DOI: https://doi.org/10.54393/pbmj.v7i03.1059

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

ISSN (P): 2709-2798, (E): 2709-278X

Volume 7, Issue 3 (March 2024)

Original Article

Isolation and Characterization of Antibiotic producing *Lysobacter*

Nimra Cheema[1] and Asma Waheed Qureshi[1*]

[1]Department of Zoology, Government College Women University, Sialkot, Pakistan

ARTICLE INFO

Keywords:

Lysobacter, Antibiotics, Rhizosphere, Reasoner's 2A Agar, Pathogens, Antibacterial

How to Cite:

Cheema, N., & Qureshi, A. W. (2024). Isolation and Characterization of Antibiotic producing Lysobacter: Isolation and Characterization of Lysobacter. Pakistan BioMedical Journal, 7(03). https://doi.org/10.54393/pbmj.v7i03.1059

***Corresponding Author:**

Asma Waheed Qureshi

Department of Zoology, Government College Women University, Sialkot, Pakistan

asma.qureshi@gcwus.edu.pk

Received Date: 7th March, 2024

Acceptance Date: 27th March, 2024

Published Date: 31st March, 2024

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]. *Streptomyces, 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

DOI: https://doi.org/10.54393/pbmj.v7i03.1059

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).

Table 1: Soil Samples Collected and Used For Isolation of *Lysobacter*

Plant Rhizosphere	Sample Number (s)	Sampling Month
Wheat (*Triticum aestivum*)	S1, S2, S3, S4, S5, S6,S7, S8, S9, S10, S11	April 2022
Clover (*Trifolium repens*)	S12, S13, S14, S15, S16, S17, S18, S19, S20, S21	April 2022
Strawberry (*Fragaria ananassa*)	S22, S23, S24, S25, S26	April 2022
Garlic (*Allium sativum*)	S27, S28, S51	April 2022
Aloe vera (*Aloe vera*)	S29, S30	April 2022
Tobacco (*Nicotiana tabacum*)	S31, S32, S33, S34, S35, S36,S37, S38, S39, S40, S41, S42, S43	May 2022
Corn (*Zea mays*)	S44, S45, S46, S47	June 2022
Chili (*Capsicum frutescens*)	S48, S49, S50	June 2022

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].

Evaluation of Antibiotic Producing *Lysobacter*

Lysobacter Inoculum preparation in Broth

Lysobacter cell suspension was prepared by the following method: Bacteria were cultured in test tubes having 5ml Nutrient Broth for two days at 28°C. After two days the test tubes were placed on a shaker for 15-20 minutes at 200rpm. After that 500µl of broth was transferred from the test tube to sterilized eppendorf. Suspensions were centrifuged at 8700 rpm for five minutes and the supernatant was discarded and the bacterial cells were aggregated as pallets in the bottom. After that 500µl of 0.9% NaCl was added to the pallet to make bacterial cells inoculum. The eppendrof having bacterial cells and 0.9% NaCl was then vortexed for 10-15 seconds to homogenize the mixture.

Screening of Antibiotic Activity

Antibacterial activity of *Lysobacter* was screened against four human pathogens i.e., *Staphylococcus aureus, E. coli, Salmonella typhi, Proteus vulgari.* To test the antibacterial activity of *Lysobacter* agar well diffusion method was used [17]. R2A agar Petri plates were prepared, and selective bacterial pathogens were inoculated on the plates, then plates were incubated for 2-3 days at 28°C. After that 8mm diameter wells were made in the plate using sterile micropipette tips. Then 50µl, 100µl, 150µl *Lysobacter* inoculum was inoculated in the wells on the same medium having test pathogens and plates were incubated for 3-7 days at 25-28°C. The zone of inhibition surrounding the colonies was measured using a measurement scale. Each strain was tested in three replicates.

Characterization of *Lysobacter* Isolates

The bacterial isolates which showed inhibition to pathogenic bacteria were then characterized by using biochemical tests.

Gram Staining

Gram Staining was used to identify the cellular morphology of antibiotics producing *Lysobacter* on soil medium under microscope. For the identification of bacteria standard Gram staining protocol was used [18]. The prepared slides were examined under a light microscope at 40X and 100X.

Catalase and Oxidase Test

The catalase test was used to determine whether bacteria contained the catalase enzyme. Oxidase test was used to check the presence of oxidase enzyme in bacteria [18].

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.

