Antibacterial activity of different plant extracts and antibiotics on pathogenic bacterial isolates from wheat field water

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Abstract: The present work was carried out to study bacterial pathogens isolated from wheat field water and also effect of some plant extracts on these bacterial pathogens. Five bacterial strains were isolated from wheat field water. Different morphological and biochemical tests were performed to identify and characterize bacterial pathogens. Among isolated strains two belonged to genus *Staphylococcus* sp., other two were *Pseudomonas* spp. and one strain belonged to genus *Salmonella* sp. Effect of various parameters such as temperature, pH, antibiotics and heavy metals of these pathogens were also studied. Optimum temperature for all bacterial strains was 37^{o}C and optimum pH was 7 except strain 3 which had pH 6. Different antibiotics with different potency were applied to check the resistance of bacterial strains against them. Among these antibiotics Cloxacillin and Teicoplanin were most potent while Oxacillin was as less potent antibiotic because three bacterial strains were resistant against it. While remaining antibiotics proved as potent. Seven heavy metals which were zinc (Zn^{+2}), copper (Cu^{+2}), Ferrous (Fe^{+2}), mercury (Hg^{+2}), Nickel (Ni^{+2}), chromium (Cr^{+2}) and cobalt (Co^{+2}) with different concentrations were applied to bacterial strains. Minimum inhibitory concentration of heavy metals for all bacterial was different. Different plant extracts (*Artemezia incise, Colebrookia oppositifolia, Rhynchosia pseudocajan)* checked for their antibacterial activity against these pathogens. These plant extracts showed antibacterial activity against antibiotic and metal resistant bacterial isolates.

Keywords: Bacterial pathogens, wheat field water contamination, plant extracts, antibiotic resistance.

INTRODUCTION

In this study the focus was to isolate pathogenic bacteria from wheat water field and after their identification and characterization, effect of different plant extracts on these pathogens was studied. This work was done because of the fact that these microorganisms are present every where in natural environment and are of economic importance as beneficial and pathogenic ones. Some bacteria play important role in cycling of carbon and many other important elements. Biological fixation of atmospheric nitrogen is also carried out by some bacteria. They also carry out the denitrification, methane production, and reduction of sulfate and decomposition of vegetal and other product residues and also play important role in biotransformation of metals (Haines *et al*., 2002). Fresh water from canals, rivers and streams are usually used for irrigation purpose may contain hazardous microorganisms such as plant pathogens which are harmful for cultivation of many crops such as Cyanobacteria are pathogenic bacteria which can decrease the plant growth due to release of toxic substances. The most well known potentially pathogenic bacteria which are present in water are *Salmonella, Shigella* and *Escherichia coli. Salmonella* are pathogenic for humans and often for animals. These enteric bacteria which are present in the intestinal tract of animals and humans are pathogenic in nature and present in large quantities in digestive tract of animals which excreted with feces of animals and contaminate the irrigated water and water present in the fields. Coliform bacteria are large group of bacteria that naturally found in soil, decaying organic matter and digestive tract of warm blooded animals and they have capability to survive longer in the water (Welch *et al*., 2002). Some of bacteria show growth promoting effect on plants. In these bacteria *Pseudomonas* spp., (Van peer and Schipper, 1989) and Scenedesmus spp., (Mazur *et al*., 2001) are include. But some species of *Pseudomonas* cause plant diseases like leaf spot, leaf stripe, wilt and similar diseases (Pelczar *et al*., 1993). Heavy metals tolerance or resistance reflects the capability of bacteria to survive in an environment with high concentration of heavy metals or heavy metals resistant bacteria are those which can accumulate high concentration of heavy metals in it without dying. Heavy metal resistant bacteria have the plasmid containing the heavy metal resistant genes and presence of these bacteria in any environment are the indicators of environmental pollution with heavy metals (Ehrlich, 1986). The ability of microorganisms to bear the affect of antibiotics is called the antibiotic activity of microorganisms. Many antibiotics have been used in last several decades in medical, veterinary and agriculture practices (Chelossi *et al*., 2003). Many bacteria are resistant against many recently used antibiotics. Resistance against antibiotics can be natural or acquired or can be transmitted by vertically or horizontally (Alanis, 2005). Also nowadays certain plants and their extracts are used to study

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antibacterial activity. In recent study *Artemezia incise, Colebrookia oppositifolia* and *Rhynchosia pseudocajan* were used for this purpose.

