Bioremediation of some harmful pollutants in soil and water

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5. SUMMARY

Part 1

This work included research points summarized as follows:

5.1. Isolation and screening of heavy metal resistant- plant growth promoting producing microorganisms.

From eight samples, which were collected during September 2020 from heavy metal contaminated fresh water (El-Batas drain, located in Tamia El-Fayoum, Egypt) and heavy metal and crude oil contaminated agricultural land (located in Tamia, El-Fayoum and El-dakhla, Alwadi Aljadid, Egypt), plant growth promoting producing heavy metal resistant bacteria were isolated using nutrient agar medium (NA) supplemented with 1 mM of Cd and 1.5 mM of Pb. A total of 54 heavy metal (Cd and Pb) resistant bacterial isolates were isolated from the eight samples. All isolates were tested of their ability to produce indole acetic acid, phosphate and zinc solubilization and growth on crude oil as a sole carbon source. Of them 49 isolates were indole acetic acid producers with different ratios of production, 17 isolates were zinc solubilizers, and 8 isolates were phosphate solubilizers. 23 isolates of all isolates had the ability to grow on B.H agar medium supplemented with crude oil.

The ability of isolates to solubilize the non-soluble phosphate (tricalcium phosphate) and the non-soluble zinc (zinc oxide) in a solid medium were tested. Isolate EG2PS1 showed the most ability to solubilize zinc and phosphate, with solubilization index values of 12.5 and 10.52, respectively, followed by isolate EGF14S2 with values of 6.8 and 6.07, respectively. For chemical determination of indoleacetic acid the isolates were cultured in Indole production broth medium containing

L- tryptophane (1 gL^{-1}), The appearance of a pink color indicates IAA production, Standard curve of IAA was conducted and measured at 530 nm and the quantity of IAA of the tested samples was calculated compared to standard curve. The most amount of IAA (chemically determined) was produced by isolate EGF17S2, followed by isolate EGF16S3 with values of 199.14 and 106.84 μg/ml, respectively.

Based on the pervious mentioned parameters, the most four promising Plant growth promoting and heavy metal resistant isolates, EG2PS1, EGF14S2, EGF16S3 and EGF17S2 were selected for more detailed taxonomic studies.

To confirm the results of indole, it was estimated by using HPLC for the most promising 4 isolates. The findings of IAA by HPLC for the selected isolates were as follows; 0.0, 5.93, 83.71, and 147.38 μg/ml by isolates EG2PS1, EGF14S2, EGF16S3, and EGF17S2, respectively. This result revealed that isolate EGF17S2, the highest IAA producer, and the results of IAA determined by the colorimetric method were inaccurate compared to HPLC

5.2. Taxonomical studies for selected strains

The isolates EG2PS1, EGF14S2, EGF16S3, and EGF17S2 were selected as the most promising PGP isolates and heavy metal resistant and characterized morphologically, physiologically, and biochemically (Phenotypic characterization), as well as genotypic characterization and more studies.

5.2.1. Morphological, Physiological and Biochemical characterization of selected strains

Cells of all isolates were Gram-negative, rods, and motile except isolate EGF16S3 was Gram positive, irregular rods, and non-motile. Furthermore, all of the selected isolates were non-spore-formers and catalase positive, while isolates EG2PS1 and EGF14S2 were oxidase positive, and isolates EGF16S3 and EGF17S2 were oxidase negative, all of the isolates were negative for hydrolysis of starch, casein, gelatin, and lipid, except isolate EG2PS1, which was positive, these isolates weren't able to utilize a wide range of organic substrates except the isolate EG2PS1 could utilize a wide range of carbon sources , the selected isolates EG2PS1 and EGF17S2 had a wide ability to form acids from the different carbon sources, compared to isolates EGF14S2 and EGF16S3, which hadn't this ability.

5.2.2. Genotypic characterization of selected strains

The nucleotide sequence of the 16S rRNA gene was also analyzed; its amplification was performed using pair of universal primers: fD1 5' AGAGTTTGATCCTGGCTCAG 3' and rD1 5' CTTAAGGAGGTGATCCAGCC 3'**,** After study and analysis of the 16S rDNA gene for the selected isolates, it was shown that these isolates belong to 3 major genera; *Pseudomonas*, *Leucobacter*, and *providencia***,** which belong to the families; Pseudomonadaceae, Microbacteriaceae and Morganellaceae, respectively**.**

The percentage of similarity of the isolated strains with the most closely related strain in the gene bank (NCBI) were 99.88, 99.53, 99.87, and 99.88 % for strains EG2PS1, EGF14S2, EGF16S3, and EGF17S2, respectively. Based on the phenotypic and genotypic characteristics, strains EG2PS1, EGF14S2, EGF16S2, and EGF17S2 can be classified as

Pseudomonas aeruginosa, *Pseudomonas putida*, *Leucobacter komagatae*, and *Providencia rettgeri*, respectively.

