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Evaluating the Potential of Chromium Resistant Bacteria Isolated from Industrial Effluents for Indole Acetic Acid (IAA) Production

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Abstract. In Pakistan, more than 800 tannery industries are present. Untreated industrial effluents lead to contamination of nearby soil, aquatic life, groundwater quality and ultimately humans by noxious heavy metals and other contaminants. The current study has designed to evaluate potential of indole acetic acid (IAA) synthesis from chromium resistant bacteria extracted from industrial effluents. A total of 20 samples of wastewater and adjacent soil were collected from 5 different tannery industries of Gujranwala and Lahore, Pakistan. Sequestration of bacteria resistant to chromium was done by spread plate method and further proceeded by screening test for IAA production. Bacteria resistant to chromium were cultured in LB media and centrifugation was done at gravitational acceleration of 18,894 g for 20 min to obtain supernatant. Salkowski reagent added to supernatant in (2:1) ratio. Five Cr resistant bacteria which confirmed maximum IAA production were further examined by applying different experimental conditions such as variation in pH, temperature, Trp (tryptophan) concentration and inoculum size. Investigations revealed that, isolated chromium resistant bacteria showed maximum IAA production at 37 °C and acidic pH. The isolated Cr resistant bacteria showed increase in IAA production by increasing Trp (tryptophan) concentration and inoculum size. Sequestration of bacteria resistant to chromium from industrial waste will be beneficial for IAA production which can be further applied for plant growth enhancement as bio-fertilizer.

Keywords: IAA, tannery effluents, heavy metals, chromium, chromium resistant bacteria, streak plate method, salkowski reagent, centrifugation, UV-Vis spectroscopy

Introduction

On the report of US-EPA chromium is among 17 heavy-metals, causing serious threats to humans. Different oxidation numbers of chromium are present, perhaps focus is on oxidation-state 3 and 6 of chromium (Rizvi *et al*., 2016). According to WHO, only a specific amount of chromium is bearable in water, soil and plants 0.1mg/L, 100mg, 1.3mg/Kg respectively (Arshad *et al*., 2019). Due to mutagenicity, carcinogenicity and teratogenicity in humans, animals and plants chromium is evaluated as cancer causing agent to human (Narayani and Shetty, 2013). Most eminent pollutant is hexavalent-chromium metal which is produced by numerous industries such as tannery industry, wood-processing industry, chrome-plating industry, metal-cleaning industry. Chromium-metal is highly permeable to cell-membrane, interacts with the genome and soluble in water (Sagar *et al*., 2011).

Under the current situation, reduction of chromium-metal intake should have to be controlled in order to hinder its presence in the food-chain and ultimately to avoid distortion in the food-web (Fatima and Ahmed, 2020). In increasing the economy of Pakistan through transporting outside, leather-industry plays a second-lead role. Leather-industry supports five hundred thousand people by providing them work facilities and giving up to five percent gross domestic product (GDP) to Pakistan (Hashmi *et al*., 2017). In developing-countries wet-chrome tanning-method uses excessive H_2O and releases almost ninety percent in the form of wastes in tannery-industries (Meneberu, 2021). Six hundred kilograms solid-waste and forty thousand cubic meter waste-water is produced during the treatment of 1000 kg raw-hide. The untreated wastes of industries are released to nearby aquatic and terrestrial areas. On a daily basis, almost eight thousand to nine thousand m3 contaminated waste-water is released from tannery-industries (Nawaz *et al*., 2021). In heavy-metals polluted areas, microbes adopt tolerance and resistance towards heavy-metals for their survival there (Yamina *et al*., 2012). Though, there is an adaptation of pathways in microbes that made them resistant-bacteria having tolerance towards noxious heavy-metals (Verma *et al*., 2001). Chromium-resistant bacteria interrupt up-take of chromium-metal (VI) and convert it into chromium-metal (III), plants are unable to consume it. In this way,

