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Antibacterial Activity of Protease and Lipase Enzymes Obtained from *Proteus mirabilis*

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Abstract: Proteases and lipases are the major metabolism enzymes. They undergo a number of chemical reactions and are responsible for many aspects of bacterial cell life and pathogenicity. However, unlike the proven ability to target proteins and lipases in other aspects of drug detection, the clinic does not have any protein antibiotics or lipase inhibitors, except for the biosynthetic peptidoglycan and transpeptidase, which is β -lactic acid. In addition, the importance of proteases in bacterial etiology makes it possible to avoid selecting targets for new antiviral drug agents. Therefore, bacterial proteins and lipases have not been studied, and they are among the best pioneers deserving continued effort in the discovery of antibacterial drugs in the 21st century.

Keywords: Proteases, Lipases, *Proteus mirabilis*, *Pseudomonas aeruginosa*, Antibacterial

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Introduction

The manufacturing of lipase by microbial cells involves the presence of lipids, including olive oil or some other inducer, such as triacylglycerols, fatty acids and tweens in the growth media (Paetzel *et al.*, 2002; Akiyama *et al.*, 2015; Emiola *et al.*, 2015; Brown and Wright, 2016; Culp and Wright, 2017). Olive product is known as the most appropriate lipid substrate because it has the drawback of including high doses of oleic acid and is much more economical (Moayeri *et al.*, 2015). Some researchers have studied crude and purified

lipases. Various factors such as pH, atmospheric pressure, metallic ions, organic solvents, among many others, may speed up or slow down lipase activity (Hedstrom, 2002; Powers *et al.*, 2002; Brik and Wong, 2003; Zambelloni *et al.*, 2015; Hauser *et al.*, 2016; Huber *et al.*, 2016).

Hence, the present investigation was designed to ascertain the optimization and growth condition of bacterial strains to enhance the production of extracellular protease and lipase enzymes. The primary aim of this study was to

evaluate protease and lipase produced by bacterial strains from samples collected and to determine the optimum enzyme activity by the selected potent bacterial isolates. There are many reports on the concomitant production of protease and lipase by *Pseudomonas* species and *Bacillus* species but there are no reports on *Proteus mirabilis* which can produce both enzymes simultaneously.

Materials and Methods

The bacterial protease and lipase enzyme obtained from *Proteus mirabilis* was used for antibacterial activity (Leung *et al.*, 2011). Three different concentrations of enzyme solution (10 μ l, 20 μ l, 30 μ l/disc) were evaluated for antimicrobial properties toward pathogenic bacterial species such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Bacterial cultures have been raised in Muller Hinton Agar and Muller Hinton broth. The content was observed using the standard approach of disseminating disc plates on agar. Then 0.1 ml of each bacterial culture was scattered to agar plate floors for antimicrobial test. All bacterial cultures were grown in Muller Hinton Broth Medium for 24 h at 37 C and loaded in Muller Hinton Agar for agar well diffusion experiments. HiMedia sterile discs (6 mm in diameter) were placed on the agar medium in different concentrations (10 μ l, 20 μ l, 30 μ l/disc) of partially purified protease and lipase enzyme (*Proteus mirabilis*) were tested separately. The Inhibition diameters were measured in millimeters (mm) on all plates. All experiments were repeated three times. Gentamicin (10 μ g/disc) was used as positive control incubated for 24 h at 37 C.

Results and Discussion

Antibacterial activity of extra-cellular protease enzyme isolated from *Proteus mirabilis* was assessed and the data are shown in Table 1. It is interesting to note that the 10 μ l concentration did not showed any inhibitory activity, whereas, 20 μ l

concentration of extra-cellular protease enzyme inhibited the growth of bacterial species tested in the present investigation. 23.67 mm zone of inhibition was observed against the *B. subtilis*, 22.00 mm zone of inhibition was observed against *S. aureus* and 14.00 mm of inhibition was noted against *Bacillus cereus* at 20 μ l concentrations. The same concentration did not show any antibacterial activity against *E. coli* and *P. aeruginosa* (Table 1). Further, the 30 μ l concentration of extra-cellular protease enzyme activity showed highest inhibition zone (28.00 mm) against *B. subtilis*; 25.67 mm inhibition zone against *S. aureus*; 16.00 mm inhibition zone against *B. cereus* and inhibition of the development of *E. coli* and *P. aeruginosa* to a lesser extent i.e., 12.00 and 11.33, respectively (Table 1; Figs. 1A-C).