Morphology of Antibiotics Producing Isolates

The colonies of the isolates were smooth, circular, raised with entire margins. The colonies varied from cream off white to yellow in color with about 0.6-0.8mm in size after

DOI: https://doi.org/10.54393/pbmj.v7i03.1059

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

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

Antibiotic Activity of *Lysobacter* Isolates

All 18 isolates screened for antibiotics production against four bacterial pathogens i.e., *Staphylococcus aureus*, *E. coli*, *Salmonella typhi*, *Proteus vulgaris* using well diffusion assay showed prominent inhibitory affect against all these pathogens. Zones of inhibition against pathogenic bacteria were measured at three sizes of bacterial inoculum. *Proteus vulgaris* shows some resistance towards bacterial isolates as the zones were not as clear (Figure 1).

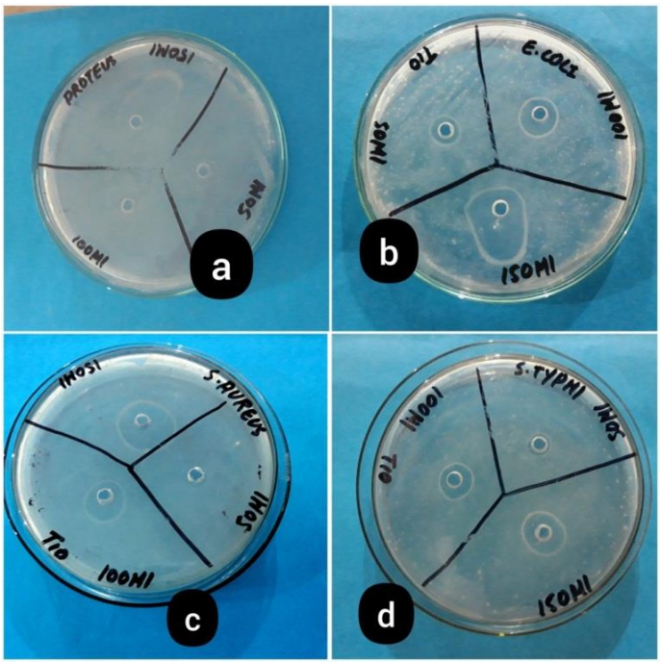

Figure 1: Zones of Inhibition by *Lysobacter* S40 against (a= *P. Vulgaris*, b=*E. coli*, c=*S. Typhi*, d=*S. Aureus*) after 24 hours Incubation of on R2A agar medium at 28°C.

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. colias* shown in figure 2.

DOI: https://doi.org/10.54393/pbmj.v7i03.1059

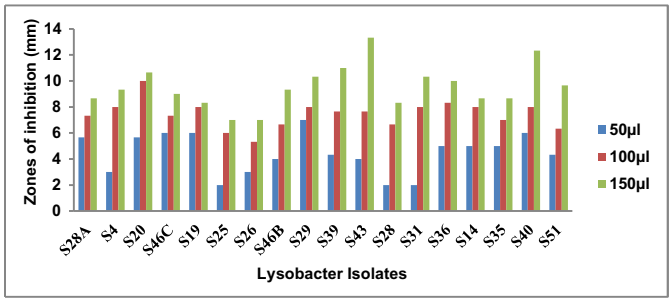

Figure 2: Antibiotic activity of *Lysobacter* strains against *E. coli*. X-axis represents bacterial isolates and Y-axis represents diameter of zones of inhibition produced by *Lysobacter* isolates against Escherichia coli at 50 µl, 100 µl and 150 µl.

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), S29 (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.

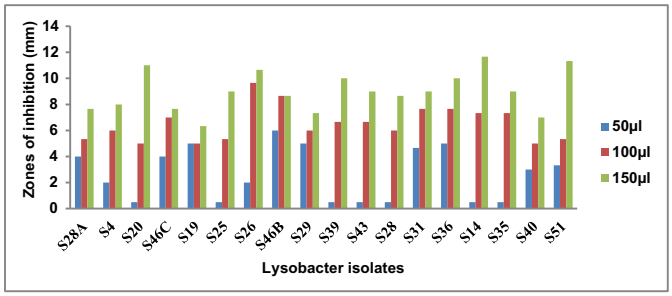

Figure 3: Antibiotic activity of *Lysobacter* isolates against *Proteus vulgaris*. X-axis represents bacterial isolates and Y-axis represents diameter of zones of inhibition produced by *Lysobacter* isolates against *P. vulgaris* at 50 µl, 100 µl and 150 µl

Antibiotic Activity of *Lysobacter* Isolates against *Salmonella typhi*

The antibiotics produced by *Lysobacter* isolates inhibit the pathogenic *S. typhi*. At 50µl inoculum concentration of *Lysobacter* the minimum diameter of inhibition zone 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=0.041), S36 (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.

