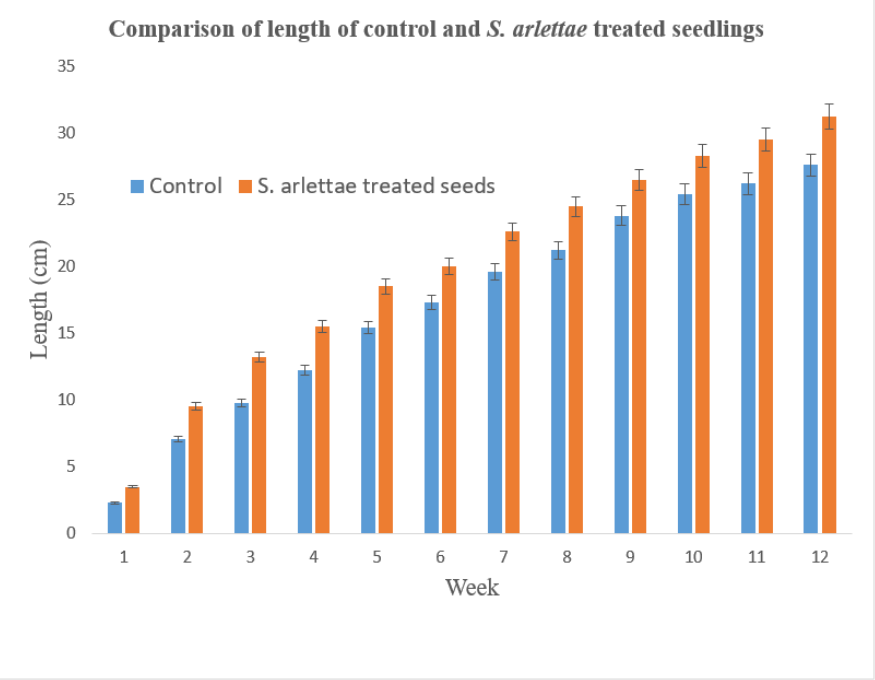

FIGURE 4.5: Comparison of length of control and *Staphylococcus arlettae* treated seedlings

lococcus arlettae stimulated shoot elongation can be recognized by its ability to show PGPP. The data was also found statistically significant when T-test was applied as $p < 0.05$. Ehen environment was stressful with increased Cr concentration biofilm formation may also contributed to its survival. There are a few microbes that can be used which can produce plant growth promoting compounds, show resistance to heavy metal and also speed up recolonization of polluted soil can be useful [177]. In a previous study, it is shown that sequence analysis was performed on 16S rDNA, bacterial strains were recognized as *Serratia fonticola*

ART-8 and *Pseudomonas putida* ART-9 specified that *Pseudomonas putida* ART-9 stimulates the growth of *Triticum aestivum* to a greater extent than *Serratia fonticola* ART-8. In addition, it was indicated that there would be more positive effects on agricultural ecosystems that is the characteristics of *P. putida* ART-9 than *Staphylococcus fonticola* ART-8 [178].

TABLE 4.3: Comparison of length of control and *Staphylococcus arlettae* treated seedlings

Week	**Length(cm)**	
	Control	***Staphylococcus arlettae* treated seeds**
1	2.3	3.5
2	7.1	9.5
3	9.8	13.2
4	12.2	15.5
5	15.4	18.5
6	17.3	20
7	19.6	22.6
8	21.2	24.5
9	23.8	26.5
10	25.4	28.3
11	26.2	29.5
12	27.6	31.2

In another study, it is demonstrated that from metal polluted chilli (*Capsicum annum*) *rhizosphere P. aeruginosa* CPSB1 recovered, which showed tolerance against heavy metals and several antibiotics. Even in the presence of higher rates of heavy metals, the bacterial strain *P. aeruginosa* CPSB1 shown its ability to promote plant growth and active biomolecules were alsodiscovered by SEM, Fourier Transform Infrared Spectroscopy and EDX, respectively [179]. Another group of researchers illustrated in a study that, pea plant growth and antagonistic activity was observed by the five strains of *Trichoderma* with known bio control against large *Pythium* ultimum inocula. When control *Pythium* was compared

to Pythium, it shown greater weight of fresh roots when strains of *Trichoderma* strains such as TH1 and T4 were used and the increase was around 62% and 57%, respectively. Control strain N47 significantly improved the shoot to root ratio as compared to *Pythium*. Pea seedlings were significantly increased with strains TH1 and T12 in the absence of *Pythium*, when comparison was done with control also shown consistent increased appearance *Pythium* was present [180].