MATERIALS AND METHODS

Bacteria were isolated from water standing in wheat fields. Nutrient agar broth were used for the growth of bacterial colonies. Different morphological tests were performed to determine the morphology of isolated bacteria (table 1). Different biochemical tests were performed for the identification and characterization of isolated bacteria (table 2 and 3).

Optimum temperature and pH of isolated bacterial strains was also studied (table 4 and 5). Antibiotic resistance of isolated bacterial strains was also studied by using disk plate method. Seven different antibiotic discs with different potency which include Ampicillin (10ug), Carbenicillin (100ug), Cloxacillin (5ug), Methicillin (5 ug), Oxacillin (1ug), Polymyxin B (300ug) and Teicoplanin (30ug) were used (table 6).

Minimum inhibitory concentration (MIC) of heavy metals and plant extracts for bacteria isolated from irrigated water was determined. Heavy metals such as zinc (Zn^{+2}), copper (Cu^{+2}), Ferrous (Fe^{+2}), mercury (Hg^{+2}), Nickel (Ni^{+2}), chromium (Cr^{+2}) and cobalt (Co^{+2}) and plant extracts of *Artemezia incise, Colebrookia oppositifolia, Rhynchosia pseudocajan* were used. The solutions of heavy metals and plant extracts were added in nutrient agar plates in various concentrations ranging from 5µl-900µl. These plates then inoculated with streak plate method by using isolated bacterial strains under aseptic conditions and incubated at 37^{o}C for next 24 hours. The growth was observed next day then the concentration of each metal was increased and colonies were streaked over it. When growth was stopped the concentration of heavy metal and plant extracts used was noted (table 7, 9).

RESULTS

Strain 1 was isolated from the irrigated water present in wheat fields. It was identified as genus *Pseudomonas* (table 8). Optimum temperature for strain 1 was 37^{o}C and optimum pH was 7 (table 4 and 5). In case of antibiotic resistance strain 1 showed resistance against Oxacillin (1µg) while showed maximum sensitivity to Teicoplanin (30µg) with 8mm zone of inhibition (table 6). While minimum inhibitory concentration was shown in (table 7, 9). All plant extracts showed antibacterial activity against this strain.

Strain 2 was identified as *Staphylococcus* sp. (table 8) this bacteria showed maximum activity on 37^{o}C and pH 7 (table.4 and 5). To check the antibiotic resistance strain 2 was sensitive to all antibiotics and showed maximum sensitivity to carbencillin (100µg) with 11.7mm of zone of inhibition (table 6). Minimum inhibitory concentration of heavy metals and plant extracts was given in (tables 7, 9).

Strain 3 belonged to genus *Salmonella* (table 8). Optimum temperature was 37^{o}C and optimum pH was 6 (table 4 and 5). To check the antibiotic resistance strain 3 showed resistance against carbenicillin (100µg) and Oxacillin (1µg) while it showed maximum sensitivity to methicillin (5µg) with 6.5mm zone of inhibition (table 6). MIC of heavy metals and plant extracts was summarized in (tables 7, 9)

Strain 4 identified as *Pseudomonas* sp. (table 8). Its optimum temperature is 37^{o}C and pH was 7 (tables 4 and 5). Strain 4 remained sensitive to all antibiotics were applied and showed maximum sensitivity to cloxacillin (5 µg) with 14mm zone of inhibition (table 6). MIC of heavy metals was shown in (table 7).

Strain 5 belonged to genus *Staphylococcus* (table 8). Optimum temperature was 37^{o}C and optimum was pH 7 (table 4 and 5). Strain 5 had resistance against oxacillin (1 µg) and showed maximum sensitivity to methicillin (5 µg) with 8.2mm zone of inhibition (table 6). Minimum inhibitory concentration was presented in (table 7).