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plants confront less destruction due to chromium-metal. Consequently, plants in chromium-contaminated places show enhanced growth. Utilization of bacteria resistant to chromium-metal can increase auxin-hormone synthesis as well as minimizing use of costly-chemicals and other reduction procedures for removing chromium-metal (Fatima and Ahmed, 2020). Few of the bacteria resistant to chromium-metal can reduce chromium-metal with the help of enzymes named reductase-enzymes. The reductase-enzyme breaks down chromium-metal (III) into chromium-metal (VI) which are present in bacteria resistant to chromium-metal. Chromate-reductase genes and proteins make these bacteria able to synthesize reductase-enzyme (Basu *et al*., 1997). IAA is responsible for regulation of plant-growth which is a growth-hormone (Gupta *et al*., 2018). IAA most important growth-hormone and almost eighty percent is synthesized by microorganisms living in the root of plants that stimulate plant-growth (Astriani *et al*., 2020). IAA is a natural hormone synthesized by different organisms and improves plant-growth. Precursor-element for IAA is tryptophan-molecule (Li *et al*., 2018). Tryptophan-molecule plays a vital role as a precursor-element for indole-acetic acid production in different plants. A few microbes synthesize indole-acetic without tryptophan-molecule but it is synthesized in large quantities with availability of tryptophan-molecule (Naveed *et al*., 2015). Indole-acetic enhances cell-division, cell-elongation and lateral-root synthesis (Astriani *et al*., 2020). The core objective is based on evaluating potential of industrially isolated chromium resistant bacteria for synthesizing indole acetic acid.

Materials and Methods

Isolation of chromium resistant bacteria. Leather-industry utilizes salts of chromium-metal (III) as tanning-agents (Adeel *et al*., 2012). Compound (chromium-sulfate) used to interlock leather collagen, poses serious dangers. About thirty to forty percent of chromium-sulfate is not utilized and their untreated disposal causes serious dangers to nearby land surface and soil areas. In many other countries as well in Pakistan, this is a serious responsibility to tackle this problem (Benjamin and Nishat, 2021). Twelve (12) samples of tannery wastewater were collected in sterilized bottles and eight (8) samples of soil were assembled in sterilized polythene bags and stored at room temperature in research laboratory of Lahore Garrison University. Serial dilution of the collected samples was done to isolate chromium resistant bacteria. For isolation of chromium resistant bacteria from soil sample, 1g of weighed soil sample was added to 10 mL sterilized distilled water 1 mL suspension was added to next test tube containing 9 mL distilled water and the same process was repeated for rest of test tubes by using a micropipette. For isolation from wastewater sample, simply 10 test tubes were taken containing 9 mL sterilized distilled water in each. 1 mL water sample was added in first test tube and the suspension was added to other test tubes to make dilutions. Autoclaved media containing 10% stock solution of chromium (10 g potassium dichromate $KMnO_4$ salt in 100 mL of distilled water) and nutrient agar was poured in petri plates and dilutions were poured on media plates by spread plate method. The whole procedure was performed under sterilized conditions in a biological safety cabinet. After complete spreading of sample (10µL in volume per plate) onto media plates, plates were placed in incubator for 24 to 48 h at temperature 37 °C. Bacterial colony morphology was noted after incubation period.

Screening of bacterial isolates for indole-acetic acid (IAA) production. For screening of IAA producing bacteria, inoculated the bacterial isolates in LB broth medium comprising 0.01g L-tryptophan (per 100 mL LB) at pH 5 and incubated for about 24-48 h. After incubation culture was centrifuged at gravitational acceleration of 18,894 g for twenty minutes to obtain supernatant and cell pellet. The supernatants were transferred to separate test tubes, treated with Salkowski reagent in 2:1. In 2mL supernatant 1mL Salkowski reagent was added and stored at a dark place for 30 min after mentioned time observed change in colour (Chen *et al*., 2020). The optical density values of treated supernatants were determined at 530 nm absorbance for IAA production and noted the results.

Characterization and optimization of IAA producing isolated bacteria. Morphological and biochemical characterization of isolated bacteria was done by performing different tests for each of the isolate. Performed tests include catalase test, oxidase test, urease test, dnase agar test, starch agar test, MR-VP test and indole test. Optimization was performed at different ranges of temperature, pH, inoculum size and L-tryptophan (substrate) concentration. For this purpose LB broth was prepared and inoculated with selected bacterial isolates at pH 5, 6, 7, 8 and 9 and incubated at constant temperature 37 °C. After incubation period of 24 to 48 h, Optical density of growth at 600 nm