TABLE 4.4: Length of seedlings treated with different concentration of chromium in absence of *Staphylococcus arlettae*

	Length of seedlings (cm)		
Weeks	**0.3g/L**	**0.6g/L**	**1.2g/L**
1	0	0	0
2	1.5	0	0
3	5.5	2.8	0
4	7.5	5.8	3
5	9.6	7.9	4.5
6	12.8	9.8	6.3
7	15.5	11.2	8.5
8	17.7	13.8	10.3
9	20.2	16.7	11.8
10	22	19.5	13
11	23.8	20.7	15.2
12	25.2	21.2	16

TABLE 4.5: Length of seedlings when treatment was performed with different concentration of chromium in presence of *Staphylococcus arlettae*

Length of seedlings (cm)			
Weeks	**0.3g/L**	**0.6g/L**	**1.2g/L**
1	3.5	1.5	0
2	7.5	3.5	3
3	10.6	6	4.2
4	13.5	9.2	5.5
5	16.8	10.8	7

TABLE 4.5: Length of seedlings when treatment was performed with different concentration of chromium in presence of *Staphylococcus arlettae*

	Length of seedlings (cm)		
6	19.5	13	8.6
7	21.8	14.8	10
8	23.8	16.2	12.5
9	25.9	18.5	14.5
10	28.7	20.7	17
11	32.1	23	18.8
12	35.9	26.4	20.5

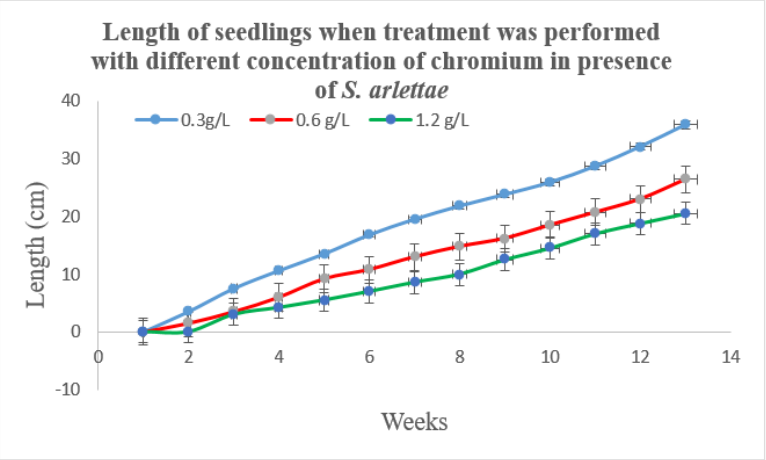

FIGURE 4.6: Length of seedlings when treatment was performed with different concentration of chromium in presence of *Staphylococcus arlettae*

4.5 Impact on Shooting Response

Shooting response of wheat seeds with different treatment groups was studied. *Staphylococcus arlettae* treated seeds showed increased number of shoots than untreated seedlings (Table 4.6). Although Cr treated seedlings had least shooting response (Table 4.7 and figure 4.7) but plants showed quite improved shooting response after treatment of *Staphylococcus arlettae*, even in the presence of Cr, with longer length of shoot and more no. of shoots (Table 4.8 and Figure 4.8) [181]. The data was also found statistically significant when T-test was applied as p<

0.05. It has also been reported that, from buds of apple a specific bacterial strain was isolated showed plant growth promoting properties which were assessed using metabolic tests. Phytochrome production by endophytic bacteria such as indole acetic acid, cytokinins and gibberellins was proved to stimulate plant growth. Bacterial proteins round about 10 to 68 μg ml-1, changed mean concentration of indole acetic acids by 5 folds. Among all of the recognized bacterial strains, Pantoea sp. (Ga_5 and D_8) were largely produced IAA. Moderate to low quantities of IAA was produced by *Pseudomonas* fluorescens group *P. vagans* (D_10), (Ga_1, Oa_2, and D_6) and *P. stutzeri* (O_16) produced [182]. Another group of researchers illustrated that from *Lolium perenne rhizosphere* there has been a bacterial strain is separated, promoted plant growth and also produced bio control agents.