DISCUSSION

Five pathogenic bacterial strains were isolated in which two bacterial strains belonging to genus *Staphylococcus*, other two strains belonging to genus *Pseudomonas*, while one strain belonging to genus *Salmonella*. These observations indicated that bacterial strains belonging to genus *Staphylococcus* and *Pseudomonas* were present abundantly in the irrigated water while genus *Salmonella* was also present in the water.

All five isolated bacterial strain showed sensitivity to all antibiotics used. So these antibiotics were proved to be potent. In these antibiotics Ampicillin (10µg) is a semisynthetic pencillin and act against wide spectrum of bacteria. It is a strong bactericidal. Polmyxin B (300µg) was also applied. It is a polypeptide antibiotic which has ability to injure the cell membrane structure thus affecting its permeability (Pelczar *et al*., 1993).

In this study, Oxacillin (1µg) proved as less potent antibiotic because three bacterial strains have resistance against it. While other antibiotics were proved as potent antibiotics because all strains sensitive to these antibiotics. Antibiotic resistant bacteria present in irrigated water can spread from animals to humans via food chain and fresh vegetables and crops which are eaten raw containing these antibiotic resistant bacteria which can enter in the body of animals and humans can cause

Table 1: Morphological Tests for Isolated Bacterial Strains

Tests	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
Gram's staining	-ve	+ve	-ve	-ve	+ve
Endospore staining	-ve	-ve	-ve	-ve	-ve
Acid Fast staining	-ve	-ve	-ve	-ve	-ve
Motility test	+ve	-ve	+ve	+ve	-ve

Table 2: Biochemical Tests for Isolated Bacterial Strains

Test	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
Catalase	+ve	+ve	+ve	+ve	+ve
Urease	+ve	-ve	-ve	+ve	-ve
Gelatin hydrolysis	+ve	+ve	-ve	+ve	+ve
Carbohydrate Fermentation	+ve	+ve	+ve	+ve	+ve
Litmus Milk	+ve	+ve	+ve	+ve	+ve
Glucose Fermentation	-ve	+ve	+ve	-ve	+ve
Fructose Fermentation	-ve	+ve	+ve	-ve	+ve
Sucrose Fermentation	-ve	+ve	+ve	-ve	+ve
Methyl red	+ve	+ve	+ve	+ve	+ve
Voges Proskauer	-ve	-ve	-ve	-ve	-ve
Citrate	-ve	-ve	+ve	-ve	-ve
Indole	-ve	-ve	-ve	-ve	-ve
Hydrogen Sulphide	-ve	-ve	+ve	-ve	-ve
MacConkey agar	-ve	-ve	-ve	-ve	-ve
Blood agar	-ve	-ve	-ve	-ve	-ve

Table 3: Selective Medium used for growth of Bacterial strains

Test	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
EMB agar	-ve	-ve	-ve	-ve	-ve
Pseudomonas Selective medium	+ve	-ve	-ve	+ve	-ve
Staphylococcus Selective medium	-ve	+ve	-ve	-ve	+ve

Table 3.1: Effect of Temperature on growth of Bacterial Strains

Temperature (^oC)	Strain 1 (O.D at 600 nm)	Strain 2 (O.D at 600 nm)	Strain 3 (O.D at 600 nm)	Strain 4 (O.D at 600 nm)	Strain 5 (O.D at 600 nm)
4	0.025	0.010	0.007	0.045	0.036
25	0.240	0.233	0.186	0.433	0.291
37	0.527	0.625	0.513	0.620	0.548

severe diseases. Antibiotic resistant bacteria become a serious healthcare problem world wide (Tenover, 2001).

Minimum inhibitory concentration (MIC) of heavy metals (zinc (Zn^{+2}), copper (Cu^{+2}), Ferrous (Fe^{+2}), mercury (Hg^{+2}), Nickel (Ni^{+2}), chromium (Cr^{+2}) and cobalt (Co^{+2}) was also observed in this research work. Different concentrations of these heavy metals were applied. Minimum inhibitory concentration of heavy metals for each strain was different.

