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ANTIBACTERIAL ACTIVITY OF ANTIBIOTICS AGAINST PATHOGENS AND THEIR GENOMIC DNA ISOLATION

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Abstract: Microorganisms like staphylococci and pseudomonads are responsible for food poisoning. These are natural residents of soil and water and cause primary skin infections. In present study, antibiotics were used against these bacteria through diffusion disc method. Two types of media (MacConky agar and nutrient agar) were used. *Pseudomonas syringae* was found to be sensitive to tobramycin, ciprofloxacin, and ampicillin while it was resistant to tetracycline, gentamycin, penicillin-G, kanamycin, and streptomycin. *Staphylococcus aureus* was sensitive to kanamycin, gentamicin, tobramycin, streptomycin, and ampicillin, while it was susceptible to the various selected antibiotics such as ciprofloxacin, tetracycline, and amoxicillin. Genomic DNA was extracted to detect the defense genes both from *P. syringae* and *S. aureus* through modified techniques for amplification of various genes which play some role in prevention of various related diseases.

Key words: disc method, enterotoxins, *Staphylococcus, Pseudomonas*

INTRODUCTION

Pseudomonas can cause food spoilage including dairy spoilage by *P. fragi*, (Pereira and Morgan, 1957), mustiness in eggs caused by *P. taetrolens* and *P. mudicolens*, (Levine and Anderson, 1932) andthe spoilage of milk, cheese, meat, and fish by *P. lundensis* (Gennari and Dragotto, 1992). On the other hand, *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis (Bartoszko-Tyczkowska *et al*., 2008).

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The use of plant extracts and phytochemicals with well known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, the number of studies had been conducted in different countries to prove such efficiency (Artizzu *et al*., 1995; Ikram and Inamal, 1984; Izzo *et al*., 1995; Kubo *et al*., 1993; Almagboul *et al*., 1995; Shapoval *et al*., 1994).

The antibiotic is a drug that kills bacteria and is used to treat infection but it is generally harmless to host. Antibiotics can kill susceptible microorganisms or inhabit their growth (Prescott *et al*., 1996). Microorganisms that produce various antibiotics are widely distributed in nature, where they play important role in regulating the microbial population of soil, water and sewage and compost process.

The problem of microbial resistance is prevailing and the view for the use of antimicrobial drugs in future is still doubtful. Therefore, there is unmet need to solve this problem through various strategies i.e. by the use of antibiotic, advancing research to understand the genetic mechanisms of resistance and to continue to develop or discover new drugs, either synthetic or natural. The ultimate goal is to offer the appropriate and efficient antimicrobial drugs to patients.

In this study, we reported the antimicrobial activity of antibiotics against human associated pathogens. The screening and selection of *P. syringae* and *S. aureus* were carried out in biotechnology lab by using selective media such as MacConky agar and nutrient agar medium. It had observed that these antibiotics had great effects on microbial growth and possess both bacteriostatic and bactericidal activity.

MATERIALS AND METHODS

Sample collection

Pseudomonas syringae and *Staphylococcus aureus* samples were collected from the microbiology laboratory of Combined Military Hospital (CMH), Muzaffarabad, Pakistan and samples were brought to the Biotechnology laboratory of Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan for further investigations and genomic DNA analysis. Samples were brought in the form of streaking on the MacConky agar plates and placed at 4°C before using for further isolation of genomic DNA, protein and antibiotic activity.

Antibacterial Test

The antimicrobial sensitivity test against *P. syringae* and *S. aureus* was performed by standardized diffusion disc technique, which is described by Bauer *et al*., 1966. Two types of media were used for the standardized diffusion disc technique *i.e.,* MacConky agar medium and nutrient agar medium to check the reproducibility of results and the effects of the microbe on selective medium. The single colony of bacterial strains was picked with yellow tip or sterile loop and dipped in 100 ml of nutrient agar medium (NAM). The broth nutrient medium was placed at 37°C for overnight. Next day, the base of sterilized plates was poured with agar medium in laminar flow and placed at room temperature for 15-20 min to solidify. After 20 min, the overnight grown culture was mixed with the MacConky agar medium and poured on already solidified plates. Two plates were used for each isolate in which antibiotics discs were placed on the surface of plates at constant distance. The inoculated plates were incubated overnight at 37°C and zone of inhibition was checked and calculated under normal and white light board.

