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RESEARCH ARTICLE

"A snapshot of amylolytic and antibacterial activity of B*acillus sp.* MTZ-1 from indigenous soils".

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Abstract

Soil is a rich habitat of microbes which possess the ability to produce hydrolytic enzymes and antimicrobial compounds. Among soil microorganisms, genus *Bacillus* is well known for production of industrial enzymes, antibiotics and pesticides. This research was aimed to explore amylolytic and antibacterial activity of *Bacillus sp.* MTZ-1 from rhizosphere of a Guava plant *(Psidium guajava)*. Amylase production was carried out through Shake flask fermentation experiments. Growth and enzyme activities of the strain were observed from 48 to 192 h by spectrophotometric analysis. Crude enzyme preparations also showed antibacterial potential at different time intervals against pathogenic bacterial strains including Vancomycin-resistant *Enterococcus* (VRE), *K. pneumoniae*, *S. aureus*, *S. paratyphi* A, *S. paratyphi* B, *S. flexneri*, *B. subtilis* and *P. mirabilis*. Amylase production was maximum at 168 h (134 U/ml) which was further declined at 192 h (76 U/ml) with increased growth. *Bacillus sp.* MTZ-1 showed strong amylolytic and antibacterial activity, however, both activities remained independent of each other. Antibacterial potential was maximum against *S. paratyphi* A and VRE (Vancomycin-resistant *Enterococcus*). Zone of inhibition was maximum (35 mm) against *S.* p*aratyphi* A at 48 h with no enzyme activity, however, it was 25 mm against VRE with maximum enzyme activity at 168-192 h. Antibacterial activity was found to be independent of the enzyme activity throughout the experiments.

INTRODUCTION

Amylases are the biological catalysts which carry out starch hydrolysis into glucose containing sugar derivatives such as dextrins and other small saccharides (Windish and Mhatre 2012).

Though amylases can be obtained through many biological sources, usually microbial enzymes are preferred industrially. Till date, variety of amylases are being used on commercial scale which have taken place of chemicals for starch hydrolysis in respective industries (Pandey et al., 2000). Microbes play a vital role in enzyme production on industrial scale. In 2010, industrial enzyme market throughout the world was at 52 billion with a chance of mean increase up to 3.3% in production rate per year. These amylases encounter tremendous role in biotechnological implications of industries like paper, food, medicine, ebullition and textile etc (Kunamneni et al., 2005). Members of genus *Bacillus* secrete several enzymes among which proteases and amylases have a key role in industries. *Bacillus sp.* of mesophilic nature are known to produce extremely alkaline and thermostable α-amylases (Saxena et al., 2007). Commercially, *B. stearothermophylus* is considered as a substituent for the yield of thermotolerant α-amylases. *Bacillus spp.*, *B. halodurans* and *B. licheniformis* has been reported to produce thermally firm and alkaline α-amylases (Setyorini et al., 2006). A recent study by Pomita (2013) described the influence of different modes of fermentation by shake flask fermentation process and observed the highest enzyme activity, favorable substrate and

nitrogen sources. Synonymous studies have been carried out further by submerged and shake flask fermentation of amylases (Sujata, 2010; Vijayalakshmi et al., 2012; Arifa and Sabita, 2011; Baby and Sissy, 2013).

Along with the production of hydrolytic enzymes, Bacillus species are potent producers of bioactive compounds during later phase of their exponential growth or at the onset of stationary period in batch fermentation (Katz and Demain 1977). Bacillus species have oppugnant potential for various disease causing microorganisms and are usually used as preventive or curing entity for various diseases of animals and plants. Their bioactive potential is accredited by antimicrobial pepetide by-products like bacteriocins, bacteriocin analogues and lipopeptides which possess potent surface-active agent properties (Abriouel et al., 2011). Investigations on antimicrobial activity of crude enzyme preparations of Bacillus species from different origins have been performed (Tooba et al., 2014; Jadhav et al., 2013; Tanja et al., 2012). In the present research, amylase producing *Bacillus sp.* MTZ-1 was investigated for the production of amylase and the antibacterial activity of crude enzyme was studied against a number of pathogenic strains.

2. Materials and methods

2.1 *Screening for amylase production*

A previously isolated *Bacillus sp.* MTZ-1 from rhizosphere area of guava plant (*Psidium guajava*) was screened for amylase production through Iodine test by applying 1% iodine solution over colonies on starch agar plates. The strain showed zone of starch hydrolysis and hence selected for further investigations.