5.2.3. Antagonistic and hemolytic properties of the selected strains

The analysis of inter-bacterial interactions revealed no evidence of antagonistic activity among the tested strains, as demonstrated by the absence of inhibition zones surrounding the bacterial growth on the agar plates. Consequently, all isolates were deemed compatible and therefore they can be utilized to construct a bacterial consortium intended for crude oil biodegradation and the bioremediation of heavy metals in contaminated soil.

Both strains EGF16S3 and EGF17S2 exhibited gamma hemolysis, indicating an absence of erythrocyte lysis, while strains EG2PS1 and EGF14S2 demonstrated alpha hemolysis, evidenced by the greenish or brownish discoloration typical of partial red blood cell breakdown. Notably, beta hemolysis—characterized by complete lysis of erythrocytes and commonly associated with high virulence—was not observed in any of the isolates, suggesting a reduced pathogenic potential.

5.3. Resistance of the selected strains to heavy metals in the growth medium

The resistance of the studied strains to heavy metal salts was determined after incubating the bacteria for 2 days in liquid L.B medium with the addition of $ZnSO_4.7H_2O$, $K_2Cr_2O_7$, $Cd(CH_3COO)_2.2H_2O$, $CuSO_4.5H_2O$, $Pb(CH_3COO)_2.3H_2O$, and $NiCl_2.6H_2O$ (final concentrations 0.0; 0.5; 1.0; 2.0; 4 and 6.0 mM), cell viability was determined by the optical density of the culture (λ=540 nm).

The strain EG2PS1 has a high resistance to cadmium in the growth medium, if compared to the previously mentioned strains and then gradually decreased to concentrations of 2.5 Mm. According to the resistance of studied strains to cadmium, we can arrange them in descending order as follows: EG2PS1, EGF14S2, EGF16S3, and EGF17S2, respectively. For chromium, all tested strains have a resistance to chromium, but with different percentages of resistance, except strain EGF17S2, which was strongly affected by the presence of chromium in the growth medium. According to the resistance of studied strains to chromium, we can arrange them in descending order as follows: EG2PS1, EGF14S2, EGF16S3, and EGF17S2, respectively. Also, all strains have resistance to copper but with different percentages of resistance. Based on the resistance of studied strains to copper, we can arrange them in descending order as follows: EG2PS1, EGF14S2, EGF16S3, and EGF17S2, respectively. For nickel, all strains have a resistance to nickel, but with different percentages of resistance. The resistance of studied strains to nickel, we can arrange them in descending order as follows: EG2PS1, EGF14S2, EGF17S2, and EGF16S3, respectively. While strain EG2PS1 showed high resistance to lead in growth medium compared to all the strains previously mentioned. Where the growth of strain EG2PS1 gradually decreased from 0.5 to 6.0 mM of lead. Based on the resistance of studied strains to lead, we can arrange them in descending order as follows: EG2PS1, EGF14S2, EGF16S3, and EGF17S2, respectively. The studied strains have resistance to zinc with different percentages of resistance. According to the resistance of studied strains to zinc, we can arrange them in descending order as follows: EG2PS1, EGF14S2, EGF16S3, and EGF17S2, respectively.

Based on the tolerance of the tested strains to heavy metals were observed to be in order of Cu > Ni > Pb > Zn > Cr > Cd, where all strains presented a resistance to the tested heavy metals with different percentages of resistance. Also, it was worth mentioning that the gram-negative strains showed high resistance to heavy metals more than gram-positive strain.