TABLE 4.6: Comparison of no. of shoots of control and Staphylococcus arlettae treated seedlings

Week	**No. of Shoots**	
	Control	***Staphylococcus arlettae* treated seeds**
1	1	1
2	1	2
3	2	3
4	2	4
5	3	4
6	4	5
7	5	5
8	6	6
9	6	7
10	7	8
11	7	10
12	8	10

Among 13 isolates obtained from the tips of the roots of isolate of *L. perenne* the only one strain (BNM 0357) showed positive results for nitrogenase (ARA test) and also produced IAA [183]. In a study it was shown that, to regulate the possibility to reduce or undo the properties of salinity on maize vegetation

which were developed in saline-sodic conditions in a field, two bacterial species of *Pseudomonas* which produce ACC-deaminase were verified independently as well as in mixtures with inorganic fertilizers. After sowing the statistics noted down at 30, 50 and 70 days intervals which exposed that both the *Pseudomonas* bacterial strains enhanced length of root and shoot, weight of fresh root and shoot, dry weight of root and shoot up to 34, 43, 35, 71, 55 and 68%, respectively, although without applying organic fertilizers, these considerations were improved as 108, 95, 100, 131, 100 and 198%, respectively [184]. In another study it was

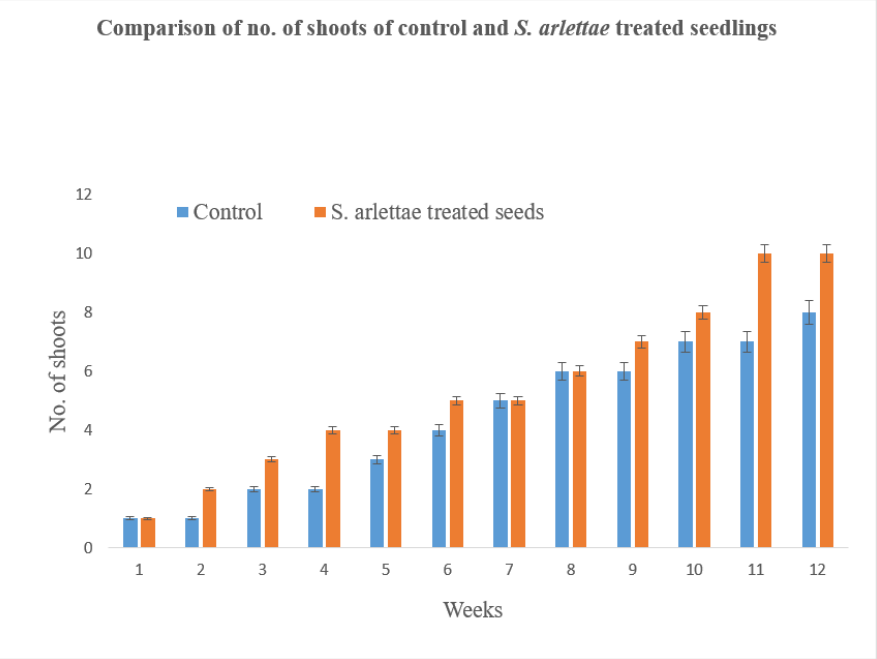

FIGURE 4.7: Comparison of no. of shoots of control seedlings and those treated with *Staphylococcus arlettae*

stated that, seven PSB isolates were separated and their effect was investigated by organizing a pot culture experiment was conducted on early development of plants. Each bacterial strain was applied to the seeds, and after inoculation period of 30 days seedlings were harvested. All strains presented enhanced effect on the plant growth. Plant length was increased which was about 45%, shoot dry weight that was upto 40% was detected in plants when treatment was performed with *Pseudomonas tolaasii* IEXb, while *Pseudomonas koreensis* SP28 and P content was enhanced remarkably when compared to untreated control groups. In combination with triple superphosphate (TSP) IEXb strain was selected and estimated

under field conditions as P fertilizer. Seedling emergence up to 8% was recorded in the presence of IEXb strain which also stimulated shoot length up to 19%, grain yield up to 44%, 18% weight was of 1000-grains, total dry biomass weight was 32% and contents of phophorous was 56% of plants such as maize [185].