absorbance of LB broth was recorded. Similar method was repeated for different temperatures (25 °C, 30 °C, 37 °C, 40 °C and 45 °C) with constant pH 5, for different concentrations (0.00049 M, 0.00098 M, 0.00147 M, 0.00196 M and 0.00245 M) of substrate (L-tryptophan) and for different inoculum sizes (1%, 2%, 3%, 4% and 5%) and optical density values were recorded. Then centrifugation of culture was done at gravitational acceleration of 18,894 g for 20 min. The supernatant was treated with Salkowski reagent in 2:1 ratio. 1mL Salkowski reagent was added in 2mL supernatant and placed in dark for 30 min to observe color change to appear. Then OD was determined at 530 nm by using UV-Vis Spectrophotometer for IAA production and results were noted for all five selected isolates. The similar method was repeated for optimization of IAA production at different ranges of temperature, pH, inoculum size and L-tryptophan (substrate) concentration.

Results and Discussion

Isolation of chromium resistant bacteria. Initially isolation was done for 20 samples by spread plate method and proceeded by IAA screening. Total seventy five (75) colonies of bacteria resistant to chromium-metal (VI) were sequestered from all twenty samples. The isolates that were confirmed positive for IAA production were purified on separated petri plates by streak plate method. Isolates were named according to sample type and location of sample. All the collected samples confirmed presence of chromium resistant bacteria (Table 1).

Screening of IAA producing chromium resistant bacteria. Isolated Cr resistant bacteria (Table 1) were screened for IAA production. Screening for IAA was done by using Salkowski reagent and supernatant of isolates obtained after incubation followed by centrifugation. Screening was done by taking 2 mL supernatant of cultured isolates and 1mL of Salkowski reagent (2:1). The treated supernatants were observed for a colour change after 30 min (Fig. 1). By using UV-Vis spectrophotometer optical density was measured at 530 nm absorbance. Results of all the isolated bacteria for screening of IAA were noted (Table 2). 4 bacterial colonies from Gujranwala soil sample were confirmed positive for IAA production, 4 bacterial colonies were confirmed positive for IAA production from soil and 4 from wastewater sample confirmed positive for IAA production collected from Lahore. Five isolates LSA3, LWG1, GSF1, GSH2 and GSI2 showed maximum IAA production were selected for further characterization.

Table 1. Isolation of chromium resistant bacteria

Isolated bacteria	Number of colonies
GWA	GWA1, GWA2, GWA3, GWA4
GWB	GWB1, GWB2, GWB3, GWB4, GWB5
GWC	GWC1, GWC2, GWC3
GWD	GWD1, GWD2, GWD3, GWD4
GSE	GSE1, GSE2, GSE3, GSE4
GSF	GSF1, GSF2, GSF3, GSF4, GSF5
GSG	GSG1, GSG2, GSG3, GSG4
GSH	GSH1, GSH2, GSH3
GSI	GSI1, GSI2
LSA	LSA1, LSA2, LSA3, LSA4
LSB	LSB1, LSB2, LSB3, LSB4
LSC	LSC1, LSC2, LSC3
LWD	LWD1, LWD2
LWE	LWE1, LWE2, LWE3, LWE4, LWE5
LWF	LWF1, LWF2, LWF3, LWF4
LWG	LWG1, LWG2, LWG3
LWH	LWH1, LWH2, LWH3, LWH4, LWH5
LWI	LWI1, LWI2, LWI3, LWI4
LWJ	LWJ1, LWJ2, LWJ3
LWK	LWK1, LWK2, LWK3, LWK4

Fig. 1. Positive screening results of Cr resistant bacteria for IAA production.

Characterization and optimization of IAA producing isolated bacteria. Morphological characterization (Table 3) was performed by observing colony morphology. A series of biochemical tests was performed for purified isolates. The tests included oxidase test, indole production test, methyl red test, voges proskauer test, dnase test, catalase test, starch agar test, urease test. Five bacterial isolates gave positive results for indole test, catalase test, starch agar test, dnase test and urease

