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EVALUATION OF EFFECT OF *BACILLUS SUBTILIS* ON SOME PATHOGENIC BACTERIA

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Abstract

The present study aimed to evaluation effect second metabolism of five *Bacillus subtilis* isolates (B.s1, B.s2, B.s 3, B.s4 and B.s 5) on five pathogenic bacteria (*Klebsiella pneumoniae, Escherichia coli*, *Pseudomonas aeruginosae, Staphylococcus epidermidis*, and *Staphylococcus aureus*,) the result showed all *B. subtilis* isolate (conc. 30, 60, and 90 mg/ml) have antibacterial activity, B.s.4 (conc. 90 mg/ml) record maximum anti-bacterial activity compare with all *B. subtilis* isolate and gentamycin10µg which caused highest Zone of inhibition (mm) against to *E. coli* 35.20 mm followed by *K. pneumoniae* 33.13 mm, *S. epidermidis* 30.10 mm, *S. aureus* 28.33mm and *P. aeruginosae* 28.13mm respectively and followed by B.s.2 which record no significantly different with antibiotic gentamicin were caused Zone of inhibition to *K. pneumoniae* 29.00 mm followed by *S. aureus* 25.66 mm, *S. epidermidis* 25.00 mm, *E. coli* 21.33 mm and *P. aeruginosa* 19.20 mm respectively.

Key words : Second metabolism, pathogenic bacteria, antibacterial activity, Zone of inhibition.

Introduction

Antibiotic resistance bacteria is a worldwide health concern (Bryce *et al.,* 2016) that showed negative effect in last year (Picão *et al.,* 2013) the indiscriminate use of antibiotics were led to development of many to anti-microbial resistant pathogens, Many pathogenic bacteria showed high resistant to several antibiotic which showed most emerging threats, the famous antibiotic resistance bacteria called ESKAPE which mean (*Enterococcus faecium*, *S. aureus*, *K. pneumonia*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species) (Santajit *et al.,* 2016) in addition An important example of antibiotic resistance bacteria *Escherichia coli* (Pormohammad *et al.,* 2019) and *Staphylococcus epidermidis* (Eladli *et al.,* 2019).

If like this happen continuously, will take place a disaster to human being, At present time, many of the antibacterial agents are used in food animal product for control of the diseases an usually used as growth promoter which enter in human food chain and leads to serious health problem in animals and human (Hao *et al.,* 2014).

Several of *Bacillus* species so importance which have potential to produce a wide different of metabolites which have antimicrobial activity and used in pharmaceutical industry and medicine to control of many humans bacterial diseases (Sansinenea, 2011) The secondary metabolites of many *Bacillus subtilis* strains shown antagonism ability to the several of pathogenic bacteria (Al-Saraireh *et al.,* 2015).

The most important antibiotic is Bacitracin, which produced by *Bacillus* species and considered the primarily active against of Gram-positive bacteria (Bisht, *et al.,* 2011) For instance of the other antibiotic produced by *Bacillus* species polymyxin, colistin, circulin (Katz and Demain, 1977) gramicidin, tyrocidine, Surfactin, mycobacillin and subtilin (Nakano and Zuber, 1990)are have highly active against many of microorganisms (Emmert and Handelsman, 1999).

The aim of this study was to evaluation of second metabolism producing by *Bacillus subtilis* strain on some pathogenic bacteria.

Materials and Methods

Antagonistic bacteria

The five isolation of *Bacillus subtilis* (B.s.1, B.s.2,

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B.s.3, B.s.4 and B.s.5) were obtained from Department of Plant protection, College of Agriculture, University of Basrah, Iraq.

Pathogenic Bacteria

Five Pathogenic Bacteria used in this study *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosae* were obtained from Al-Sadr Teaching Hospital, Basra, Iraq.

Extraction of *Bacillus subtilis* secondary metabolites

Each Bacterial isolate were grown in Erlenmeyer flasks 500-ml containing 250 ml nutrient broth and incubated at 28C (110 rpm/min on a shaker) for 4 days, each culture was centrifuged at 15,000 rev min^{-1} for 10 min in a refrigerated the supernatants which containing to the metabolites was filtered through a 0.22-›3 membrane filter, were mixed with solvents ethanol and chloroform separately (1:1), the separating funnel used to separate the solvent layer as crude extracts which stored in sterile vials.