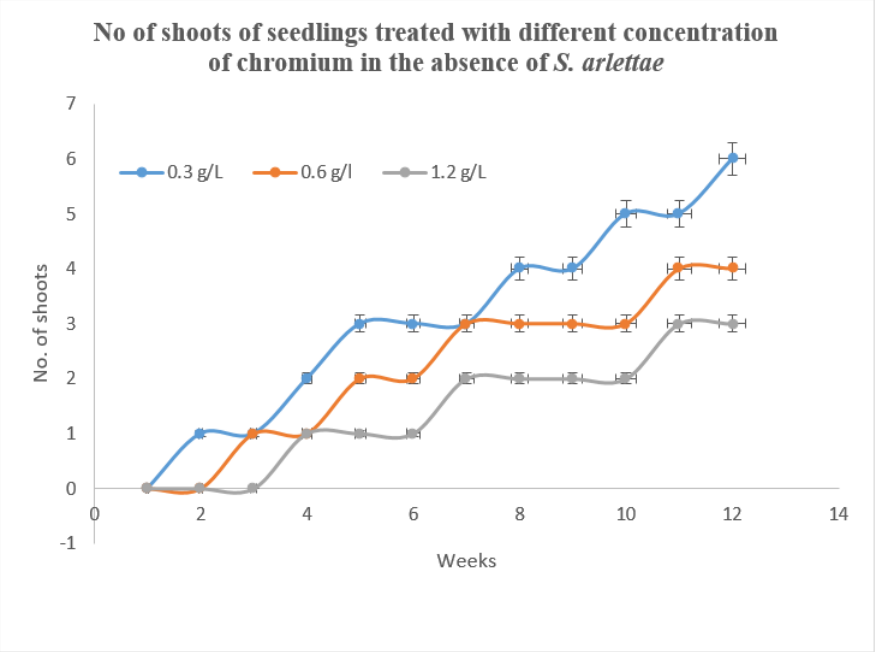

FIGURE 4.8: No. of shoots of seedlings treated with different concentration of chromium in absence of *Staphylococcus arlettae*

TABLE 4.7: No. of shoots of seedlings treated with different concentration of chromium in the presence of *Staphylococcus arlettae*

Weeks	**No. of shoots**		
	0.3g/L	**0.6g/l**	**1.2g/L**
1	0	0	0
2	1	0	0
3	1	1	0
4	2	1	1
5	3	2	1
6	3	2	1
7	3	3	2
8	4	3	2
9	4	3	2

TABLE 4.7: No. of shoots of seedlings treated with different concentration of chromium in the presence of *Staphylococcus arlettae*

