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Short Communication

Antagonistic Activity of Bacterial Strains Isolated from Human Producing Biological Control Agents

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Abstract. In present research the antagonistic activity of human bacterial pathogens *viz*., *Streptococcus pyogenes, Staphylococcus epidermidis, Klebsiella pneumonia, Staphylococcus aureus, Serratia marcescens, Escherichia coli* and *Pseudomonas aeruginosa* were analysed. Significant amount of lactic acid production was shown by *S. pyogenes, S. epidermidis* and *P. aeruginosa* (63 mg/mL, 54 mg/mL, and 54 mg/mL), while *S. pyogenes* and *S. epidermidis* also produced significant quantity of hydrogen peroxide (1.70 mg/mL and 1.605 mg/mL). An impressive diversity of spots of chemical constituents was also obtained through thin layer chromatography (TLC) from broth culture of bacterial strains. The antagonistic activity may be indicated the potency of lactic acid and hydrogen peroxide production to inhibit the microbes.

Keywords: lactic acid, hydrogen peroxide, antagonistic activity, biological control agent

Microorganisms are not only the cause of infections; they can also produce organic substances that can cure infections (Jensen and Fencial, 2000) and various bioagents have been isolated from bacteria, fungus, and algae (Abdel-Fattah *et al*., 2011; Galal *et al*., 2011). Screening of bioactive compounds is based on some major factors like selection of a proper microorganism, isolation and culture methods and the detection and identification of their metabolites (Alwathnani and Perveen, 2012; Reddy *et al*., 2011).

Seven bacterial strains i.e., *Steptococcus pyogenes*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Staphylococcus aurues*, *Serratia marcescens*, *E. coli* and *P. aeruginosa* were isolated from different clinical samples of human. Most of the strains were catalase positive and indole negative (Awan *et al*., 2013). Antagonistic activity of human isolated pathogens was analysed through three agar disc diffusion systems such as phosphate buffer culture disc (PBCD) method, culture agar disc (CAD) method, and cell free supernatant disc (CFSD) method (Drummond and Waigh, 2000; Colle and Marr, 1989), zone of inhibition was measured in mm (Fig. 1).

Trivedi *et al*. (2008) and Trivedi and Sa (2008) demonstrated the antagonistic activity of *Pseudomonas* spp. against two phytopathogenic fungi, *Fusarium oxysporium* and *Alternaria alternata*. Similarly, in current research *P. aureginosa* results indicated that PBCD is much better method than rest of two methods. It has been analysed that *P. aureginosa* showed maximum activity against *S. pyogenes*, moderately high activity against *S. epidermidis* and moderately low against all other tested pathogens, respectively (Fig. 1A). Present results are consistent with Sindhu and Dadarwal (2001). The obtained results of TLC were found similar to that detected by Kumar *et al*. (2005) (Table 1).

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Table 1. Thin layer chromatography of microbes by using various solvent systems

Human microbes	UAW 1	UAW 2	UAW 3	UAW 4	UAW 5
Pseudomonas aeruginosa	S1= 0.211 S2= 0.923	S1= 0.961 S2= 0.980	no result	S1= 0.608	no result
Staphyloccocus epidermidis	S1= 0.063 S2= 0.80 S3= 0.87	no result	no result	S1= 0.225	no result
Staphylococcus aureus	S1= 0.319 S2= 0.382 S3= 0.531	no result	S1= 0.833	S1= 0.394	S1= 0.969
Klebsiella pneumonia	no result S2= 0.292 S3= 0.365	no result	S1= 0.121	no result	S1= 0.6
Escherichia coli	no result	S1= 0.971	S1= 0.0444	S1= 0.4	no result
Streptococcus pyogenes	no result S2= 0.680	S1= 0.319	no result	no result	no result
Serratia marcescens	S1= 0.088 S2= 0.888	S1= 0.782	no result	no result	S1= 0.875

Note: spots on TLC indicated by S1, S2, S3 and solvent systems were labeled as UAW 1; EtoAc: EthoH 9:1; UAW 2: EtoAc: MeoH 4:1; UAW3: EtoAc: Pet.ether 4:1; UAW 4: EtoAc: Pet.ether 1:4; UAW5: Hex: Acetone 7:3.