5.4. Study of the ability of the selected strains to degrade crude oil

The activity of isolates against hydrocarbons was studied by Petri plates with solid Bushnell-Haas medium and, by liquid Bushnell-Haas medium with the addition of 1% crude oil as the sole carbon source. For fractionation different solvents were used to elute different fractions of paraffins, naphthenes, mono-, bi- and polycyclic aromatic hydrocarbons, alcohol-benzene resins.

all strains could grow using crude oil as a sole carbon source, the strains EG2PS1 and EGF16S3 showed their high ability to degrade crude oil and showed strong growth under the crude oil drop, followed by strains EGF17S2 and EGF14S2. Microscopic examination for all strains revealed that the center of the droplets was occupied by microorganisms, and microbial cells in the vicinity of the oil droplets were found to be very motile and did not form any aggregates.

A study of the degradative activity of these microorganisms toward oil (1%) showed that strain EG2PS1 could destroy 49.0 % of crude oil as the largest strain capable of degrading crude oil, followed by strains EGF16S3 and EGF17S2, which were capable of degrading 32.8 and 28.3 % of crude oil, respectively. The lowest percentage of degradation of crude oil was recorded by strain EGF14S2, which was 25.2 %. Fractional analysis of residual oil indicated that all main fractions for all strains were subjected to microbial degradation: paraffins, naphthenes, mono-, bi- and poly-cyclic aromatic hydrocarbons, as well as alcohol-benzene tars.

It is known that paraffins (alkanes) are the main component in crude oil, their content in the oil we used is approximately 70%, The strain EG2PS1 better degraded paraffins, followed by strains EGF16S3, EGF17S2, and EGF14S2, respectively. GLC analysis of the utilization of alkane fractions by the selected strains compared to the control sample shows that all strains degrade paraffins (C14-C28), but with different percentages of degradation. Results of GLC of paraffin fractions reveal that the strain EGF17S2 biodegraded the lower molecular weight hydrocarbons (C14 to C22) more efficiently than the higher molecular weight hydrocarbons (C23 to C28). the highest degradation rate of the bacteria was in the range of low- and medium-chain alkanes of C9–C18.

In conclusion, all strains could utilize (as sole carbon and energy sources) and biodegrade (as indicated by the quantitative GLC analysis) all components of crude oil with different percentages.

5.5. Part 2

5.5.1. Effect of bacterial strains on leaves tissue water content, membrane stability, and photosynthetic pigments of Phaseolus plants grown in Cd- contaminated soil

Cd stress significantly decreased the relative water content (RWC), membrane stability index (MSI), chlorophyll value (SPAD), chlorophyll a fluorescence (F_v/F_m and F_v/F_0), and performance index (PI) of Phaseolus plants. Relative to control, Cd-contaminated soil with 5 and 10 ppm decreased RWC by 3.2%, and 7.2%, MSI by 18.3%, and 24.6%, SPAD by 9.2%, and 21.5%, F_v/F_m by 2.4%, and 3.7%, and PI by 10.4%, and 20.8%. The inoculation of Cd- contaminated soil by bacterial strains significantly increased RWC, MSI, SPAD, F_v/F_m, F_v/F_0, and PI by 17.5%, 5.1%, 31.4%, 35.3%, 33.3%, and 9.03% for strain EGF17S2, and by

20.3%, 6.4%, 34.3%, 50.0%, 50.0%, and 9.8% for bacterial consortium, respectively, as compared by un-inoculated soil. Cd- contaminated soil with 5 ppm, and inoculated soil with bacterial consortium led to an increases by 7.5%, 18.8%, 22.5%, 2.5%, 38.24%, 25.0%, 31.3% and 33.7% for RWC, MSI, SPAD, F_v/F_m, F_v/F_0, and PI, respectively, compared to (5 ppm of Cd and un-inoculated plants with bacterial strains). Also, under Cd contaminated soil (10 ppm), inoculated soil with bacterial consortium positively increased RWC, MSI, SPAD, F_v/F_m, F_v/F_0, and PI by 15.5%, 124.5%, 26.2%, 13.9%, 51.6, and 50.0%, respectively, compared to (10 ppm of Cd +without bacteria) treatment.