2.2 *Amylase production*

A 50 ml of growth medium (Bhaskara et al., 2011) containing (g/ml): starch (10); peptone (10); yeast extract (20); KH_2PO_4 (0.05); $MnCl_2.4H_2O$ (0.015); $MgSO_4.7H_2O$ (0.25); $CaCl_2.2H_2O$ (0.05); $FeSO_4.7H_2O$ (0.01), was prepared and inoculated with a loop full of selected bacterial strain *Bacillus sp.* MTZ-1. The inoculum was developed for 24 h at 37°C in shaker incubator. After 24 h of incubation, aseptic transfer of 15 ml growth medium was done to 150 ml production medium with the same composition. Production of amylase was carried out by shake flask fermentation process**.** Controls of production medium was also prepared i.e. without inoculation of bacterial culture for the analysis and comparison of bacterial growth status in test sample (production medium inoculated with bacterial culture). Samples were collected from 48 to 192 h at regular intervals of 24 h and centrifuged at 2000 rpm for 20 minutes. The samples were placed at 4 $^{\circ}$C into refrigerator for 5 minutes and supernatant of the filtrate was used as source of crude enzyme.

2.3 *Estimation of growth*

Growth of the strain in fermentation medium from 48 h to 192 h was measured by optical densities against uninoculated medium (blanks) at 660 nm through UV-visible spectrophotometer.

2.4 *Estimation of enzyme activity*

Enzyme activity from 48 h to 192 h was observed by performing amylase assay.

Enzyme Assay: Presence of amylase was determined by modified **Fischer and Stein Spectrophotometric method** (Fisher and Stein 1961). Crude enzyme preparations (cell free culture supernatant), Tris buffer (pH 8), soluble starch solution 1% (0.5ml) and 1 ml DNS (3,5-Dinitrosalicylic acid) was added in sample tubes while DNS was added at first step in blanks. All tubes of control and test samples were placed in water bath at 35°C for 1h. After the addition of 1ml DNS in the test samples all the tubes were left for 5 minutes in boiling water bath followed by cooling at room temperature. All tubes were then centrifuged for removal of palette from the bottom at 2000 rpm for 10 minutes. Enzyme activity was detected at 540 nm by spectrophotometer. One enzyme unit was defined as the quantity of enzyme required to release 1µmol of maltose per ml per minute (U/ml).

2.5 *Comparative analysis of antibacterial activity and enzyme activity of crude enzyme preparations*

Antibacterial activity of crude enzyme preparations samples of 48,72 and 94 h was observed against a number of pathogens i.e. Vancomycin-resistant *Enterococci, S. paratyphi* A, *S. paratyphi* B, *S. aureus, S. flexneri, K. pneumoniae, P. mirabilis an*d *B. subtilis* through **Well diffusion method** which was compared with the enzyme activity.

3. Results and discussion

3.1 ***Screening for amylase production***

As a result of screening for amylase production, the strain *Bacillus sp.* MTZ-1 showed maximum zone of hydrolysis and was selected for further study Figure 1.

3.2 ***Estimation of growth***

There was a gradual increase in growth observed from 96 to 192 h. Growth rate was directly proportional to the incubation period. Maximum growth was recorded at 192 h while minimum at 48 h. As shown in Figure 2., it was noticed that amylase production was not associated with the growth of the strain *Bacillus sp.* MTZ-1. On the contrary, studies (Natasa et al., 2011; Rani et al., 2003; Hamer, 1995) showed direct relationship between enzyme activity and growth of the organism.

3.3 ***Estimation of enzyme activity***

Enzyme activity was found to be maximum at 48 h (250 U/ml), which is in accordance with the previous findings (Pomita et al., 2013; Prabhakaran and Hewitt, 2009; Vijayalakshmi et al., 2012). While at 72 h no enzyme activity was detected which might be due to the production of antimicrobials and other metabolites which suppressed the enzyme production as shown in Figure 3. There was a gradual increase in enzyme activity from 96 to 168 h followed by a sudden decrease at 192 h Figure 2. The reason for this dwindling in enzyme activity might be the inception of decline phase of cells.