Evaluation effect of antibacterial activity of *Bacillus subtilis* crude extracts

In this study, the Agar well diffusion method used to evaluation the antimicrobial activity of *Bacillus subtilis* isolates crude extracts, each pathogenic bacteria (*P. aeruginosae*, *S. aureus*, *K. pneumoniae*, *S. epidermidis*, and *E. coli*) were spread on plates continuing Muller Hinton agar (MHA) and then 100 µL from each concentration of crude extract (30, 60, 90, used dimethyl sulfoxide as a solvent) were placed to each well (each well 6 mm diameter were made by use sterile cork borer) Gentamycin 10 µg and DMSO used as a control, the antibacterial activities were recorded after an incubation at 37 °C for 24–48 h.

Statistical analysis

In the percent study, all the experiments were applied with three replications, and analyses of variance with comparison means, were tested by duncan Test ($P < 0.05$) used SPSS Statistics program (version 23).

Results

All *B. subtilis* isolate selected in current study showed different degree of antibacterial activity (Table 1) the result recorded minimum effect in Concentration 30 mg/ml for all the crude extract, followed by Concentration 60 mg/ml showed different antibacterial effect, while the maximum antibacterial activity were in conc. 90 mg/ml by B.s.4 which showed significantly different compare with all *B. subtilis* isolate and Gentamicin, caused Zone of inhibition (mm) against of *E. coli* (35.20 mm) followed by *K. pneumoniae* (33.13 mm), *S. epidermidis* (30.10 mm), *S. aureus* (28.33mm) and *P. aeruginosae* (28.13mm) respectively (Figure 1), followed by B.s.2 which showed no significantly different with Gentamicin were recorded Zone of inhibition of *K.*

Table 1: Antibacterial activity of different concentration of *B. subtilis* isolates crude extracts.

Types of bacteria	Zone of inhibition (mm)					standard
	Conc. 30 mg/ml of *B. subtilis* crude extract.					
	B.s.1	B.s.2	B.s.3	B.s.4	B.s.5	Gentamycin10 µg
S. aureus	0^{a}	14.00^{b}	0^{a}	17.16^{c}	0^{a}	20.00^{d}
S. epidermidis	11.16^{b}	11.36^{b}	0^{a}	15.33^{c}	0^{a}	23.10^{d}
E. coli	11.90^{a}	11.00^{a}	11.00^{a}	16.56^{b}	11.03^{a}	20.66^{c}
P. aeruginosa	0^{a}	14.23^{b}	0^{a}	17.33^{c}	12.66^{d}	19.16^{e}
K. pneumoniae	10.00^{b}	12.66^{c}	0^{a}	15.10^{d}	0^{a}	25.10^{e}
	Conc. 60 mg/ml of *B. subtilis* crude extract.					
S. aureus	11.16^{a}	19.00^{b}	10.66^{a}	21.10^{c}	11.40^{a}	20.00^{bc}
S. epidermidis	13.53^{ab}	14.73^{b}	11.00^{a}	18.33^{c}	10.36^{a}	23.10^{d}
E. coli	16.06^{b}	15.23^{ab}	13.13^{a}	23.10^{c}	15.00^{ab}	20.66^{c}
P. aeruginosa	0^{a}	18.70^{d}	12.66^{b}	21.16^{e}	15.66^{c}	19.16^{d}
K. pneumoniae	13.00^{a}	17.33^{b}	9.90^{c}	19.33^{b}	12.13^{a}	25.10^{c}
	Conc. 90 mg/ml of *B. subtilis* crude extract.					
S. aureus	14.33^{ab}	25.66^{d}	16.10^{b}	28.33^{e}	14.00^{a}	20.00^{c}
S. epidermidis	18.00^{b}	25.00^{c}	17.06^{ab}	30.10^{d}	14.66^{a}	23.10^{c}
E. coli	17.73^{a}	21.33^{b}	18.33^{a}	35.20^{c}	18.66^{a}	20.66^{b}
P. aeruginosa	10.66^{a}	19.20^{b}	18.01^{b}	28.13^{c}	18.30^{b}	19.16^{b}
K. pneumoniae	20.03^{b}	29.00^{c}	17.33^{a}	33.13^{d}	18.00^{a}	25.10^{e}

Each values are average of 3 replicates, in each horizontal row followed by same letter means not differ significantly, ($p<0.05$), analyzed by Duncan Test.