5.5.2. Effect of bacterial strains on plant growth characteristics of Phaseolus plants grown in Cd-contaminated soil

Phaseolus plants grown under cadmium contaminated soil with 10 ppm, the plant height, stem diameter, leaves no, plant fresh weight, plant dry weight and leaves area were significantly decreased by 13.5%, 13.4%, 27.2%, 25.0%, 25.9%, and 32.7%, respectively, as compered by control (0 ppm Cd). Whereas soil application with bacterial strains such as; strain EG2PS1, strain, 29, strain EGF16S3, strain EGF17S2, and bacterial consortium significantly increased plant height, stem diameter, leaves no, plant fresh weight, plant dry weight and leaves area compared to the control plants. Under Cd contaminated soil (10 ppm), inoculated soil with bacterial consortium led to an increases in plant height by 42.7%, stem diameter by 46.5%, leaves no by 57.4%, plant fresh weight by 41.4%, plant dry weight by 27.5% and leaves area by 70.8%, compared to (10 ppm of Cd and +without bacteria) treatment

5.5.3. Effect of bacterial strains on the yield of Phaseolus plants grown in Cd-contaminated soil

Plants grown under Cd- contaminated soil at the concentrations of 5 and 10 ppm, pods number/plant were decreased by 18.4 and 35.1%, and pods yield/plant by 29.4 and 45.6%, respectively, as compared by control (0 ppm Cd). On the other hand, inoculation Cd- contaminated soil by bacterial strains increased pods number/plant and pods yield/plant by 18% and 7.9% for strain EG2PS1, by 18.9% and 15.9% for strain EGF14S2, by 27.1%, and 21.6% for strain EGF16S3, by 24.7% and 26.5% for strain EGF17S2, by 35.7% and 34.1% for bacterial consortium, respectively, as compared by un-inoculated soil. Under Cd contaminated soil at 5ppm, inoculated soil with bacterial consortium led to an increases by 31.3% and 33.7% for pods number/plant, and pods yield/plant compared to (5 ppm of Cd + without bacteria) treatment. Also, under Cd contaminated soil (10 ppm), inoculated soil with bacterial consortium led to an increases in pods number/plant, and pods yield/plant by 53% and 34% compared to (10 ppm of Cd + without bacteria) treatment.

5.5.4. Effect of bacterial strains on leaves and pods Cd content of Phaseolus plants grown in Cd-contaminated soil

Plants grown under Cd- contaminated soil at the concentrations of 10 ppm, Cd were increased by 46.9% and 52.6% for leaves and pods, respectively, as compared by 5ppm Cd treatment. The inoculation of Cd- contaminated soil by bacterial strains significantly decreased the Cd content in leaves and pods by 34.4%, and 32.1% for strain EG2PS1, by 42.2% and 39.3% for strain EGF14S2, by 46.9%, and 46.4% for strain EGF16S3, by 43.8% and 39.3% for strain EGF17S2, by 48.4% and 46.4% for bacterial consortium, respectively, as compared by un-inoculated soil. The Cd- contaminated soil with 5 ppm, and inoculated soil with bacterial consortium decreed the Cd content in leaves and pods by 50.0% and 50.0% compared to (5 ppm of Cd + without bacteria) treatment. Also, under Cd- contaminated soil (10 ppm), inoculated soil with bacterial consortium decreased the Cd content in leaves and pods by 48.2% and 47.7% compared to (10ppm of Cd + without bacteria) treatment.

5.5.5. Effect of bacterial strains on osmolyte accumulation of Phaseolus plants grown in Cd-contaminated soil

Under 5 and 10 ppm of Cd - contaminated soil, the accumulation of TSS increased significantly by 44.0% and 49.8%, free proline by 50.5% and 59.7%, as compared to 0 ppm of Cd. The inoculation of Cd-contaminated soil by bacterial strains significantly decreased TSS and free proline by 12.1% and 5.4% for strain EG2PS1, by 15.0% and 10.6% for strain EGF14S2, by 23.1% and 11.1% for strain EGF16S3, by 27.8%, and 17.1% for strain EGF17S2, and by 34.6% and 20.9% for bacterial consortium, respectively, as compared by un-inoculated soil. Cd- contaminated soil with 5 ppm, and inoculated soil with bacterial consortium positively decreased TSS and free proline by 27.7% and 17.1%, respectively, compared to (5 ppm of Cd + without bacteria) treatment. In Cd contaminated soil (10 ppm), inoculated soil with bacterial consortium significantly decreased TSS and free proline by 37.3% and 24.5%, compared to (10 ppm of Cd + without bacteria) treatment.

