Isolation and Characterization of Antibiotic producing *Lysobacter*

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**ABSTRACT**

Lysobacter species, known for their cosmopolitan distribution across diverse habitats, are promising sources of antibiotics and bioactive compounds. They showcase lytic activity against a wide range of microorganisms including human pathogens. **Objective:** To isolate and characterize the antibiotic producing *Lysobacter* bacteria. **Methods:** A total of 51 rhizosphere soil samples were collected from district Sialkot. The duration of this study was 7 months from April to October 2022. Out of these samples 18 antibiotics producing *Lysobacter* bacteria were isolated. These isolates were characterized morphologically and biochemically by standard methods. Antibiotic activity of *Lysobacter* was evaluated against gram negative and positive pathogenic bacteria. Four pathogens i.e., *E. coli*, *S. aureus*, *S. typhi* and *P. vulgaris* were used in this study to evaluate antibiotic activity of *Lysobacter*. **Results:** The most sensitive pathogen towards antibiotics produced by *Lysobacter* isolates was *E. coli* while the *P. vulgaris* showed some resistance. All antibiotics producing *Lysobacter* isolates were gram negative and rod shaped. The colonies of isolates were circular, mucoid and color ranges from cream white to pale yellow. All strains were catalase and oxidase positive except S14 that was oxidase negative. **Conclusions:** The results of this study revealed that the antibiotics producing *Lysobacter* isolate are effective inhibitors for both gram negative and gram positive human pathogens.

**INTRODUCTION**

The most promising source of antibiotics in recent decades has been bacteria and bacteria will continue to be a significant source of novel bioactive natural compounds in the future [1]. *Streptomycetes, Bacillus, Cephalosporium,* and *Penicillium* are some of the significant bacteria that can produce antibiotics [2]. In the last decade, many *Lysobacter* species have been identified and mostly isolates from Asian soil [3]. *Lysobacter* species are cosmopolitan in distribution [4]. These species can be found in many diverse habitats such as soil and water habitats[5]. Studies describing the microbial communities in the agroecosystem have made it evident that *Lysobacter* species are frequent inhabitants of agricultural soils [6]. Some members have also been isolated from air and as well as from oil, human skin, and mural paintings [7]. Members of this genus have also been found in extreme environments. Such as *Lysobacter enzymogenes* can occupy hydrothermal vents [8]. Studies of the characterization of microorganisms communities dwelling in the agroecosystems have also indicated that *Lysobacter* species are common inhabitants of agricultural soils [9]. The colonies of *Lysobacter* are mostly mucoid and their color ranges from cream-colored pink, or yellow-brown [4]. Due to the gliding motility of the genus *Lysobacter* colonies are very slimy and may spread to the solid media and become very thin. Wrinkled colonies with a dry surface have also been observed in some strains of *Lysobacter* [10]. All members of the genus *Lysobacter* are gram negative rods [4]. A typical *Lysobacter* rod measures about 0.4-0.6×2-5µm [9]. However, many of the *Lysobacter* population's cells are extremely long and filamentous, with sizes that can be measured up to 70cm [10]. *Lysobacter* is aerobic and...
the optimum growth temperature for their growth is 28 °C [9]. Lysobacter is originally famous as the home of antibiotics that is genetically usable in bioengineering [11, 12]. This genus shows lytic activity against many microorganisms including gram positive and negative bacteria as well as fungi, oomycetes, nematodes and unicellular algae as they named so because of their lytic characteristics [3]. Lysobacter also produces cephabacins, phenazines and Lactivicin antibiotics [11-14]. Members of the genus Lysobacter also have great potential antibiotic compounds against human pathogens [15]. The main aim of this research was to isolate antibiotics producing Lysobacter bacteria from soil, to characterize the isolates and check their antibiotic activity against selective bacterial pathogens.

**METHODS**

**Sample Collection**

Soil samples were collected from the rhizosphere soil of various plants from agricultural fields of the Sialkot district of Punjab. Collected soil samples were sieved to remove roots or rotten leaves, stored in plastic seal bags, and transported to the lab within 24 hours. The soil was stored at -20°C and further used (Table 1).

**Isolation and Purification of Bacterial Isolates**

To isolate Lysobacter the serial dilutions of soil that were prepared in distilled water and plated on the Reasoner’s 2A agar. The plates were incubated at 28°C for three to seven days. The identified colonies were subsequently purified by streak plate method [16].

**Isolated Bacterial Strains with Antibiotics Production**

A total 42 bacterial isolates were obtained by serial dilutions of 51 soil samples collected from rhizosphere of eight plants. All the isolates were purified by repeated streaking on R2A agar. Out of these bacteria 18 were antibiotics producing Lysobacter identified using well diffusion assay against four pathogenic bacteria.