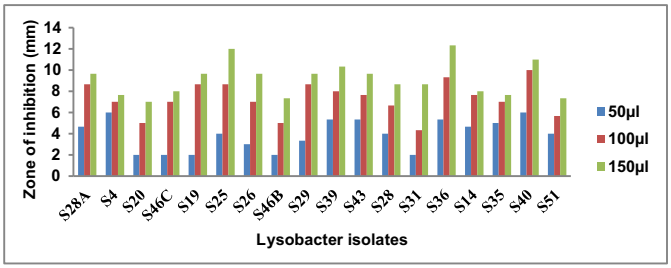

Figure 4: Antibiotic Activity of *Lysobacter* Isolates against *Salmonella typhi*. X-axis represents bacterial isolates and Y-axis represents diameter of zones of inhibition produced by *Lysobacter* isolates against *S. typhi* at 50 µl, 100 µl and 150 µl.

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.

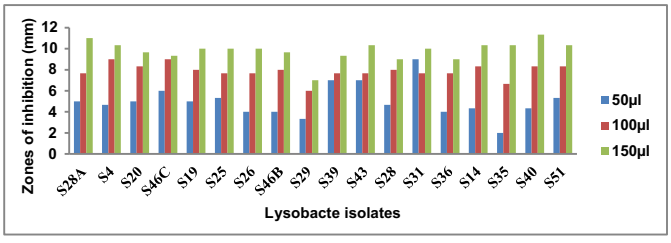

Figure 5: Antibiotic activity of *Lysobacter* isolates against *Staphylococcus aureus*. X-axis represents bacterial isolates and Y-axis represents diameter of zones of inhibition produced by *Lysobacter* isolates against *S. aureus* at 50 µl, 100 µl and 150 µl

DOI: https://doi.org/10.54393/pbmj.v7i03.1059

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 10.33mm, 9.33mm, 9.66mm, 11mm, 9mm, 10mm, 10.33mm respectively at 150µl inoculum concentration. These 7 *Lysobacter* isolates showed maximum antibiotic activity against S. aureus compared to other *Lysobacter* isolates. S43 (11.33mm), S29 (10.33mm) , S37(10.33mm) and S40(12.33mm) 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 12mm, 12.33mm and 10.33mm respectively. S14 (11.66mm), S19 (10.66mm), S20 (11mm) and S51 (11.33mm) *Lysobacter* isolates showed maximum antibiotic activity against *P. vulgaris* compared to other *Lysobacter* isolates.

DISCUSSION

Lysobacter genus is getting more attention in the biotechnological fields of the world because of its antibiotic production properties [19]. In our research we isolated 18 antibiotics producing *Lysobacter* isolates from the rhizosphere soil of plants. Out of these strains six were isolated from the rhizosphere of a tobacco plant. The isolation of *Lysobacter* from the tobacco rhizosphere indicates that this plant has a rich diversity of Lysobacter isolates. These findings agreed with to a study in which *Lysobacter tabacisoli* was isolated from the rhizosphere of the tobacco plant [20]. 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 11.33mm 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 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

DOI: https://doi.org/10.54393/pbmj.v7i03.1059

R2A agar [29]. The colonies of S28A isolate in our study were shinny and transparent yellow in color when cultured on R2A agar after 24 hours. Similar colony morphology was observed in *Lysobacter spongiicola* that was isolated from sea sponge specimen [30]. S25 isolated from rhizosphere of strawberry formed cream colored mucoid colonies. *Lysobacter ginsengisoil* has same morphology, because it also produced creamy mucoid colonies after incubation at 25-30 ºC 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 negative for both catalase and oxidase tests. The strain was isolated from ginseng field soil sample and produce light yellow colonies similar to most of our isolated strains [15].

CONCLUSIONS

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, Eschersia 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.

Authors Contribution

Conceptualization: GS
Methodology: NI
Formal analysis: SARN
Writing-review and editing: GS, JA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Elbendary AA, Hessain AM, El-Hariri MD, Seida AA, Moussa IM, Mubarak AS, *et al.* Isolation of antimicrobial producing Actinobacteria from soil samples. Saudi Journal of Biological Sciences. 2018 Jan; 25: 44-6. doi: 10.1016/j.sjbs.2017.05.003.

[2] Muleta A and Assefa F. Isolation and screening of antibiotic producing actinomycetes from rhizosphere and agricultural soils. African Journal of Biotechnology. 2018 May; 17: 700-15. doi: 10.5897/AJB 2017.16080.