Table 2. IAA screening results of isolated colonies

Isolated bacteria	Screening results	Isolated bacteria	Screening results	Isolated bacteria	Screening results
GWA1	-	GSG1	-	LWE4	-
GWA2	-	GSG2	-	LWE5	-
GWA3	-	GSG3	-	LWF1	-
GWA4	-	GSG4	++	LWF2	-
GWB1	-	GSH1	-	LWF3	+
GWB2	-	GSH2	+++	LWF4	-
GWB3	-	GSH3	-	LWG1	+++
GWB4	-	GSI1	-	LWG2	-
GWB5	-	GSI2	+++	LWG3	-
GWC1	-	LSA1	++	LWH1	-
GWC2	-	LSA2	-	LWH2	-
GWC3	-	LSA3	+++	LWH3	-
GWD1	-	LSA4	-	LWH4	-
GWD2	-	LSB1	-	LWH5	-
GWD3	-	LSB2	+	LWI1	-
GWD4	-	LSB3	-	LWI2	-
GSE1	++	LSB4	-	LWI3	-
GSE2	-	LSC1	-	LWI4	-
GSE3	-	LSC2	+	LWJ1	-
GSE4	-	LSC3	-	LWJ2	-
GSF1	+++	LWD1	+	LWJ3	-
GSF2	-	LWD2	-	LWK1	-
GSF3	-	LWE1	+	LWK2	-
GSF4	-	LWE2	-	LWK3	-
GSF5	-	LWE3	-	LWK4	-

+ =Indicates production of IAA; ++ =Indicates relatively good production of IAA; +++ =Indicates efficient production of IAA; - =Indicates no production of IAA

Table 3. Morphological characterization of purified isolated colonies

Purified isolates	Shape	Size	Colour	Margin	Elevation	Texture
LSA3	Round	Small	Pink	Entire	Flat	Smooth
LWG1	Irregular	Medium	Off-white	Undulate	Raised	Rough
GSF1	Irregular	Small	White	Undulate	Raised	Rough
GSH2	Round	Medium	Off-white	Entire	Flat	Smooth
GSI2	Irregular	Small	White	Undulate	Raised	Rough

Table 4. Biochemical tests

Purified isolates	Gram staining	Indole test	MR test	VP test	DNase agar test	Oxidase test	Catalase test	Starch agar test	Urease test
LSA3	+	+	-	+	+	-	+	+	+
LWG1	+	+	-	+	+	-	+	+	+
GSF1	+	+	-	+	+	-	+	+	+
GSH2	-	+	+	-	+	-	+	+	+
GSI2	+	+	-	+	+	-	+	+	+

test. Only GSH2 showed positive result for methyl red test, and except GSH2 showed negative results for Voges proskauer test (Table 4). Optimal conditions for IAA producing Cr resistant bacteria were noted by observing (Fig. 2) the effect of varying pHs (5, 6, 7, 8 and 9), (Fig. 3) temperatures (25 °C, 30 °C, 37 °C, 40 °C and 45 °C), (Fig. 4) L-tryptophan (substrate) concentrations (0.00049 M, 0.00098 M, 0.00147 M, 0.00196 M and 0.00245 M) and (Fig. 5) inoculum sizes (1%, 2%, 3%, 4% and 5%) for growth of bacteria and

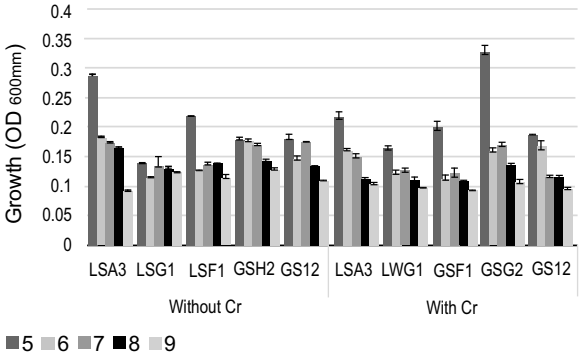

Fig. 2. Effect of different pH on growth of IAA producing Cr resistant bacteria after 24-48 h with constant temperature of 37 °C.

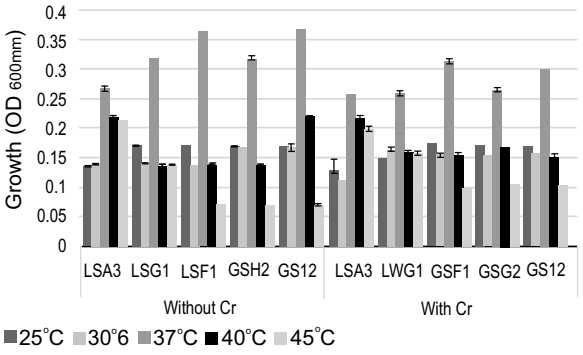

Fig. 3. Effect of different temperatures on growth of IAA producing Cr resistant bacteria after 24-48 h with constant pH 5.