**Statistical Analysis**

Collected data of inhibition zones were analyzed statistically using SPSS version 23.0 (Statistical program for social sciences).

**RESULTS**

### Isolated Bacterial Strains with Antibiotics Production

A total 42 bacterial isolates were obtained by serial dilutions of 51 soil samples collected from rhizosphere of eight plants. All the isolates were purified by repeated streaking on R2A agar. Out of these bacteria 18 were antibiotics producing Lysobacter identified using well diffusion assay against four pathogenic bacteria.
24 hours incubation on R2A Agar. (Table 2)

**Biochemical Characterization of Lysobacter Isolates**

Characterization of the isolated bacteria was carried out by standard biochemical tests.

**Gram Staining**

The microscopy revealed that all Lysobacter strains were gram negative rods. The bacteria did not possess spores. (Table 2)

**Catalase Test**

All the strains that produce antibiotics were catalase positive as they form bubbles with 3% hydrogen peroxide. S20 and S37 produce bubbles in large amount immediately after colony touches the hydrogen peroxide showing the strongest activity of the catalase enzyme. All other strain also produces bubble with 10 to 15 seconds. (Table 2)

**Oxidase Test**

All antibiotics producing bacterial isolates were tested positive for Oxidase test except S14 as they turn the oxidase test strip purple when colony was placed on the surface of strip using clean inoculating loop. No color change was observed in case of S14. (Table 2)

**Table 2: Morphology of Antibiotics Producing Isolates on R2A Agar Medium**

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Colony Morphology</th>
<th>Cellular Morphology</th>
<th>Gram Stain</th>
<th>Catalase +/-</th>
<th>Oxidase +/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>S28A</td>
<td>Transparent yellow shiny</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S4</td>
<td>Pale yellow swarming</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S20</td>
<td>Pale yellow</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S46C</td>
<td>Yellow mucoid, circular</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S19</td>
<td>Pale yellow, mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S25</td>
<td>Cream mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S26</td>
<td>Yellow mucoid, circular</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S46B</td>
<td>Cream mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S29</td>
<td>Cream colored, shiny</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S39</td>
<td>Pale yellow</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S43</td>
<td>Honey yellow, mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S28</td>
<td>Off white cream, mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S37</td>
<td>Yellow mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S36</td>
<td>Cream colored, transparent</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S14</td>
<td>Pale yellow, circular with entire margins</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>S35</td>
<td>Cream colored, mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S40</td>
<td>Yellow cream colored</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>S51</td>
<td>Off white cream, mucoid</td>
<td>Rod shaped</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Figure 1: Zones of Inhibition by Lysobacter S40 against (a= P. Vulgaris, b= E. coli, c= S. Typhi, d= S. Aureus) after 24 hours incubation of on R2A agar medium at 28 ºC.

**Antibiotic Activity of Lysobacter Isolates against Escherichia coli**

The antibiotics produced by the isolated Lysobacter strains were efficient enough to inhibit the growth of E. coli and produce a zone of inhibition around wells. The minimum zone formed at 50µl inoculum concentration was 2mm by S25, S28, S37 and the maximum at this concentration was 7mm by S29. At 100µl Lysobacter inoculum concentration the minimum inhibition zone measured was 5.33mm by S26 and maximum was 10mm by S20. At 150µl inoculum concentration the minimum zone measured was 7mm by S25 and S26 and the maximum was 13.33mm by S43. The efficacy of antibiotics produced by S28A (p=0.014), S20 (p=0.03), S46C (p=0.013), S19 (p=0.009), S26 (p=0.048), S46B (p=0.049), S29 (p=0.013), S51 (p=0.049), S39 (p=0.008), S36 (p=0.034), S14 (p=0.013), S35 (p=0.023) and S40 (p=0.042) was significant against E. coli. The efficacy of antibiotics produced by S4 (p=0.07), S25 (p=0.082), S43 (p=0.092), S28 (p=0.096) and S37 (p=0.112) was insignificant against E. coli as shown in figure 2.
measured was 2mm by S20, S46C, S19, S46B, and S28 and the maximum was 6mm by S4 and S40. At 100µl of the minimum diameter of inhibition zone measured was 4.33mm by S28 and the maximum measured was 10mm by S40. At 150µl the minimum diameter of inhibition zone was 7mm by S20 and maximum 12.33mm by S36. The efficacy of antibiotics produced by S28A (p=0.038), S4 (p=0.005), S39 (p=0.036), S43 (p=0.026), S28 (p=0.047), S14 (p=0.024), S35 (p=0.015), S40 (p=0.028) and S51 (p=0.028) was significant. On contrary the efficacy of antibiotics produced by S20 (p=0.085), S46C (p=0.093), S19 (p=0.10), S25 (p=0.071), S26 (p=0.077), S46B (p=0.09), S29 (p=0.067) and S37 (p=0.125) was insignificant as shown in figure 4.