5.5.6. Effect of bacterial strains on non-enzymatic and enzymatic antioxidants of Phaseolus plants grown in Cd-contaminated soil

The content of GSH, AsA, and the activity of CAT, and SOD in leaves of Phaseolus plants enhanced in 5 and 10 ppm Cd-contaminated soil, compared to the control (0 ppm Cd), with an increase of 20.8% and 23.1% in GSH, 8.2% and 10.2 in AsA, 2.3% and 55.9% in CAT, 24.5%

and 25.9% in SOD. The application of bacterial strains reduces GSH, AsA, CAT and SOD by 16.8%, 11.6%, 6.3%, and 5% for strain EGF14S2, and by10.6%, 11.11%, 13.8% and 5.1% for bacterial consortium.

5.5.7. Effect of bacterial strains on nutrient contents of Phaseolus plants grown in Cd-contaminated soil

Cd- contaminated soil with 5 ppm led to a significant reductions of 5.8%, 26.0% and 8.8% for N, P and K in leaves and 3.1%, 20.0% and 12.8% for pods. Also, Cd- contaminated soil with 10 ppm led to significant reductions of 13.8%, 37.5% and 13.6% for N, P and K in leaves and 8.6%, 48.8% and 16.0% for pods. The application of bacterial strains enhanced remarkably the leaves uptake of N, P and K by 13.4%, 32.8% and 13.66 for strain EG2PS1, and by 14.3%, 21.9% and 12.4 for strain EGF14S2, and by 14.3%, 23.4% and 23.8 % for strain EGF16S3, and by 11.3%, 34.4%, and 15.6% for strain EGF17S2, and by 17.3%, 53.1% and 25.3% for bacterial consortium, respectively as compared with control. Treating Cd-contaminated soil with bacterial strains enhanced remarkably the pods uptake of N, P and K by 1.7%, 22.82 and 12.3% for strain EG2PS1, and by 7.9%, 44.4% and 11.5% for strain EGF14S2, and by 8.5%, 53.3% and 16.5% for strain EGF16S3, and by 11.63%, 46.7%, and 16.7% for strain EGF17S2, and by 13.7%, 54.4% and 22.4% for bacterial consortium, respectively as compared to (without bacterial strains) treatment.

5.5.8. Effect of bacterial strains on leaf anatomical structure of Phaseolus plants grown in Cd-contaminated soil

Under 5 ppm Cd, the MH, MW, MVBH, MVBW, LBT, STT, and PT in Phaseolus plants were decreased by 6.0%, 10.2%, 10.5%, 9.2%, 13.0%, 10.4 and 11.1%, respectively, as compared by control (0 ppm). Also, under 10 ppm Cd, the MH, MW, MVBH, MVBW, LBT, STT, and PT in Phaseolus plants were decreased by 13.5%, 14.1%, 14.6%, 16.1%, 21.1%, 17.8 and 22.4%, respectively, as compared by control (0 ppm). Compared to the control, inoculated Phaseolus plants grown under Cd-contaminated soil with strain EGF16S3 resulted in an increase of 17.9% in MH, 11.4% in MW, 18.3% in MVBH, 22.2% in MVBW, 31.4% in LBT, 55.6% in STT, and 44.6% in PT. Whereas, bacterial consortium increased the MH, MW, MVBH, MVBW, LBT, STT, and PT in Phaseolus plants by 27.7%, 12.8%, 33.8%, 25.6%, 47.9%, 78.3%, and 58.2%, respectively over the control.

5.6. Part 3

5.6.1. Impacts of bacterial consortium on leaf integrity and photosynthetic efficiency of faba bean plants grown under petroleum contaminated soil

Relative to control (without Bac, and Pet), the contaminated soil by petroleum decreed RWC by 5.5%, 11.2% and 14.4%, MSI by 1.2%, 13.6% and 20.7%, SPAD by 16.3%, 22.3% and 29.5%, *Fv/Fm* by 3.7%, 4.9% and 9.8%, *Fv/F0* by 15.8%, 25.0% and 39.6%, and PI by 51.4%, 60.1% and 72.3% at concentrations of 1% Pet, 2% Pet, and 3% Pet, respectively. On the other hand, faba bean plants treated with bacterial consortium increased the RWC, MSI, SPAD, *Fv/Fm*, *Fv/F0*, and PI by 4.0%, 14.9%, 11.8%, 2.4%, 41.2%, and 21.7% as compared with control (without Bac, and Pet), respectively. Treated plants grown under petroleum contaminated soil with 3% Pet+Bac caused an increase of 8.7% in RWC, 12.6% for MSI, 13.2% for SPAD, 13% and 8.1% for *Fv/Fm*, 6.1% for *Fv/F0* and 105.7% for PI, respectively