Genomic DNA extraction

The genomic DNA was extracted from *P. syringae* and *S. aureus* through the modified technique (Doyle and Doyle, 1990; Aziz *et al*., 2003; Millar *et al*., 2000; Badri and Sariah, 2009) as given in Fig. 1.

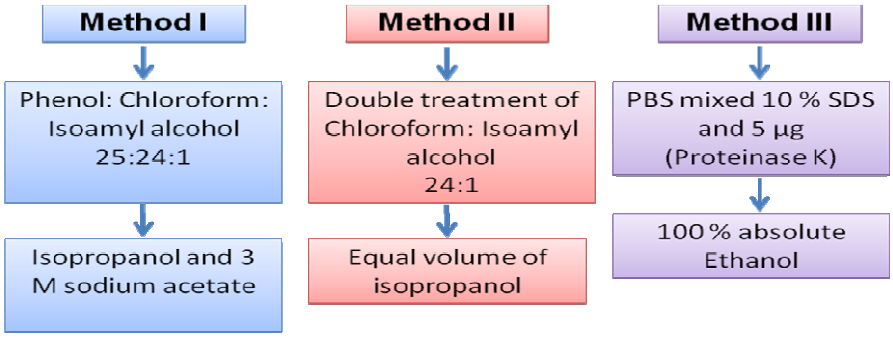

Fig. 1 Modified protocols for isolation of Genomic DNA from *P. syringae* and *S. aureus*.

RESULTS

In the present study, *P. syringae* and *S. aureus* were isolated from the human infected samples such as urine and pus, respectively. It was found that *P. syringae* was sensitive against tobramycin, ciprofloxacin, and ampicillin as shown on both MacConky agar and nutrient agar medium plates (Figure 2A and 2B). The concentration and antibacterial activity of selected antibiotics against *P. syringae* had shown in Table I.

It was investigated that *S. aureus* was catalase-positive and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci. A small percentage of *S. aureus* can also be differentiated from most other staphylococci by the coagulase test: *S. aureus* is primarily coagulase-positive that causes clot formation, whereas most other *Staphylococcus* species are coagulase-negative that they do not produce coagulase (Mattews *et al*., 1997; Ryan and Ray, 2004).

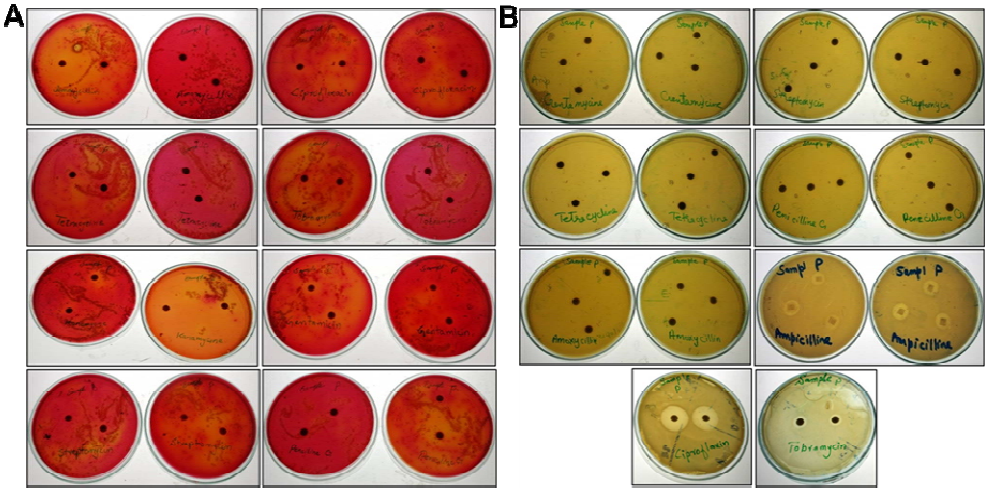

Fig. 2 Antibiotics sensitivity test against *P. syringae* on (A) MacConky agar (B) nutrient agar .