Antibiotic Activity of Lysobacter Isolates against Proteus vulgaris

The pathogenic P. vulgaris showed some resistance towards Lysobacter isolates as the zones of inhibition produced were diffused. At 50µl the lowest zone of inhibition was 0.5mm that was barely noticeable and maximum at this concentration was 6mm by S46B. The minimum zone of inhibition at 100µl was 5mm by S40 and the maximum was 9.66mm by S19. At 150µl concentration the minimum zone of inhibition measured was 6.33mm by S25 and the maximum was 11.33mm by S51. The efficacy of antibiotics produced by S28A (p=0.034), S46C (p=0.031), S25 (p=0.007), S46B (p=0.013), S28 (p=0.012), S37 (p=0.031), S36 (p=0.035) and S40 (p=0.049) against P. vulgaris was significant. On contrary the efficacy of antibiotics produced by S4 (p=0.09), S20 (p=0.212), S26 (p=0.18), S19 (p=0.113), S39 (p=0.17), S43 (p=0.168), S28 (p=0.17), S14 (p=0.184), S35 (p=0.154) and S51 (p=0.10) was insignificant as shown in figure 3.

Antibiotic Activity of Lysobacter Isolates against Staphylococcus aureus

The Lysobacter isolates produced antibiotics that were powerful enough to inhibit the pathogenic S. aureus. At 50µl the minimum diameter of zone of inhibition was 2mm by S35 and the maximum diameter measured was 9mm by S37. At 100µl the minimum diameter of zone of inhibition measured was 6mm by S29 and the maximum diameter measured was 9mm by S4. At 150µl the minimum diameter of zone of inhibition measured was 7mm by S29 and the maximum was 11.33mm by S40. The efficacy of antibiotics produced by S28A (p=0.045), S4 (p=0.43), S20 (0.031), S46C (p=0.017), S19 (p=0.034), S26 (p=0.054), S46B (p=0.05), S29 (p=0.038), S39 (p=0.007), S43 (p=0.015), S28 (p=0.031), S37 (p=0.012), S36 (p=0.044), S14 (p=0.049), S40 (p=0.059) and S51 (p=0.031) was significant. The efficacy of antibiotics produced by S25 (p=0.30) and S35 (p=0.120) was insignificant as shown in figure 5.
Discussion

Present study 51 different soil samples of the rhizosphere isolates showed maximum antibiotic activity against bacteria can be found in diverse habitats [22]. In the Meibomian gland dysfunction. This indicated that these isolated from Meibomian gland secretions of patient with plants. On contrary a novel strain of main inhabitants of rhizosphere soils associated with strains from the greenhouse cultivated with lettuce [21]. Weon et al., isolated two Lysobacter strains from the greenhouse cultivated with lettuce [21] supporting our findings that the Lysobacter isolates are the main inhabitants of rhizosphere soils associated with plants. On contrary a novel strain of Lysobacter was isolated from Meibomian gland secretions of patient with Meibomian gland dysfunction. This indicated that these bacteria can be found in diverse habitats [22]. In the present study 51 different soil samples of the rhizosphere of plants 42 bacterial isolates were purified and screened for antibiotics production against human pathogen bacteria. The study showed resemblance to a study by Liu et al., who isolated a Lysobacter strain named Lysobacter capsici from the rhizosphere of green pepper. This strain was able to produce antibiotics and it was screened against two bacteria that were Bacillus megaterium and Xanthomonas oryzae [23]. An isolated strain of Lysobacter enzymogenes was effective against fungal and oomycetes pathogens [24]. In another study on Lysobacter enzymogenes the enzymes and toxins produced were able to cause death and disintegration of several nematode pathogens i.e., Caenorhabditis elegans, Heterodera schachtii, Meloidogyne javanica [25]. So it can be concluded that the antibiotics producing Lysobacter isolates are not only effective against bacterial pathogens but as well as against fungal pathogens and nematodes so these isolates are effective biological control agents. Lysobacter strains isolated during a study were able to inhibit the growth of bacteria similar to our study. The diameter of the zone against S. aureus was 22 mm. The screening method used in this study was the disc diffusion method. The inhibition of the pathogen could be the result of the same kind of antibiotics production by Lysobacter [8]. On contrary in our research the maximum zone of inhibition against S. aureus was 11.33 mm evaluated through well diffusion assay. This difference in diameters of inhibition zone may be due to unlike incubation conditions for the bacteria. P. vulgaris was the only pathogen that showed resistance towards antibiotics produced by isolates. It can be called as semi-resistant as light zones appeared around wells. Although it was shown earlier by Ryazanova et al., that the enzyme produced by Lysobacter were able to lyse the cells of gram negative Proteus vulgaris. These enzymes were more efficient at inhibition of S. aureus and C. cerevisiae [26]. The culture growth media used in our study was R2A agar which was the optimum growth media for the Lysobacter strains and effective for the production of antibiotics produced by these isolates. Antibacterial activity of Lysobacter was evaluated against Xanthomonas campestris and Pectobacterium atrosepticum on different culture media. The pathogen X. campestris was inhibited by the Lysobacter but not the other bacterial pathogen. Results of the study revealed that the antibiotic activity of Lysobacter isolates is culture media dependent with R2A agar being the optimum media because on this media Lysobacter showed maximum inhibitory activity [17]. Lysobacter capsici isolated during a research inhibited the growth of both gram negative and gram positive bacteria except the pathogenic E. coli bacteria. The enzyme isolated from L. capsici did not show any inhibitory activity towards E. coli. Although results indicated that the inhibitory activity of L. capsici enzyme against pathogenic S. aureus was