Weeks	No. of shoots		
	0.3g/L	**0.6g/l**	**1.2g/L**
10	5	3	2
11	5	4	3
12	6	4	3

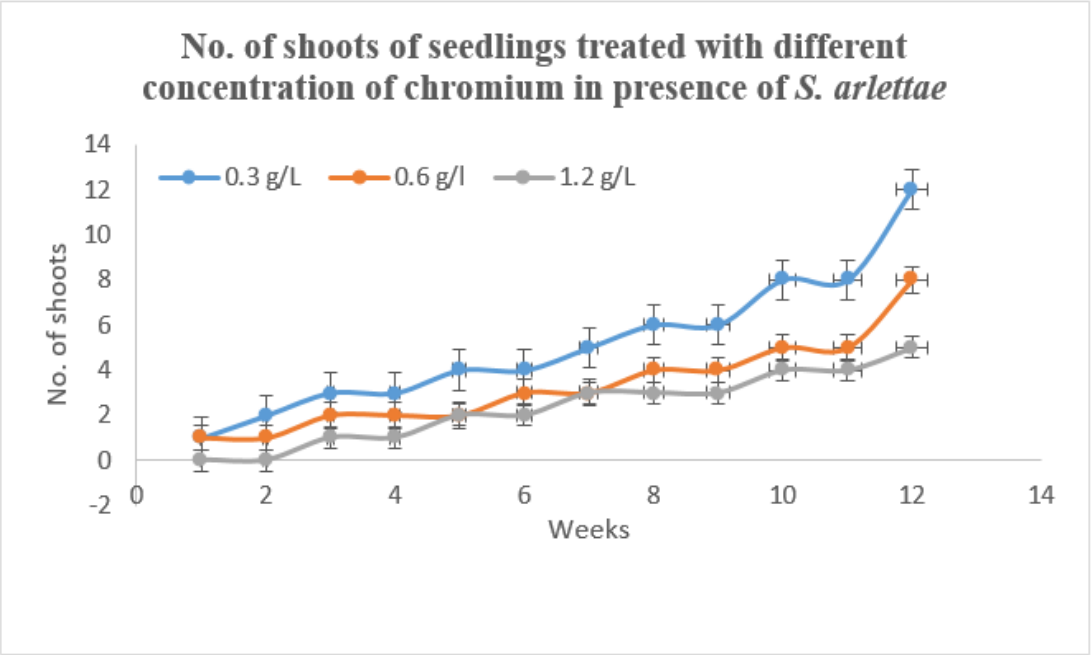

FIGURE 4.9: No. of shoots of seedlings treated with different concentration of chromium in presence of *Staphylococcus arlettae*

4.6 Impact on Rooting Response

Rooting response of different treatment groups (Cr and *Staphylococcus arlettae* treated) was also studied. *Staphylococcus arlettae* treated plants showed healthier rooting response than untreated seedlings (Table 4.9). Although Cr treated seedlings has the slightest rooting response but after treatment of *Staphylococcus arlettae*, plants showed quite improved rooting response with longer root length, even in the presence of Cr (Table 4.9 and Figure 4.9) [181]. The data was also found

FIGURE 4.10: Growth of shoots

statistically significant when T-test was applied as $p<0.05$.In an earlier study, on growth of wheat seedlings growth retarding effect of different concentrations of chromium was observed. First point of contact for those lethal Cr were roots of plants so the toxic chromium species in the growth media the toxicity of chromium was more profound in roots.

As compared to shoot growth the adverse effects were noted on the growth of roots. *Miscanthus sinensis, Sorghum bicolor, Triticum aestivum* and *Vigna radiate* shown more growth inhibition by metals and greater sensitivity of root weight when compared to shoot dry weight. That showed Cr interfered chemical and metabolic pathways resulted in damage to plants as shown by the adverse results such as reduced root growth and *phytomass chlorosis*, impairment of photosynthetic, growth arresting and finally death of plant happened [186].

In another study it was showed that, black pepper cuttings from cultivar *Bragantina* showed 76.63 and 27.75% root response when IBA was applied in 4000 mg kg-1 in talc and when it was absent. Improved rooting ability was resulted by

the ability of auxins to increase the sugar content of the cuttings. IBA is responsible to activate sugar metabolic pathways result in energy release and protein production also activates peroxidases which in results divide cells and differentiate at very early stage of root primordial. More soluble sugars like glucose and starch is in less quantity was observed with rooting of olive cuttings treated with IBA (2000 mg L-1) was 76% [187]. It is reported in another study that *Agrobacterium tumefaciens* strain A281 × 200 along with co-culture of shoots shown rooting response about 33-78% and it was 0% when *Agrobacterium tumefaciens* was not present.

With the different bacterial strains when co-culture period was increased, it also increased frequency of root response. Rooting response was elongated when strain A281 × 200 was utitlized elongation although most strains had similar effects on root elongation [188]. Another study illustrated that when treated with chemicals, percent rooting demonstrated significant gains in cuttings of elm (ulmus laevigata royle) over control. In terms of rooting treatment with T6 with concentration of 8000 ppm IBA and 2000 ppm p-HBA and 5% sucrose + 5% captan was given showed up improved results. It showed that cuttings with great phenol contents can result in maximum root response [189].