[3] de Bruijn I, Cheng X, de Jager V, Expósito RG, Watrous J, Patel N, *et al.* Comparative genomics and metabolic profiling of the genus Lysobacter. BMC Genomics. 2015 Dec; 16: 1-6. doi: 10.1186/s12864-015-2191-z.

[4] Lee YS, Anees M, Hyun HN, Kim KY. Biocontrol potential of *Lysobacter* antibioticus HS124 against the root-knot nematode, Meloidogyne incognita, causing disease in tomato. Nematology. 2013 Jan; 15: 545-55. doi: 10.1163/15685411-00002700.

[5] Park JH, Kim R, Aslam Z, Jeon CO, Chung YR. *Lysobacter* capsici sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus Lysobacter. International Journal of Systematic and Evolutionary Microbiology. 2008 Feb; 58: 387-92. doi: 10.1099/ijs.0.65290-0.

[6] Weon HY, Kim BY, Kim MK, Yoo SH, Kwon SW, Go SJ *et al. Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea. International Journal of Systematic and Evolutionary Microbiology. 2007 Mar; 57(3): 548-51. doi: 10.1099/ijs.0.64473-0.

[7] Yan ZF, Trinh H, Moya G, Lin P, Li CT, Kook MC, Yi TH *et al. Lysobacter* rhizophilus sp. nov., isolated from rhizosphere soil of mugunghwa, the national flower of South Korea. International Journal of Systematic and Evolutionary Microbiology. 2016 Nov; 66: 4754-9. doi: 10.1099/ijsem.0.001422.

[8] Chen DM, Yang HJ, Huang JG, Yuan L. *Lysobacter enzymogenes* LE16 autolysates have potential as biocontrol agents—*Lysobacter* sp. autolysates as biofungicide. Journal of Applied Microbiology. 2020 Dec; 129: 1684-92. doi: 10.1111/jam.14752.

[9] Brescia F, Pertot I, Puopolo G. *Lysobacter*. InBeneficial Microbes in Agro-Ecology 2020 Jan (pp. 313-338). Academic Press. doi: 10.1016/B978-0-12-823414-3.00016-2.

[10] Dworkin M. The Prokaryotes: Vol. 6: Proteobacteria: Gamma Subclass. Springer Science & Business Media; 2006 Oct. doi: 10.1007/0-387-30746-X.

[11] Ling J, Zhu R, Laborda P, Jiang T, Jia Y, Zhao Y, Liu F *et al.* LbDSF, the *Lysobacter* brunescens quorum-sensing system diffusible signaling factor, regulates anti-xanthomonas XSAC biosynthesis, colony morphology, and surface motility. Frontiers in Microbiology. 2019 Jun; 10:454267. doi: 10.3389/fmicb.2019.01230.

DOI: https://doi.org/10.54393/pbmj.v7i03.1059

[12] Yu M and Zhao Y. Cell permeability, β-lactamase activity, and transport contribute to high level of resistance to ampicillin in Lysobacter enzymogenes. Applied Microbiology and Biotechnology. 2020 Feb; 104: 1149-61. doi: 10.1007/s00253-019-10266-7.

[13] Zhao Y, Qian G, Ye Y, Wright S, Chen H, Shen Y, Liu F, Du L *et al.* Heterocyclic aromatic N-oxidation in the biosynthesis of phenazine antibiotics from Lysobacter antibioticus. Organic Letters. 2016 May; 18: 2495-8. doi: 10.1021/acs.orglett.6b01089.

[14] Odhiambo BO, Xu G, Qian G, Liu F. Evidence of an unidentified extracellular heat-stable factor produced by Lysobacter enzymogenes (OH11) that degrade Fusarium graminearum PH1 hyphae. Current Microbiology. 2017 Apr; 74: 437-48. doi: 10.1007/s00284-017-1206-1.

[15] Siddiqi MZ and Im WT. *Lysobacter pocheonensis* sp. nov., isolated from soil of a ginseng field. Archives of Microbiology. 2016 Aug; 198: 551-7. doi: 10.1007/s00203-016-1214-8.

[16] Lee JC and Whang KS. *Lysobacter telluris* sp. nov., isolated from Korean rhizosphere soil. Archives of Microbiology. 2021 Jan; 203: 287-93. doi: 10.1007/s00203-020-02032-5.

[17] Gómez Expósito R, Postma J, Raaijmakers JM, De Bruijn I. Diversity and activity of *Lysobacter* species from disease suppressive soils. Frontiers in Microbiology. 2015 Nov; 6: 166241. doi: 10.3389/fmicb.2015.01243.