3.4 ***Comparative analysis of antibacterial activity and enzyme activity of crude enzyme preparations***

Antibacterial activity of crude enzyme preparations was observed in exponential and stationary phase of growth and bioactive potential of crude enzyme was observed against many pathogenic bacteria such as Vancomycin-Resistant *Enterococci* (VRE), *S. paratyphi A*, *S. paratyphi B*, *S. aureus*, *S. flexneri*, *K. pneumoniae*, *P. mirabilis* and *B. subtilis.* Zones of growth inhibition against all mentioned pathogenic bacterial strains have been shown in Figure 5. Maximum zones of growth inhibition were recorded against *S.paratyphi A* and Vancomycin-Resistant *Enterococci* (VRE) with zone sizes of 35 and 23 mm as shown in Figure 6 (A) and (B), respectively. Studies suggest that antibiotic production at the beginning of spore formation in Bacillus species is assumed to be responsible for the transformation of vegetative phase into sporulation phase (Katz and Demain 1977). Evidence in the favor of this study is the dependence of *tyc* A gene (Tyrocidin synthesizing genes) transcription in *B.subtilis* on the by-products of stage 0 genes which control sporulation (Marahiel et al., 1989).

Comparison of enzyme activity and antibacterial activity has been shown in Figure 3 and Figure 4. Antibacterial activity against *K. pneumoniae* was maximum at 72 h with zone of inhibition of 18 mm when there was no enzyme activity, while minimum at 96 h with low enzyme activity. Similarly, antibacterial activity against Vancomycin-Resistant *Enterococci* (VRE) gradually increased from 48 to 96 h. Contrary to this, enzyme activity was found maximum at 48 h and minimum at 72 h which showed that antibacterial potential was independent of the amylase production Figure 4.

3.5 ***Conclusion***

The current study showed that *Bacillus sp.* MTZ-1 possess amylolytic as well as antibacterial activity against many pathogenic bacterial strains. Studies for the optimization of amylase production need to be carried out along with the purification of amylase. Furthermore, identification and purification of antimicrobial compounds produced by the strain can be simulated because of their natural origin and less side effects as compared to synthetic drugs.

Figure 1. Zone of hydrolysis around the streak of *Bacillus sp.* MTZ-1

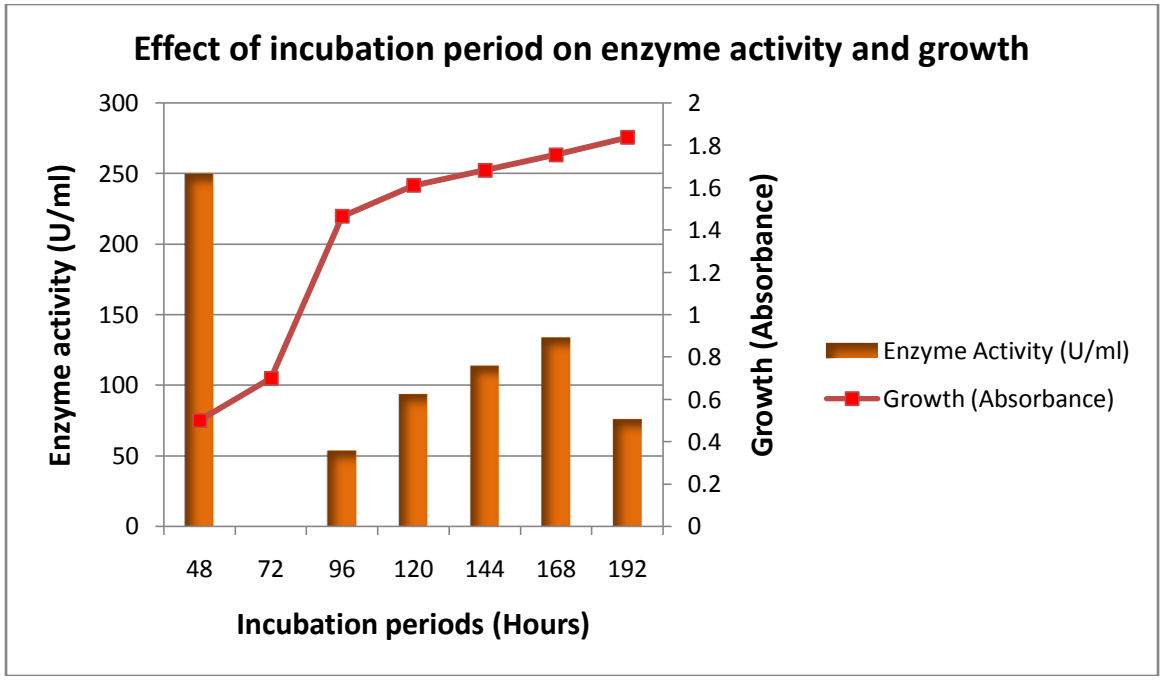


Figure 2. Effect of incubation period on enzyme activity and growth.