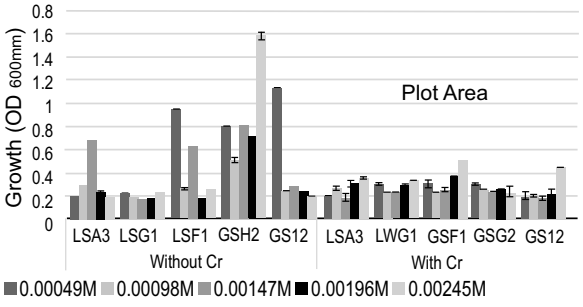

Fig. 4. Effect of different tryptophan concentrations on growth of IAA producing Cr resistant bacteria.

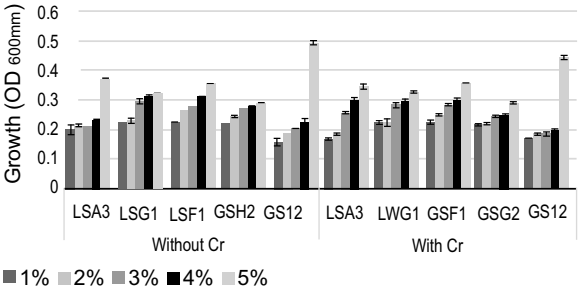

Fig. 5. Effect of different inoculum sizes on growth of IAA producing Cr resistant bacteria.

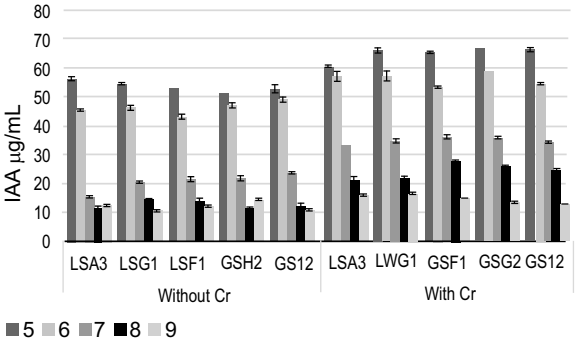

Fig. 6. Effect of different pH on IAA production of IAA producing Cr resistant bacteria.

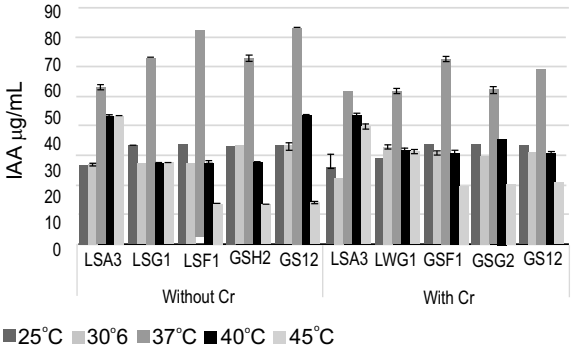

Fig. 7. Effect of different temperatures on IAA production of IAA producing Cr resistant bacteria.

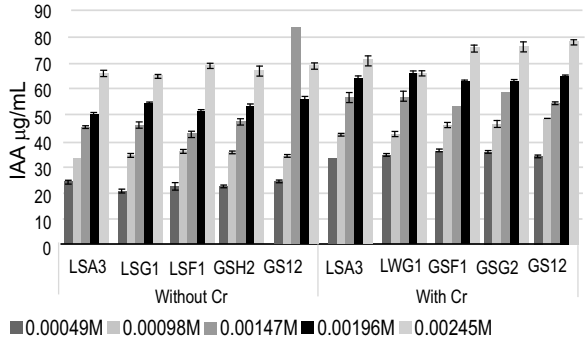

Fig. 8. Effect of different tryptophan concentrations on IAA production of IAA producing Cr resistant bacteria.