[18] Chauhan A, Jindal T, Chauhan A, Jindal T. Biochemical and molecular methods for bacterial identification. Microbiological Methods for Environment, Food and Pharmaceutical Analysis. 2020: 425-68. doi: 10.1007/978-3-030-52024-3_10.

[19] Pidot SJ, Coyne S, Kloss F, Hertweck C. Antibiotics from neglected bacterial sources. International Journal of Medical Microbiology. 2014 Jan; 304: 14-22. doi: 10.1016/j.ijmm.2013.08.011.

[20] Xiao M, Zhou XK, Chen X, Duan YQ, Alkhalifah DH, Im WT, *et al. Lysobacter tabacisoli* sp. nov., isolated from rhizosphere soil of Nicotiana tabacum L. International Journal of Systematic and Evolutionary Microbiology. 2019 Jul; 69: 1875-80. doi: 10.1099/ijsem.0.003164.

[21] Weon HY, Kim BY, Baek YK, Yoo SH, Kwon SW, Stackebrandt E, *et al.* Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils. International Journal of Systematic and Evolutionary Microbiology. 2006 May; 56: 947-51. doi: 10.1099/ijs.0.64095-0.

[22] Bai H, Lv H, Deng A, Jiang X, Li X, Wen T *et al. Lysobacter oculi* sp. nov., isolated from human Meibomian gland secretions. Antonie Van Leeuwenhoek. 2020 Jan; 113: 13-20. doi: 10.1007/s10482-019-01289-1.

[23] Liu Y, Qiao J, Liu Y, Liang X, Zhou Y, Liu J *et al.* Characterization of *Lysobacter capsici* strain NF87-2 and its biocontrol activities against phytopathogens. European Journal of Plant Pathology. 2019 Nov; 155: 859-69. doi: 10.1007/s10658-019-01817-9.

[24] Nian J, Yu M, Bradley CA, Zhao Y. *Lysobacter enzymogenes* strain C3 suppresses mycelium growth and spore germination of eight soybean fungal and oomycete pathogens and decreases disease incidences. Biological Control. 2021 Jan; 152: 104424. doi: 10.1016/j.biocontrol.2020.104424.

[25] Chen J, Moore WH, Yuen GY, Kobayashi D, Caswell-Chen EP. Influence of Lysobacter enzymogenes strain C3 on nematodes. Journal of Nematology. 2006 Jun; 38: 233.

[26] Ryazanova LP, Ledova LA, Tsurikova NV, Stepnaya OA, Sinitsyn AP, Kulaev IS *et al.* Effect of the proteolytic enzymes of Bacillus licheniformis and the lysoamidase of Lysobacter sp. XL1 on Proteus vulgaris and Proteus mirabilis cells. Applied Biochemistry and Microbiology. 2005 Sep; 41: 490-4. doi: 10.1007/s10438-005-0088-3.

[27] Afoshin AS, Konstantinov MA, Toropygin IY, Kudryakova IV, Vasilyeva NV. B-lytic protease of *Lysobacter capsici* VKM B-2533T. Antibiotics. 2020 Oct; 9: 744. doi: 10.3390/antibiotics9110744.

[28] Begunova EA, Stepnaya OA, Tsfasman IM, Kulaev IS. The effect of the extracellular bacteriolytic enzymes of *Lysobacter* sp. on gram-negative bacteria. Microbiology. 2004 May; 73: 267-70. doi: 10.1023/B:MICI.0000032235.06143.5e.

[29] Kim I, Choi J, Chhetri G, Seo T. *Lysobacter helvus* sp. nov. and *Lysobacter xanthus* sp. nov., isolated from Soil in South Korea. Antonie Van Leeuwenhoek. 2019

[30] Aug; 112: 1253-62. doi: 10.1007/s10482-019-01256-w.
Romanenko LA, Uchino M, Tanaka N, Frolova GM, Mikhailov VV. *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. International Journal of Systematic and Evolutionary Microbiology. 2008 Feb; 58: 370-4. doi: 10.1099/ijs.0.65391-0.

[31] Jung HM, Ten LN, Im WT, Yoo S, Lee ST. *Lysobacter ginsengisoli* sp. nov., a novel species isolated from soil in Pocheon Province, South Korea. Journal of Microbiology and Biotechnology. 2008; 18: 1496-9.

[32] Choi JH, Seok JH, Cha JH, Cha CJ. *Lysobacter panacisoli* sp. nov., isolated from ginseng soil. International Journal of Systematic and Evolutionary Microbiology. 2014 Jul; 64: 2193-7. doi: 10.1099/ijs.0.062034-0.