5.6.2. Impacts of bacterial consortium on osmoprotectants, enzymatic, and non-enzymatic activity of faba bean plants grown under petroleum contaminated soil

Under 1% Pet -induced stress the levels of TSS, free proline, CAT, SOD, AsA and GSH in *V. faba* plants were 11.93 (mg g^{-1} FW), 21.76 (mg g^{-1} FW), 0.145 (u mg^{-1} protein), 0.127 (u mg^{-1} protein), 0.144 (μmol g^{-1} FW) and 0.203 (μmol g^{-1} FW), respectively. Relative to control, 2% Pet decreed of 30.2% in TSS, 30.8% in free proline, 22.2% in CAT, 3.8% in SOD, 31.3% in AsA and 31.3% in GSH. Whereas 3% Pet decreased of 44.82% in TSS, 339.7% in free proline, 34.1% in CAT, 46.4% in SOD, 36.8% AsA and 43.6% in GSH, respectively over the control. Treating petroleum contaminated soil with bacterial consortium led to an increases of TSS, proline, CAT, SOD, AsA and GSH by ٢٠.٢%, ٩.٥%, ٣٣.٥%, ٢٦.٦%, ٩.٠%, and ١٩.٨% as compared with control. Moreover, the treated plants grown under petroleum contaminated soil at 3% Pet + Bac significantly increased TSS, proline, CAT, SOD, AsA and GSH by 26.7%, 18.1%, 15.5%, 22.3%, 9.2%, 33.6%, respectively as compared to (3% Pet) treatment.

5.6.3. Impacts of bacterial consortium on stomatal performance of faba bean plants grown under petroleum contaminated soil

Relative to control (without Bac, and Pet), the contaminated soil by petroleum decreed stomata density by 4.7%, 9.6% and 11.7%, stomata aperture area by 39.2%, 49.8% and 61.9% at concentrations of 1% Pet, 2% Pet, and 3% Pet, respectively. On the other hand, faba bean plants treated with bacterial consortium increased the stomata density, and stomata aperture area by 7.6% and 10.2%, and as compared with control (without Bac, and Pet), respectively. Whereas the treated plants grown under petroleum contaminated soil at (3% Pet+Bac) caused an increase of 6.2% in stomata density, and 42.9% in stomata aperture area as compared by 3% Pet treatment.

5.6.4. Impacts of bacterial consortium on agronomic traits of faba bean plants grown under petroleum contaminated soil

2% Pet and 3% Pet decreased plant height by 12.1% and 17.7%, leaf number by 32.2% and 45.9%, stem diameter by 14.4% and 23.6%, and plant dry matter by 29.3% and 47.9%, respectively, over the control. The treatment (3% Pet+Bac) increased growth attributes; plant height, leaf number, stem diameter and plant dry matter by 4.91%, 11.1%, 23.9% and 55.8% as compared with (3% Pet), respectively

5.6.5. Impacts of bacterial consortium on nutrient acquisition of faba bean plants grown under petroleum contaminated soil

Relative to control treatment (without Bac, and Pet), the contaminated soil by petroleum decreased N by 11.8%, 28.6% and 34.8%, P by 33.3%, 41.1% and 54.7%, K by 11.13%, 21.0% and 28.8%, at concentrations of 1% Pet, 2% Pet, and 3% Pet, respectively. Inoculation soil with bacterial consortium significantly enhanced leaf N, P, and K^+ content by 19.7%, 19.4%, and 14.7%, compared to the control treatment (without Bac and Pet).

5.6.6. Residual crude oil in soil after harvesting

The results indicate that the application of the bacterial consortium significantly enhanced crude oil degradation in soil compared to untreated controls. Based on the residual crude oil content in the soil following the harvest of bean plants, the degradation percentages were 89.2%, 84.95%, and 72.56% for the treatments Pet1%+Bac, Pet2%+Bac, and Pet3%+Bac, respectively. These findings suggest that increasing the concentration of petroleum in the soil resulted in a corresponding decrease in biodegradation efficiency. In contrast, the reduction percentages in crude oil content for the untreated treatments Pet1%, Pet2%, and Pet3% were considerably lower, at 15.9%, 9.9%, and 8.3%, respectively. Based on the results, it can be concluded that enhancing the soil with a bacterial consortium capable of crude oil biodegradation and stable coexistence within the soil environment is significant.