Figure 6: Antibiotic activity of Lysobacter isolates against Staphylococcus aureus. X-axis represents bacterial isolates and Y-axis represents diameter of zones of inhibition produced by Lysobacter isolates against S. aureus at 50 µl, 100 µl and 150 µl.

Comparison of Antibiotic Activity of Isolates against Different Pathogens

All Lysobacter strains were able to inhibit the growth of bacterial pathogens by production of antibiotics. The maximum diameter of zones of inhibition by S4, S46C, S46B, S28A, S28, S26, S35 against S. aureus was 10.33 mm, 9.33 mm, 9.66 mm, 11 mm, 9 mm, 10 mm, 10.33 mm respectively at 150 µl inoculum concentration. These 7 Lysobacter isolates showed maximum antibiotic activity against S. aureus compared to other Lysobacter isolates. S43 (11.33 mm), S29 (10.33 mm), S37 (10.33 mm) and S40 (12.33 mm) showed maximum antibiotic activity against E. coli compared to other Lysobacter strains. Against S. typhi the maximum inhibition was shown by S25, S36, and S39, and diameter of zone of inhibition were 12 mm, 12.33 mm and 10.33 mm respectively. S14 (11.66 mm), S19 (10.66 mm), S20 (11 mm) and S51 (11.33 mm) Lysobacter isolates showed maximum antibiotic activity against P. vulgaris compared to other Lysobacter isolates.
prominent [27]. The reason of enzyme being ineffective toward E. coli may be due to resistance developed in pathogen with time or could be the incubation conditions for the test. Another research finding concluded that Lysobacter isolates were able to lyse both gram negative and positive bacteria including E. coli [28]. This may perhaps because of production of enzymes and antibiotics of same chemical composition that were produced by isolates in our study. The isolate S43 from our study was isolated from a soil sample collected from rhizosphere of tobacco. The morphological characterization of this strain was similar to Lysobacter helvus isolated from soil had similar colonies morphology with honey yellow color on R2A agar [29]. The colonies of S28A isolate in our study were shiny and transparent yellow in color when cultured on R2A agar after 24 hours. Similar colony morphology was observed in Lysobacter spongicola that was isolated from sea sponge specimen [30]. S25 isolated from rhizosphere of strawberry formed cream colored mucoid colonies. Lysobacter ginsengisoi has same morphology, because it also produced creamy mucoid colonies after incubation at 25-30 °C on R2A agar [31]. S14 from our research did not show any change in color when colony was placed on oxidase test strip. Similar to S14 strain, Lysobacter panacisoli isolated from soil and cultured on R2A agar producing bright yellow colored colonies was also negative for oxidase test [32]. Our results were contradictory to a research in which Lysobacter pocheonensis that was isolated from tobacco. The morphological characterization of this strain was similar colonies morphology with honey yellow color on R2A agar [31].

C O N C L U S I O N S

It can be concluded from our findings that soil is a rich source of antibiotics producing Lysobacter bacteria. These bacteria have antibacterial activity towards tested human pathogenic bacteria i.e., Salmonella typhi, Staphylococcus aureus, Escheria coli and Proteus vulgaris. All the isolated strains produced antibiotics efficiently on R2A agar media that is the optimum growth culture media for Lysobacter. These applications of antibiotics production may be helpful in controlling human as well as animal pathogens.

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The authors declare no conflict of interest.

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R E F E R E N C E S


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