Table 4.8: Rooting response of different treatment groups

Treatment	**Root Length**
Untreated	5.5
Staphylococcus arlettae treated	7.5
Cr+*Staphylococcus arlettae* treated	
0.3 g/l	7.5
0.6 g/l	5.2
1.2 g/L	1.5
Cr+ treated	
0.3 g/l	4.5
0.6 g/l	1.2
1.2 g/L	0.2

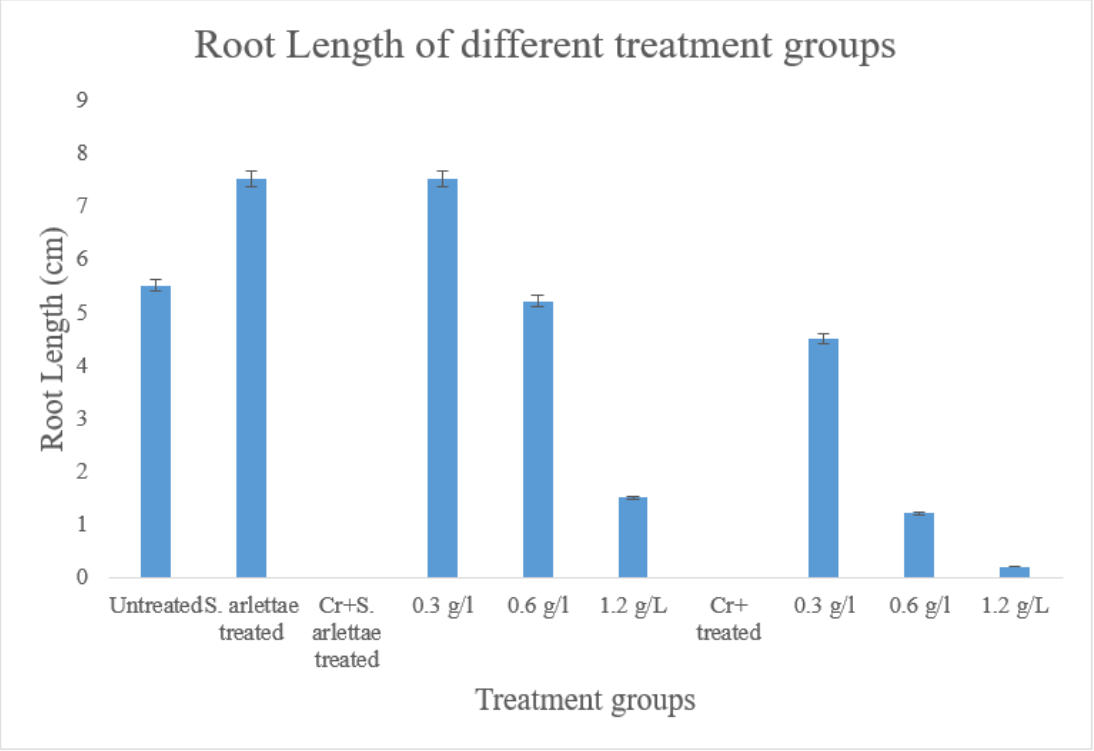

FIGURE 4.11: Rooting responses of different treatment groups

4.7 Impact on Overall Growth of the Plant

By examining the fresh and dry weight of the seedlings overall impact of *Staphylococcus arlettae* and Cr on the growth of plant was observed. As compared to untreated group *Staphylococcus arlettae* treated seedlings showed increased fresh and dry weight of biomass (Table 4.10). Similarly as compared to control untreated plants Cr treated seedlings showed decreased fresh and dry weight, but fresh and dry weight of biomass was significantly improved with treatment of *Staphylococcus arlettae*, even in the presence of Cr, (Table 4.10) [159].

Previously it is reported that, on micropropagated banana plantlets the combined effects of *arbuscular mycorrhizal* fungus *Glomus manihotis* and *Bacillus* species such as *rhizobacteria* was observed. Developmental parameters such as total fresh and dry weight, aerial dry weight, length of shoot and surface area of leaves with

combined inoculated plants showed, considerably improved growth as compared to non-treated control plants. When both strains were applied collectively, mineral contents of leaf were also increased such as nitrogen, phosphorous and potassium content. No positive results were achieved with *G. manihoti*s although results for species of *Bacillus* were different and all the growth parameters were developed progressively than the controls. As compared with the controls total fresh weight was almost doubled when strain was applied such as 1.7 times. With the control treatment dry weight of aerial parts was also improved by 1.5 times [190].