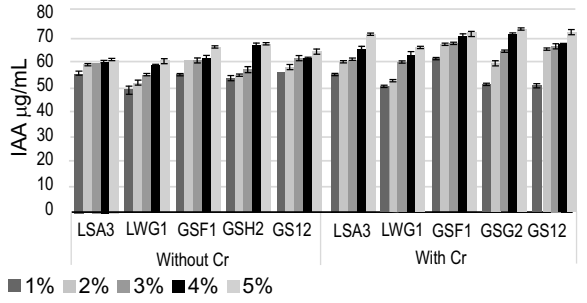

Fig. 9. Effect of different inoculum sizes on IAA production of IAA producing Cr resistant bacteria.

optical density values were recorded. Evaluation of IAA production potential was done observing in (Fig. 6) and the effect of varying pH (5, 6, 7, 8 and 9), in (Fig. 6) temperatures (25 °C, 30 °C, 37 °C, 40 °C and 45 °C) in (Fig. 7) L-tryptophan (substrate) concentrations (0.00049 M, 0.00098 M, 0.00147 M, 0.00196 M and 0.00245 M) in (Fig. 8) and inoculum sizes (1%, 2%, 3%, 4% and 5%), in (Fig. 9). Optimal conditions of IAA producing Cr resistant bacteria were performed in the presence and absence of Cr stress which is the presence or absence of Cr stock solution (10 % Cr stock solution prepared by adding 10 g Cr in 100 mL distilled water) in media.

In different industries, there is a significant use of chromium metal. Chromium having valency of six extremely noxious because it is easily soluble in water and can interact with proteins, DNA and RNA. Microbes have adopted different pathways to cope-up with metal stress. Metal tolerant microbes show efficient function in systems to treat wastewater. Bacteria resistant to chromium show capability of synthesizing important compounds, solubilizing phosphate, nitrogen fixing, toxin synthesis (Javaid and Sultan, 2013). IAA is a hormone which increases growth of plants by increasing division of cells, expansion of cells, development of leaves, fruit and root. Many microbes reported synthesizing indole-acetic acid like fungi, algae and bacteria (Bunsangiam *et al*., 2021).

In current study, isolation of chromium resistant bacteria from (12) wastewater and adjacent (8) soil samples was followed by screening of IAA production by using Salkowski's reagent. Among 75 different bacterial colonies, 10 bacterial colonies from all twenty samples were confirmed positive for IAA production. Study was further proceeded to determine optimal conditions for bacterial isolates having maximum IAA production during screening results for IAA production. Bacterial isolates were observed at different conditions (different temperature, pH, tryptophan concentration and inoculum size) to note the maximum production of IAA and to note the optimum conditions for best growth. Study revealed that IAA producing chromium resistant bacteria showed maximum IAA production at 37 °C with pH 5 and IAA production was increased by increasing tryptophan concentration and inoculum size.

Conclusion

This study concluded that chromium resistant bacteria isolated from wastewater and adjacent soil samples of tannery industries have potential to produce IAA. Different bacteria show different potential for IAA production. Maximum production of IAA by Cr resistant bacteria was recorded at 37 °C with acidic pH (5) and showed increase in IAA production with increase in tryptophan concentration and inoculum size. Results revealed that chromium resistant bacteria isolated from industrial effluents have potential to produce IAA both with and without chromium stress. The current study was based to evaluate IAA potential further research needed to extract and purify IAA from isolated chromium resistant bacteria and utilize to promote plants growth.

References

Adeel, S.S., Wajid, A., Hussain, S., Malik, F., Sami, Z., Haq Hameed, A., Channa, R.A. 2012. Recovery of chromium from the tannery wastewater by use of bacillus subtilis in Gujranwala. *Pakistan Journal of Pharmacy and Biological Sciences*, **2278-3008:** 36-45.

Arshad, M., Javaid, A., Manzoor, M., Hina, K., Ali, A.M., Ahmed, I. 2019. Isolation and identification of chromium-tolerant bacterial strains and their potential to promote plant growth. *Journal of EDP sciences*, **96:** 1-50.

Astriani, M., Zubaidah, S., Abadi, A.L., Suarsini, E. 2020. Pseudomonas plecoglossicida as a novel bacterium for phosphate solubilizing and indole-3-acetic acid producing from soybean rhizospheric soils of East Java, Indonesia. *Biodiversitas Journal of Biological Diversity*, **21:** 578-586.