Heavy metals are generally toxic and harmful for both Gram negative and Gram positive bacteria and mercury (Hg^{+2}) is very toxic for bacteria, humans and other animals (Mahler *et al*., 1981). Heavy metal resistance mechanism in bacteria includes transformation of toxic material resulting in release of metal ions (Williams *et al*., 1993).

Nies, 2000 explained that microbial detoxification of heavy metals by enzymatic action can occur by transfer of metal ions into less toxic or non-toxic by the process of enzymatic oxidation or reduction. Detoxification of heavy metals in absence of enzyme can also be done by precipitation using inorganic metabolic products such as sulfides, carbonates and phosphates (Macaskie *et al*., 1987). Isolation of heavy metal resistant bacterial strains in the irrigated water is a proof of heavy metal polluted environment and incidence of heavy metals resistant bacteria indicated the environmental contamination with these heavy metals (Roane and Kellogg, 1996).

Table 4: Optimum temperature for Isolated Bacterial Strains

Bacterial Strains	Optimum temperature
Strain 1	37°C
Strain 2	37°C
Strain 3	37°C
Strain 4	37°C
Strain 5	37°C

Table 4.1: Effect of pH on Bacterial Strains

pH	Strain 1 (O.D at 600 nm)	Strain 2 (O.D at 600 nm)	Strain 3 (O.D at 600 nm)	Strain 4 (O.D at 600 nm)	Strain 5 (O.D at 600 nm)
4	0.076	0.040	0.026	0.131	0.091
5	0.180	0.064	0.052	0.008	0.024
6	0.262	0.149	0.163	0.182	0.278
7	0.570	0.389	0.021	0.514	0.322
8	0.318	0.116	0.144	0.213	0.225

Table 5: Optimum pH for Isolated Bacterial Strains

Bacterial Strains	Optimum pH
Strain 1	7
Strain 2	7
Strain 3	6
Strain 4	7
Strain 5	7

Table 6: Antibiotics Resistance of Isolated Bacterial Strains

Antibiotics	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
Ampicillin (10 µg)	1.5mm	10.5mm	1.25mm	7.5mm	2mm
Carbencillin (100 µg)	6.50mm	11.75mm	R	12.5mm	3.5mm
Cloxacillin (5 µg)	1.5mm	6mm	2.5mm	14mm	7mm
Methicillin (5 µg)	5.2mm	6.2mm	6.5mm	9.2mm	8.2mm
Oxacillin (1 µg)	R	5mm	R	6.5mm	R
Polymyxin B (300 µg)	2.2mm	2mm	2mm	2.7mm	3.5mm
Teicoplanin (30 µg)	8mm	6.7mm	2.5mm	12mm	7mm

Table 7: MIC determination of Heavy Metals

Strains	Zn^{+2} (µg)	Cu^{+2} (µg)	Fe^{+2} (µg)	Hg^{+2} (µg)	Ni^{+2} (µg)	Cr^{+2} (µg)	Co^{+2} (µg)
Strain 1	700	500	700	900	700	700	700
Strain 2	500	500	700	700	800	700	700
Strain 3	500	500	500	700	800	700	700
Strain 4	500	500	700	900	700	500	900
Strain 5	500	600	700	900	700	600	800

Table 8: Identification of Isolated Bacterial strains

Strain 1	*Pseudomonas* sp.
Strain 2	*Staphylococcus* sp.
Strain 3	*Salmonella* sp.
Strain 4	*Pseudomonas* sp.
Strain 5	*Staphylococcus* sp.

Table 9: Minimum inhibitory concentration of plant extract against bacteria from wheat field