Objectives of this study

1. To isolate and screen the crude oil degrading, heavy metal resistance and plant growth promoting bacteria from heavy metal and crude oil affected soil and water samples.

2. To identify and classify the most promising crude oil degradable, heavy metal resistance and plant growth promoting bacterial isolates.

3. To determine the ability of the selected strains to grow on different concentrations of heavy metals and degrade crude oil and fractionate the residual crude oil and residual parafines.

4. To measure the ability of the selected strains for alleviation of the different concentration of cadmium (heavy metal) as abiotic stress on *Phaseolus vulgaris*.

5. To measure the ability of the selected strains consortium for mitigation of abiotic stress represented in different concentrations of crude oil on *Vicia faba*.

Abstract

A total of 54 heavy metal (Cd and Pb) resistant bacterial isolates were isolated from the eight samples, which collected from the heavy metal contaminated fresh water (El-Batas drain, located in Tamia El-Fayoum, Egypt) and heavy metal and crude oil contaminated agricultural land (located in Tamia, El-Fayoum, and El-dakhla, Alwadi Aljadid, Egypt). Of them 49 isolates were indole acetic acid producers with different ratios of production, 17 isolates were zinc solubilizers, and 8 isolates were phosphate solubilizers. 23 isolates of the all isolates had the ability to grow on B.H agar medium supplemented with crude oil. Based on the phosphate and zinc solubilization, indole production and growth on crude oil as sole carbon source, the isolates EG2PS1, EGF14S2, EGF16S3, and EGF17S2 were selected as the most promising heavy metal-resistant and plant growth promoting isolates. Based on phenotypic and genotypic (16S rRNA gene) characterization, the selected isolates EG2PS1, EGF14S2, EGF16S3, and EGF17S2 were identified as *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Leucobacter komagatae*, and *Providencia rettgeri*, respectively. According to the tolerance of the tested strains to heavy metals, all of strains except strain EGF17S2 had multiple resistances to three and more heavy metals. The degradative activity of these microorganisms toward oil (1%) showed that strain EG2PS1 could destroy 49.0 % of crude oil as the largest strain capable of degrading crude oil, followed by strains EGF16S3 and EGF17S2, which were capable of degrading 32.8 and 28.3% of crude oil, respectively. The lowest percentage of degradation of crude oil was recorded by strain EGF14S2, which was 25.2 %. The paraffins (alkanes) are the main component in crude oil, their content in the oil we used is approximately 70%, where strain EG2PS1 better degraded paraffins, followed by strains

EGF16S3, EGF17S2, and EGF14S2, respectively. In the Cd experiment, soil was subjected to three artificially contaminated Cd^{+2} concentrations (0, 5, and 10ppm of $CdSO_4$), and six treatments of bacteria strains were used, i.e., control (without), EG2PS1, EGF14S2, EGF16S3, EGF17S2, and mix strain. The Cd experiment consisted of 18 treatments, each containing 5 pots. The results showed that bacteria strains improved agronomic traits, physiological responses, anatomy structure, and nutrient status while Cd uptake was decreased in the leaves and pods of phaseolus plants grown under Cd concentrations. In the petroleum contaminated experiment, soil was subjected to three artificially contaminated petroleum concentrations [1, 2 and 3% plus control (without petroleum)], and two treatments of bacteria were used, i.e., without bacteria, and with bacteria. This study consisted of 8 treatments, each containing 5 pots with total 40 pots. The findings reflected that, inculcation petroleum contaminated soil with bacterial consortium improved faba bean growth, stomatal performance, leaf integrity, photosynthetic efficiency and nutrient acquisition. The application of bacterial consortium significantly enhanced crude oil degradation in soil compared to untreated controls. Based on the residual crude oil content in the soil, the degradation percentages were 89.2%, 84.95%, and 72.56% for the treatments Pet1%+Bac, Pet2%+Bac, and Pet3%+Bac, respectively. These findings suggest that increasing the concentration of petroleum in the soil resulted in a corresponding decrease in biodegradation efficiency.

Key words: PGP bacteria, Cd, plant growth promoting, heavy metal bioremediation, petroleum biodegradation, photosynthetic efficiency, faba bean, phaseolus stomatal performance, leaf integrity, photosynthetic efficiency and nutrient acquisition