Basu, M., Bhattacharya, S., Paul, A.K. 1997. Isolation and characterization of chromium-resistant bacteria from tannery effluents. *Journal of Bulletin of Environmental Contamination and Toxicology*, **58:** 535-542.

Benjamin, E.S., Nishat, A.M. 2021. Impacts of tanneries wastewater on the vicinal flora of Sheikhupura and Kasur. *Pakistan Sciendo's Journal*, **32:** 90-97.

Bunsangiam, S., Thongpae, N., Limtong, S., Srisuk, N. 2021. Large scale production of indole-3-acetic acid and evaluation of the inhibitory effect of indole-3-acetic acid on weed growth. *Scientific Reports*, **11:** 13094.

Chen, J., Li, N., Han, S., Sun, Y., Wang, L., Qu, Z., Zhao, G. 2020. Characterization and bioremediation potential of nickel-resistant endophytic bacteria isolated from the wetland plant Tamarix chinensis. *Journal of Federation of European Microbiological Societies*, **367:** 1-98.

Fatima, H., Ahmed, A. 2020. Indole-3-acetic acid synthesizing chromium-resistant bacteria can mitigate chromium toxicity in Helianthus annuus L. *Journal of Plant Soil and Environment*, **66:** 216-221.

Gupta, P., Rani, R., Chandra, A., Varjani, S.J., Kumar, V. 2018. Effectiveness of plant growth-promoting rhizobacteria in phytoremediation of chromium stressed soils. In: *Waste Bioremediation*, Z. Zakaria (ed), pp. 301-312, *Energy,* Environment and Sustainability. Springer, Singapore.

Hashmi, G.J., Dastageer, G., Sajid, M.S., Ali, Z., Malik, M.F., Liaqat, I. 2017. Leather industry and environment: Pakistan scenario. *International Journal of Applied Biology and Forensics*, **1:** 20-25.

Javaid, M., Sultan, S. 2013. Plant growth promotion traits and Cr (VI) reduction potentials of Cr (VI) resistant streptomyces strains. *Journal of Basic Microbiology*, **53:** 420-8.

Li, M., Guo, R., Yu, F., Chen, X., Zhao, H., Li, H., Wu, J. 2018. Indole-3-Acetic acid biosynthesis pathways in the plant-beneficial bacterium arthrobacter pascens ZZ21. *International Journal of Molecular Sciences*, **19:** 443.

Meneberu, H. 2021. Evaluation and characterization of wastewater from tannery industries and their impact on water quality of the receiving river. *Ethiopia International Journal of Scientific and Academic Research (IJSAR)*, **1:** 2-21.

Narayani, M., Shetty, K.V. 2013. Chromium-resistant bacteria and their environmental condition for hexavalent chromium removal. *Critical Review in Journal of Environmental Science and Technology*, **43:** 955-1009.

Naveed, M., Qureshi, M.A., Zahir, Z.A., Hussain, M. B., Sessitsch, A., Mitter, B. 2015. L- Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by burkholderia phytofirmans PsJN. *Annals of Microbiology*, **65:** 1381-1389.

Nawaz, A.R., Anwar, U., Ahmad, S. 2021. Assessing the economic impact of tanneries, pollutants in Pakistan. *Journal of Economic Impact*, **3:** 98-106.

Rizvi, Z.F., Kanwal, W., Faisal, M. 2016. Chromate-reducing profile of bacterial strains isolated from industrial effluents polish. *Journal of Environmental Studies*, **25:** 2121-2128.

Sagar, S., Dwivedi, A., Yadav, S., Tripathi, M., Kaistha, D.S. 2011. Hexavalent chromium reduction and plant growth promotion by Staphylococcus arlettae Strain Cr11. *Journal of Chemosphere*, **86:** 847-852.

Verma, T., Srinath, T., Gadpayle, R.U., Ramteke, P.W., Hans, R.K., Garg, S.K. 2001. Chromate tolerant bacteria isolated from tannery effluent. *Journal of Bioresource Technology*, **78:** 31-35.

Yamina, B., Tahar, B., Laure, F.M. 2012. Isolation and screening of heavy metal resistant bacteria from wastewater: a study of heavy metal co-resistance and antibiotics resistance. *Journal of Water Science Technology*, **66:** 2041-2048.