EVALUATION OF EFFECT OF BACILLUS SUBTILIS ON SOME PATHOGENIC BACTERIA

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Abstract

The present study aimed to evaluate the effect of second metabolism of five Bacillus subtilis isolates (B.s1, B.s2, B.s3, B.s4 and B.s5) on five pathogenic bacteria (Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, 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) recorded the maximum antibacterial activity compared with all B. subtilis isolate and gentamicin 10 µg which caused the highest Zone of inhibition (mm) against E. coli 35.20 mm followed by K. pneumoniae 33.13 mm, S. epidermidis 30.10 mm, S. aureus 28.33 mm, and P. aeruginosa 28.13 mm respectively and followed by B.s.2 which recorded 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 antimicrobial resistant pathogens, Many pathogenic bacteria showed high resistant to several antibiotics which showed most emerging threats, the famous antibiotic resistance bacteria called ES KAPE 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, cirulin (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,
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 aeruginosa 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 28°C (110 rpm/min on a shaker) for 4 days, each culture was centrifuged at 15,000 rpm min⁻¹ for 10 min in a refrigerated supernatants which containing to the metabolites was filtered through a 0.22-mm 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.

<table>
<thead>
<tr>
<th>Types of bacteria</th>
<th>Zone of inhibition (mm)</th>
<th>standard Gentamycin10 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. 30 mg/ml of B. subtilis crude extract.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B.s.1</td>
<td>B.s.2</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0²</td>
<td>14.00b</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>11.16b</td>
<td>11.36b</td>
</tr>
<tr>
<td>E. coli</td>
<td>11.90a</td>
<td>11.00a</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0²</td>
<td>14.23b</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>10.00b</td>
<td>12.66c</td>
</tr>
<tr>
<td></td>
<td>Conc. 60 mg/ml of B. subtilis crude extract.</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>11.16a</td>
<td>19.00b</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>13.53ab</td>
<td>14.73b</td>
</tr>
<tr>
<td>E. coli</td>
<td>16.06b</td>
<td>15.23ab</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0²</td>
<td>18.70a</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>13.00a</td>
<td>17.33b</td>
</tr>
<tr>
<td></td>
<td>Conc. 90 mg/ml of B. subtilis crude extract.</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>14.33ab</td>
<td>25.66a</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>18.00b</td>
<td>25.00a</td>
</tr>
<tr>
<td>E. coli</td>
<td>17.73a</td>
<td>21.33b</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>10.66a</td>
<td>19.20b</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>20.03b</td>
<td>29.00a</td>
</tr>
</tbody>
</table>

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.
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 Bacilyoscin (Tamehiro, et al., 2002), iturin, surfactin, bacillomycin D and fengycin produce by Bacillus sp. which have the ability to inhibition of pathogenic bacteria (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.

**References**