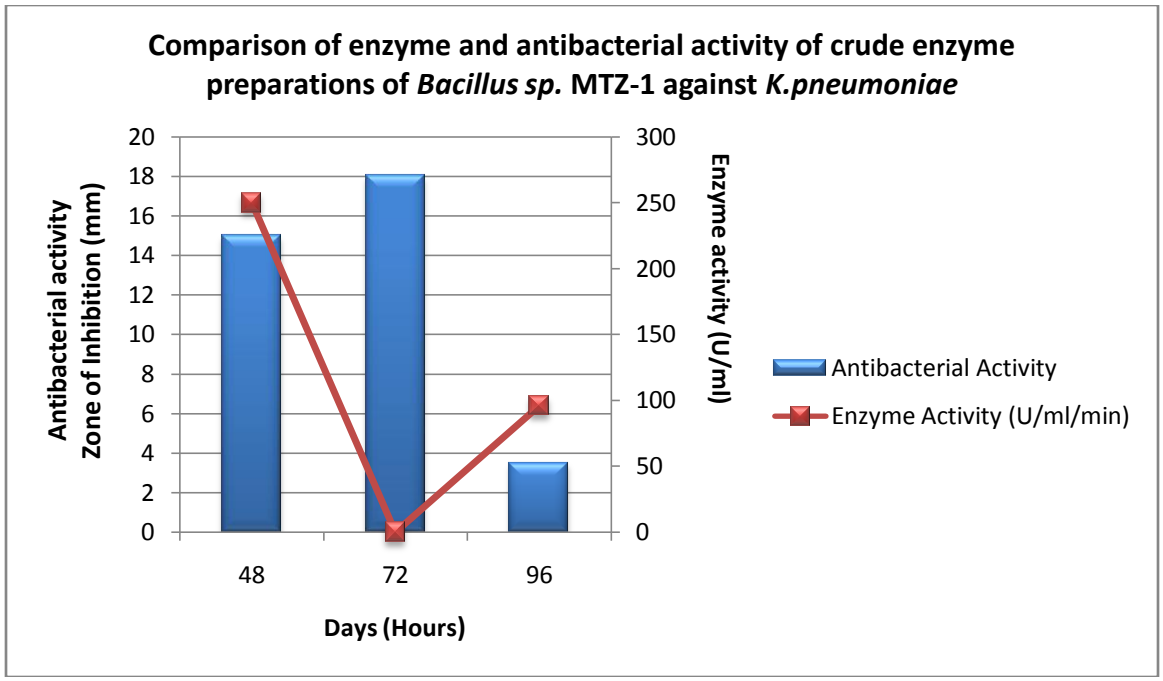


Figure 3. Comparison of enzyme and antibacterial activity of crude enzyme preparations preparations of *Bacillus sp.* MTZ-1 against *K.pneumoniae.*