Further, it was observed that *S. aureus* was sensitive to kanamycin, gentamicin, tobramycin, streptomycin, and ampicillin, respectively (Fig. 3A and 3B). The clear and reproducible results were shown on the

MacConky agar medium plates as shown in Fig. 3A, whereas it was also analyzed that *S. aureus* was susceptible to various selected antibiotics such as ciprofloxacin, tetracycline, and amoxicillin, respectively (Fig. 3A and 3B). It had shown that Gram-negative bacteria, most *Pseudomonas spp.* are naturally resistant to penicillin, tetracycline, gentamycin, penicillin G, kanamycin, and streptomycin, respectively. Their resistance to most antibiotics is attributed to efflux pumps which pump out some antibiotics before the antibiotics are able to act. Result was consistent with the previous literature as described by Ryan and Ray, 2004. The hypothesis regarding to the treatment of choice for *S. aureus* infection is penicillin antibiotic and the combination therapy with gentamicin was used to treat serious infections like endocarditis (Bayer *et al*., 1998) but its use is controversial because of high risk of damage to kidneys (Cosgrove *et al*., 2009).

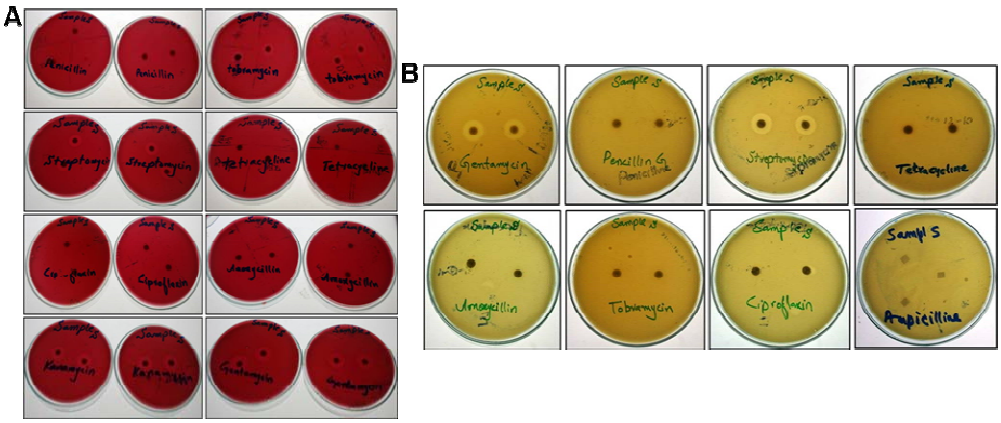

Fig. 3 Antibiotics sensitivity test against *Staphylococcus aureus* on (A) MacConky agar (B) nutrient agar medium.

It was also analyzed that mminoglycoside antibiotics such as kanamycin, gentamicin, streptomycin, *etc.* were once effective against Staphylococcal infections (Fig. 3A and 3B; Carter *et al*., 2000). The concentration and antibacterial activity of selected antibiotics against *Staphylococcus aureus* hadshown in Table I. It was consistent with the previous literature that antibiotics were used against the pathogenic and unwanted microbial growth during cloning and expression of heterologous

genesand were very effective (Wajid *et al*., 2008; Andleeb *et al*., 2008; Wajid *et al*., 2009).

We wanted to know which types of genes were helpful or had role in defense mechanism for bacterial growth in the presence of antibiotics. So, on the basis of the isolation of these defense genes, we isolated the genomic DNA from *Pseudomonas syringae* and *Staphylococcus aureus* by using three different methods. The clear and good quality bands of genomic DNA were not observed in case of I method and it had confirmed that the method II and III showed better extraction (Fig. 4). However, the genomic DNA extraction was modified by using proteinase K digestion, phenol/chloroform extraction and ethanol precipitation for amplification of various isolated genes as shown in Fig. 1 (Aziz *et al*., 2003; Doyle and Doyle, 1990; Badri and Sariah, 2009).