Plant extracts	Minimum inhibitory concentration of plant extract against bacteria from wheat field water (mg^{-1})					Name of plants
	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	
TR-AI-A	350	250	200	300	350	*Artemezia incisa* Aqueous fraction
TR-CO-W	300	250	300	300	350	*Colebrookia oppositifolia* aqueous fraction
TR-AI-C	200	150	250	200	200	*Artemezia incisa* Chloroform fraction
TR-AI-H	300	200	250	300	150	*Artemezia incisa* hexane fraction
TR-AI-E	150	200	200	250	250	*Artemezia incisa* ethyl acetate fraction
TR-AI-B	200	150	150	300	200	*Artemezia incisa* Butanol fraction
TR-Rh-B	350	400	300	300	300	*Rhynchosia pseudocajan* butanol fraction
TR-Rh-C	150	150	200	300	200	*Rhynchosia pseudocajan* chloroform fraction
TR-Rh-E	300	400	250	200	200	*Rhynchosia pseudocajan* ethyl acetate fraction
TR-Rh-H	250	150	350	300	200	*Rhynchosia pseudocajan* hexane fraction
TR-Rh-w	250	150	350	200	300	*Rhynchosia pseudocajan* aqueous fraction
TR-C0-E	300	200	200	250	300	*Colebrookia oppositifolia* ethyl acetate fraction
TR-CO-C	300	300	300	300	350	*Colebrookia oppositifolia* chloroform fraction
Ethyl alcohol, acetic acid	300	250	350	250	250	

Effect of different plant extracts was also studied in recent research work. All plant extracts showed antibacterial activity against these pathogenic, metal resistant bacteria. Different plant extracts solutions prepared in solvents like ethanol, choloroform, water, ethyl acetate and hexane *etc.* showed different MIC values. So, these plant extracts can be used against the pathogenic and antibiotic resistant bacteria which are causing health problems.

REFERENCES

Alanis AJ (2005). Resistance to antibiotics: Are we in the post-antibiotic era? *Arch.*, pp.697-705.

Chelossi, EC, Vezzulli L and Milano A *et al*. Antibiotics resistance of benthic bacteria in fish-farm and control of the sediments of Western Mediterranean. *Aquaculture*, **219**: 83-97.

Ehrlich HL (1986). Interactions of Heavy metals and Microorganisms. *In*: Mineral Exploration: Biological Systems and Organic Matter, Vol. **5**, (Carlisl D, Berry WL, Kapln IR and Watterson JR, Eds.) Englewood Cliffs, Prentice-Hall, Inc., New Jersey.

Haines JR, Herrmann R and Lee K *et al* (2002). Microbial population analysis as a measure of ecosystem restoration. *Biorremed. J.*, **6**(3): 283-296.

Macaskie LE, Watese JM and Dean ACR (1987). Cadmium accumulation by a Citrobacter sp. Immobilized on gel and solid supports: Applicability to the treatment of liquid wastes containing heavy metal cations. *Biotechnol. Bioeng.*, **30**: 66-73.

Mahler J, Levinson HS and Wang Y *et al* (1981). Cadmium and Mercury-Resistant Bacillus strains from a salt marsh and from Boston Harbor. *Appl. Environ. Microbial*, **52**: 1293-1298.

Mazur H, Konop A and Synak R (2001). Indole-3-acetic acid in the culture medium of two axenic green microalgae. J. Appl. Phycol 13: 35-42, 2001.

Nies DH (2000). Heavy metal-resistant bacteria as extremophiles. Molecular physiology and biotechnological use of *Ralsronia sp*. CH. 34. *Extreophiles*, **4**(2): 77-82.

Pelczar MJ, Chan ES and Krieg NR (1993). Microbiology 5th ed. McGraw Hill Inc., New York, pp.1-20.

Roane TM and Kellogg ST (1996). Characterization of bacterial communities in heavy metal contaminated soils. *Can. J. Microbiol.*, **42**: 593-603.

Tenover FC. Mechanism of antimicrobial resistance in Bacteria. *American Journal of Infection Control*, **34**(1): 3-10.

Van Peer R and Schippers B (1989). Plant growth response to bacterization with selected Pseudomonas spp. Strains and rhizoshere microbial development in hydroponics cultures. *Can. J. Microbial.*, **35**: 456-463.

Welch P, David J and Clark W *et al* (2000). Microbial quality of water in rural communities of Trinidad. *Rev. Panam. Salud. Publica.*, **9**: 172-180.

Williams JR, Morgan AG and Rouch DA *et al* (1993). Copper resistant enteric bacteria from United Kingdom and Australian. Piggeries. *Appl. Environ. Microbiol.*, **59**: 2531-2537.