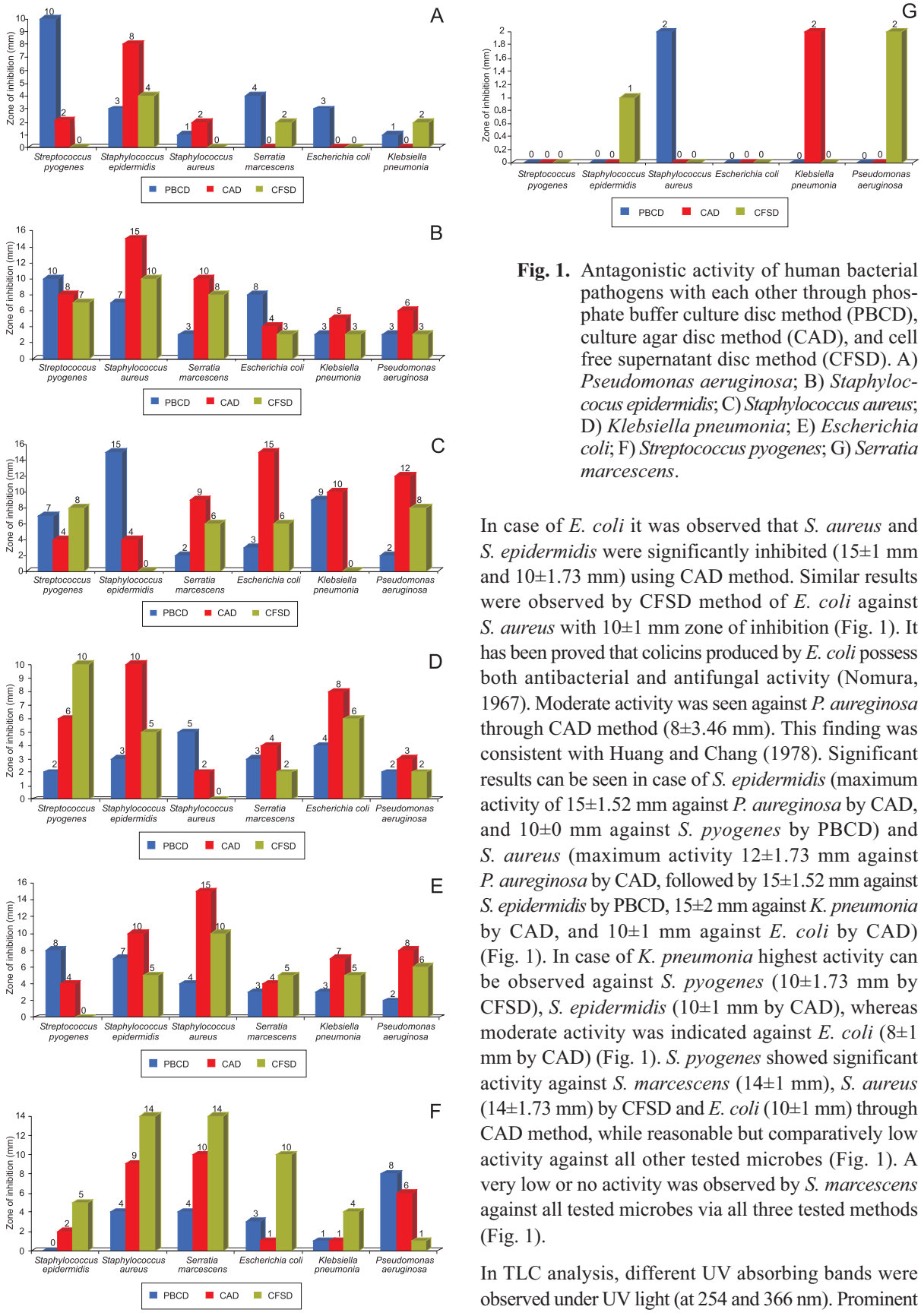

Fig. 1. Antagonistic activity of human bacterial pathogens with each other through phosphate buffer culture disc method (PBCD), culture agar disc method (CAD), and cell free supernatant disc method (CFSD). A) *Pseudomonas aeruginosa*; B) *Staphylococcus epidermidis*; C) *Staphylococcus aureus*; D) *Klebsiella pneumonia*; E) *Escherichia coli*; F) *Streptococcus pyogenes*; G) *Serratia marcescens*.

In case of *E. coli* it was observed that *S. aureus* and *S. epidermidis* were significantly inhibited (15±1 mm and 10±1.73 mm) using CAD method. Similar results were observed by CFSD method of *E. coli* against *S. aureus* with 10±1 mm zone of inhibition (Fig. 1). It has been proved that colicins produced by *E. coli* possess both antibacterial and antifungal activity (Nomura, 1967). Moderate activity was seen against *P. aureginosa* through CAD method (8±3.46 mm). This finding was consistent with Huang and Chang (1978). Significant results can be seen in case of *S. epidermidis* (maximum activity of 15±1.52 mm against *P. aureginosa* by CAD, and 10±0 mm against *S. pyogenes* by PBCD) and *S. aureus* (maximum activity 12±1.73 mm against *P. aureginosa* by CAD, followed by 15±1.52 mm against *S. epidermidis* by PBCD, 15±2 mm against *K. pneumonia* by CAD, and 10±1 mm against *E. coli* by CAD) (Fig. 1). In case of *K. pneumonia* highest activity can be observed against *S. pyogenes* (10±1.73 mm by CFSD), *S. epidermidis* (10±1 mm by CAD), whereas moderate activity was indicated against *E. coli* (8±1 mm by CAD) (Fig. 1). *S. pyogenes* showed significant activity against *S. marcescens* (14±1 mm), *S. aureus* (14±1.73 mm) by CFSD and *E. coli* (10±1 mm) through CAD method, while reasonable but comparatively low activity against all other tested microbes (Fig. 1). A very low or no activity was observed by *S. marcescens* against all tested microbes via all three tested methods (Fig. 1).

In TLC analysis, different UV absorbing bands were observed under UV light (at 254 and 366 nm). Prominent

coloured bands were observed by staining with anisaldehyde/H_2SO_4. The most promising diversity of coloured spots was seen in the crude extracts of three pathogens.

All the tested strains produced considerable amount of lactic acid and comparatively little amount of hydrogen peroxide (Table 2). This study revealed that the antagonistic activity perhaps indicated the potency of lactic acid and hydrogen peroxide production to inhibit the microbes.

Table 2. Estimation of biological control compounds production by microbes

Bacterial isolates	Production of control agents (mg/mL)	
	Lactic acid	Hydrogen peroxide
Pseudomonas aeruginosa	54	0.535
Staphyloccocus epidermidis	54	1.605
Staphylococcus aureus	9	0.535
Klebsiella pneumonia	7.2	1.070
Escherichia coli	1.8	1.070
Streptococcus pyogenes	63	1.70
Serratia marcescens	2.7	1.070

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