Table I: The concentration and antibacterial activity of antibiotics against *Staphylococcus aureus* and *Pseudomonas syringae.*

Sr.No	Name of antibiotics	Concentration of antibiotics used	*Pseudomonas syringae*		*Staphylococcus aureus*	
			MacConky agar medium	Nutrient agar medium	MacConky agar medium	Nutrient agar medium
1	Tobramycin	10µg	Resistant	Sensitive	Resistant	Resistant
2	Penicillin G	10µg	Resistant	Sensitive	Resistant	Resistant
3	Ciprofloxacin	5µg	Resistant	Sensitive	Resistant	Resistant
4	Streptomycin	10µg	Resistant	Resistant	Sensitive	Sensitive
5	Kanamycin	10µg	Resistant	Resistant	Sensitive	Resistant
6	Gentamicin	10µg	Resistant	Resistant	Sensitive	Sensitive
7	Tetracycline	10µg	Resistant	Resistant	Resistant	Resistant
8	Amoxicillin	25µg	Resistant	Resistant	Resistant	Resistant
9	Ampicillin	1mg/ml	Resistant	Sensitive	Resistant	Resistant

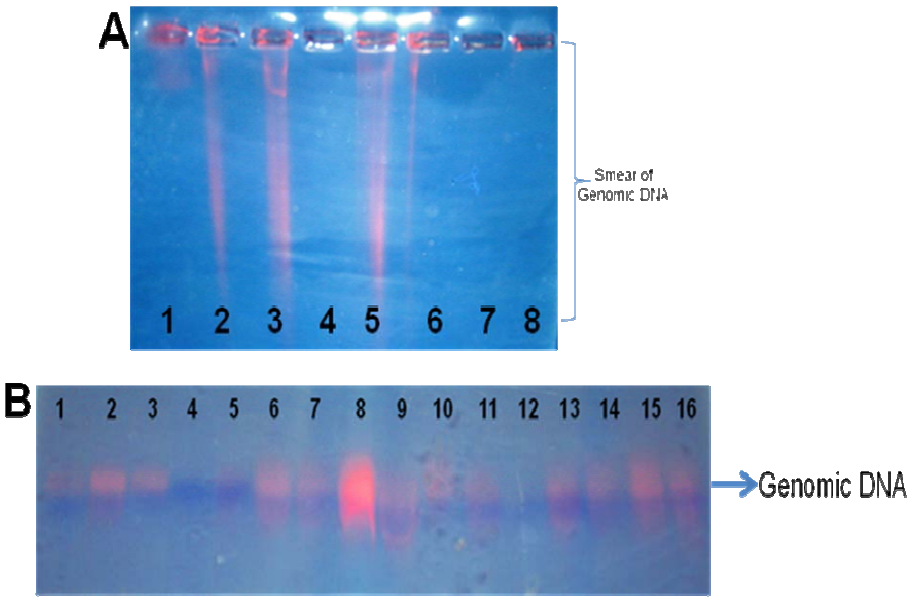

Fig. 4 (A) Genomic DNA extraction from *Pseudomonas syringae* (Lane 1-4) and *Staphylococcus aureus (Lane 5-8)* by using method I. (B) Genomic DNA extraction from *P. syringae* (1-4) and *S. aureus* (Lane 5-8) by using method II. Genomic DNA extraction from *P. syringae,* (Lane 9-12) and *S. aureus,* (Lane 13-16) by using method III.

Conclusions

We concluded that these antibiotics could be of considerable interest for the development of new drugs. The modified genomic DNA extraction protocols may be useful for sequence analysis and for finding the different genes which may play an important role in replication, act as a protein precursors and transporters.

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