In another study it was reported that, the growth impact of phosphate solubilizing bacteria such as TV14B *Stenotrophpmonas maltophilia P*, nitrogen fixing + phosphate solubilizing bacteria such as TV113C K*luyvera cryocrescens NP*, nitrogen fixing bacteria such as TV83D *Bacillus atrophaeus N*, binary combination T and V83D *B. atrophaeus* + TV119E *Bacillus*-GC group and phosphate solubilizing bacteria such as TV119E *Bacillus*-GC group P shown positive results under salinity stress was observed on growth of *Ceyhan*-99. On plant growth parameters TV14B *Staphylococcus maltophilia P* bacteria had positive impacts at diverse salinity concentrations. It is concluded that improved height of plant and phosphorus in the soil and dry weight parameters was observed by the TV119E Bacillus-GC group P bacteria in concentration of 100mM was used. By the treatment with TV83D B. atrophaeus N bacteria parameters such as length of roots, phosphorous contents and amount of nitrogen were all increased when salt concentration was 125mM in the soil compared to the control Treatments of TV54A *C. turbata N*, TV113C *K. cryocrescens* NP and TV83D *B. atrophaeus* +TV119E *Bacillus*-GC group NP bacteria increased phophorous and nitrogen content were observed by applying different salt concentrations [191]. Another group of researchers illustrated, the effect of pea seeds pollination with *Rhizobium* bacteria and spraying with vitamin B12, molybdenum and boron, as well as their mutual effect on vegetative growth characteristics, wet and dry weights was observed by taking the two treatments protocol by using (Rh + B12, Rh + Mo, and Rh. B), respectively. The results revealed that in comparison with the control the highest values were noted in vegetative growth traits such as length of plants, leaves count and fresh

and dry weight. The mutual effect on each other and different treatments with the *Rhizobium* and the two elements boron and molybdenum and vitamin B12, significantly affect the dry and wet weights of peas plants, although the most effective treatment which resulted in an increase in wet and dry weights was with (Rh + V.B12) (36.96 g- 52.03 g) after that treatment (Rh + Mo) (45.20 g-33.80 g) was performed resulted in increase in dry and wet weight, and followed by the treatment (Rh + B) (45.00 g - 33.76 g) respectively gave the best results [192].

TABLE 4.9: Wet weight and dry weight of biomass

Treatments	**Wet weight(g)**			**Dry weight(g)**		
Untreated (Control)	0.9			0.21		
Staphylococcus arlettae treated seeds	1.5			0.7		
Staphylococcus	Cr Concentrations			Cr Concentrations		
arlettae and Cr	0.3g/L	0.6g/L	1.2g/L	0.3g/L	0.6g/L	1.2g/L
treated seeds	1.49	0.9	0.2	0.3	0.07	0.01
Cr treated seeds	0.32	0.18	0.05	0.02	0.01	0.002

Chapter 5

Conclusions and Recommendations

Cross reactivity of *Staphylococcus arlettae* with the changing concentrations of chromium to heavy metals was established and it revealed that in the presence of high concentrations of heavy toxic metals such as (0.3g/L, 0.6g/L and 1.2g/L) the bacterial strain is proficient for improved growth. It is concluded that in absence as well as presence of Cr when seeds were treated with *S. arlettae* presented increased percentage of germination. It was found that Cr is deadly lethal at different periods of plant growth and development in wheat (*Triticum aestivum*) but *S. arlettae* treated seeds significantly mitigated this rapid deterioration in growth and may be recognized as its ability to reduce Cr cytotoxicity. It was observed that *S. arlettae* treated seeds resulted in an proliferation in length of shoot as compared to the control untreated seeds in the nonexistence of Cr and also indicated healthier response of rooting. Moreover, fresh and dry weight of biomass was also considerably observed with treatment of *S. arlettae*, even in the presence of high concentrations of toxic metal Cr, which indicates that PGPR bacteria promote plant growth not only by supplying nutrients to the plant, but also by phytohormones production, stress resistance managment, or avoiding pathogen-induced diseases of plants.

Thus, in general study concludes that the progression of the bio fertilizers and the advancement of bacterial culture inocula of S. arlettae in the field is an environmentally friendly approach to meet the overall necessity to lift crops yields.

Moreover, advancements in new advances prompting the improvement of bio fertilizer timeframes of realistic usability, facilitating their dispersion and application in the fields, are basic for their utilization to be reached out later on.

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