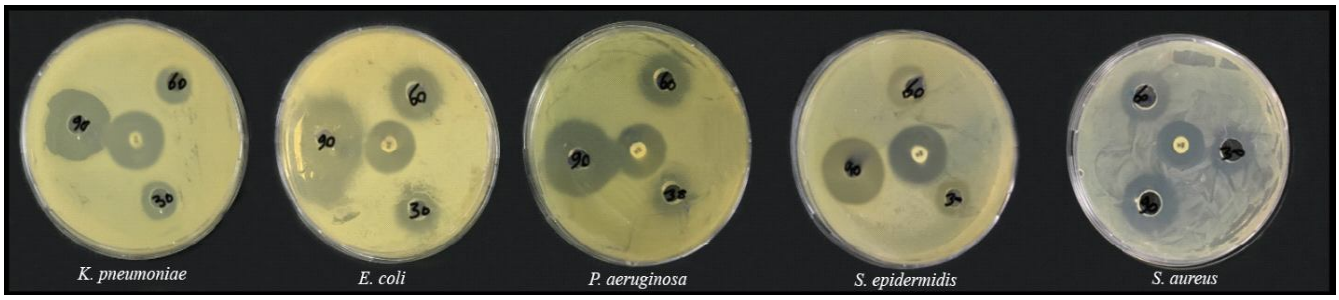

Fig. 1: Shown Zone of inhibition by of B.s.4. crude extracts (conc. 30, 60 and 90 mg/ml) against pathogenic bacteria.

pneumoniae (29.00 mm) followed by *S. aureus* (25.66 mm), *S. epidermidis* (25.00 mm), *E. coli* (21.33 mm) and *P. aeruginosa* (19.20 mm) respectively.

Discussion

The current research interest to evaluate the second metabolism of *Bacillus subtilis* isolates antagonism of pathogenic bacteria, the five *B. subtilis* isolates showed different ability of inhibition of pathogenic bacteria, and B.s.4 (conc. 90 mg/ml) showed the higher inhibition to the pathogenic bacteria, This confirms with Amin *et al.,* (2012) study which showed the high antimicrobial activity of *B. subtilis* isolates on some pathogenic bacteria, and agreement with Ramachandran *et al.,* (2014) his study showed the highest potential of *B. subtilis* applications in biocontrol of drug-resistant pathogens.

Many of Bacillus species produce several of secondary metabolites including polypeptides, lipopeptides, fatty acids, macrolactones, and polyketides, which have broad range of biological activities such as antimicrobial (Mondol *et al.,* 2013; Harwood *et al.,* 2018) bacitracin one of important antibiotic which produced by *Bacillus* sp. (Rukmini *et al.,* 2015), In addition the antibiotic Bacilysocin (Tamehiro, *et al.,* 2002), iturin, surfactin, bacillomycin D and fengycin produce by *Bacillus* sp. which have the ability to inhibition of pathogenic bactria (Xu *et al*., 2018).

Conclusion

B. subtilis produce second metabolism have antagonism ability to pathogenic bacteria, in last year, many pathogenic bacteria become resistant to antibiotic (Coates *et al.,* 2002) Therefore, this led to increasing the Looking for new antibacterial and this so important in pharmaceutical industry (Schmidt, 2004) this study result showed the second metabolism of the five *B. subtilis* isolates have different potential of antagonism of pathogenic bacteria, and B.s 4 isolate showed the maximum of inhibition of pathogenic bacteria compared with the other isolates in the different concentration.

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