Fig. 1: (A) Zone of inhibition observed with the protease enzymes against from *Bacillus subtilis*, (B) Zone of inhibition observed with the protease enzymes against from *Bacillus cereus*, (C) Zone of inhibition observed with the protease enzymes against from *Staphylococcus aureus*, (D) Zone of inhibition observed with the lipase enzymes against from *Bacillus subtilis*, and (E) Zone of inhibition observed with the lipase enzymes against from *Staphylococcus aureus*.

Antimicrobial properties of the partially purified enzyme lipase:

Antibacterial activity of extra-cellular lipase enzyme isolated from *P. mirabilis* was analyzed and the data are shown in Table 2. It is remarkable to note that the 10 μ l of lipase enzyme against *B. cereus* showed 10.33mm the inhibitory activity, whereas, 20 μ l concentration of extra-

Table 1: Antibacterial properties of the slightly purified enzyme protease

Partially purified Protease enzyme	Control 10 µl	10 µl	20 µl	30 µl	F value	P value
<i>Bacillus subtilis</i>	26.67±0.88 ^c	0.00±0.0	23.67±0.88 ^b	28.0±0.58 ^c	367.902	<0.001**
<i>Escherichia coli</i>	28.00±0.58 ^c	0.00±0.00	0.00±0.00	12.00±0.58 ^b	1056.00	<0.001**
<i>Staphylococcus aureus</i>	24.00±0.58 ^c	0.00±0.0	22.00±0.58 ^b	25.67±0.67 ^c	521.700	<0.001**
<i>Bacillus cereus</i>	17.33±0.67 ^c	0.00±0.0	14.00±0.58 ^b	16.00±0.58 ^c	230.800	<0.001**
<i>Pseudomonas aeruginosa</i>	24.67±0.88 ^c	0.00±0.0	0.00±0.00 ^a	11.33±0.88 ^b	353.905	<0.001**

Values represent mean ± S.E of three replicates. Values with different alphabet in the column differs statistically, i.e., significant at $p < 0.005\%$

Table 2: Antibacterial activity of partially purified lipase enzyme

Partially purified lipase enzyme	Control 10µl	10 µl	20 µl	30 µl	F value	P value
<i>Bacillus subtilis</i>	26.67±0.88 ^c	0.00±0.0	22.33±0.33 ^b	25.33±0.88 ^c	376.244	<0.001**
<i>Escherichia coli</i>	26.00±1.15 ^c	0.00±0.00	0.00±0.00	10.67±0.33 ^b	418.769	<0.001**
<i>Staphylococcus aureus</i>	25.67±1.20 ^c	0.00±0.00	22.00±0.58 ^b	25.00±0.58 ^c	282.737	<0.001**
<i>Bacillus cereus</i>	17.00±0.58 ^b	10.33±0.33 ^{ab}	8.67±4.37 ^a	15.00±0.58 ^{ab}	3.060	0.092
<i>Pseudomonas aeruginosa</i>	24.33±0.88 ^c	0.00±0.0	0.00±0.0	12.00±0.58 ^b	487.300	<0.001**

Values represent mean ± S.E of three replicates. Values with different alphabet in the column differs statistically, i.e., significant at $p < 0.005\%$

cellular lipase enzyme inhibited the growth of bacterial species tested in the present investigation. 22.33mm zone of inhibition was observed against the *B. subtilis*, besides, 22.00mm zone of inhibition was observed against *S. aureus* and 8.67mm of inhibition was noted against *B. cereus* at 20 µl concentration. The same concentration did not show any antibacterial activity against *E. coli* and *P. aeruginosa* (Table 2). Further, the 30 µl concentration of extra-cellular lipase enzyme activity showed highest zone of inhibition (25.33 mm) towards *B. subtilis*; 25.00 mm diameter of inhibition zone towards *S. aureus*; 15.00 mm zone of inhibition against *B. cereus* and the growth of *P. aeruginosa* and *E. coli* were inhibited to a lesser zone of inhibition i.e., 12.00 mm and 10.67 mm, respectively (Table 2; Figs. 1D, E).

Conclusion

The isolated lipase and protease enzymes have been evaluated for antibacterial activities. Perusal

of the data clearly indicates that there was a remarkable antibacterial activity. Earlier to this study there exists no report to support the present findings. Thus, the present investigation is first report regarding the antibacterial activity of lipase and protease enzymes.

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