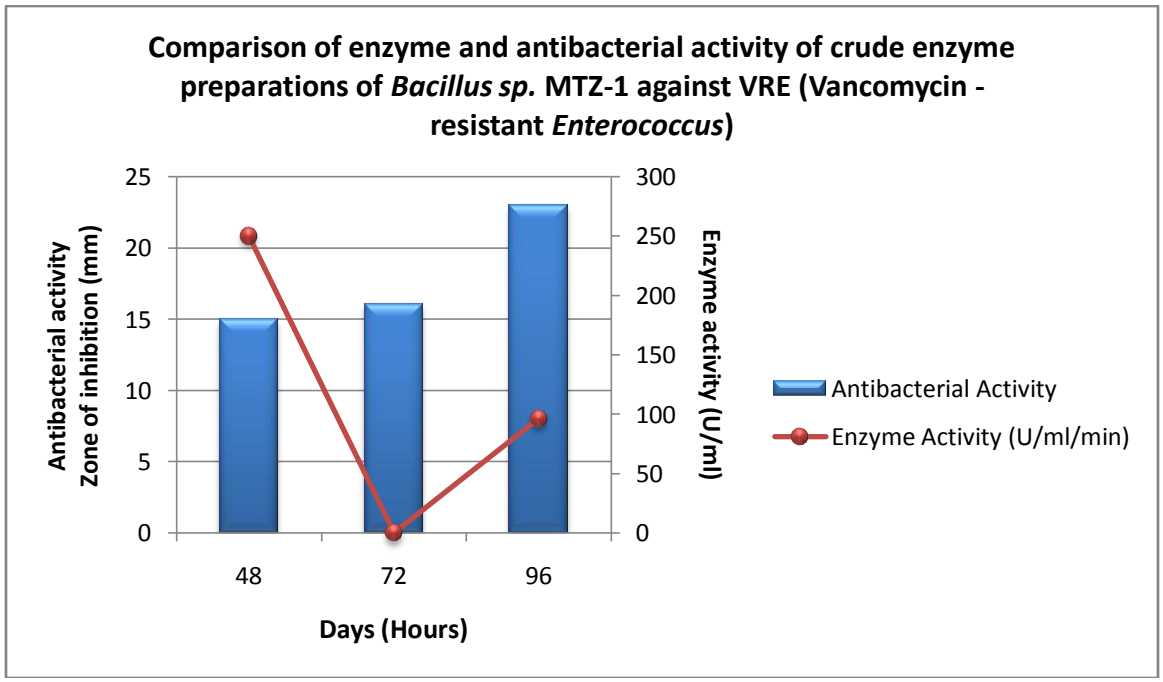


Figure 4. Comparison of enzyme and antibacterial activity of crude enzyme preparations preparations of *Bacillus sp.* MTZ-1 against VRE (Vancomycin-resistant *Enterococcus*).

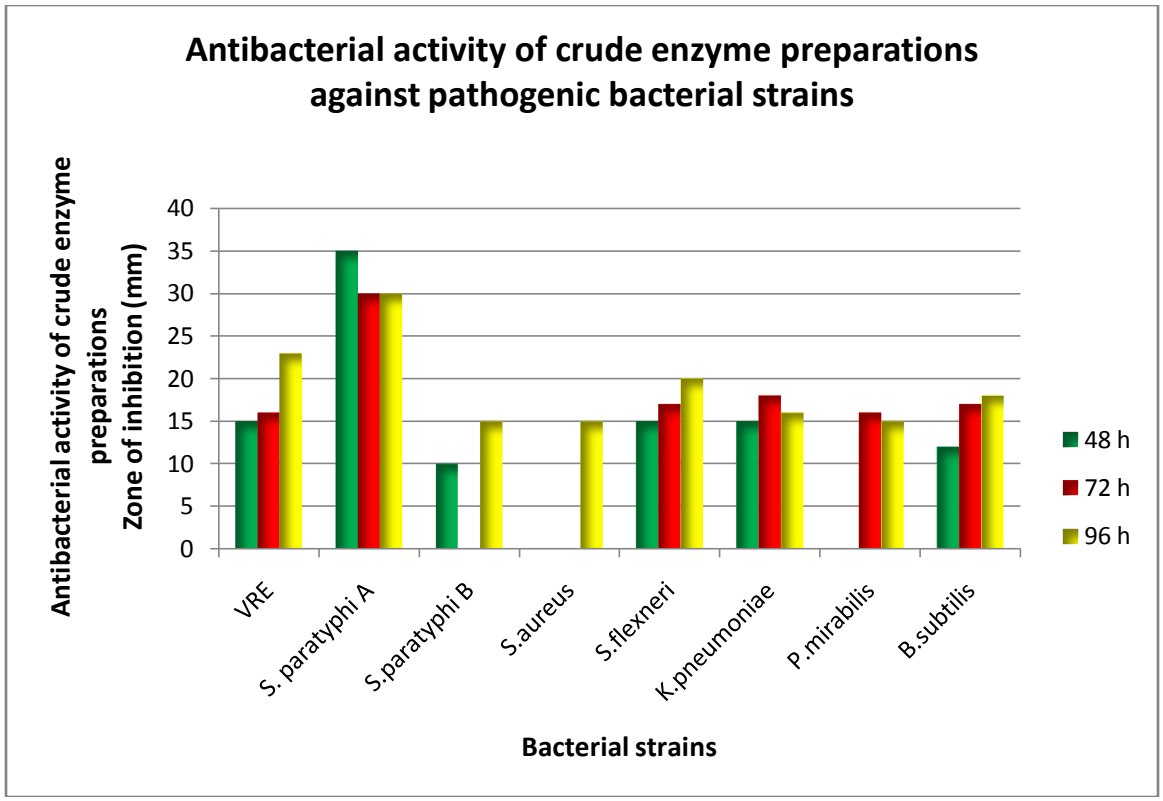
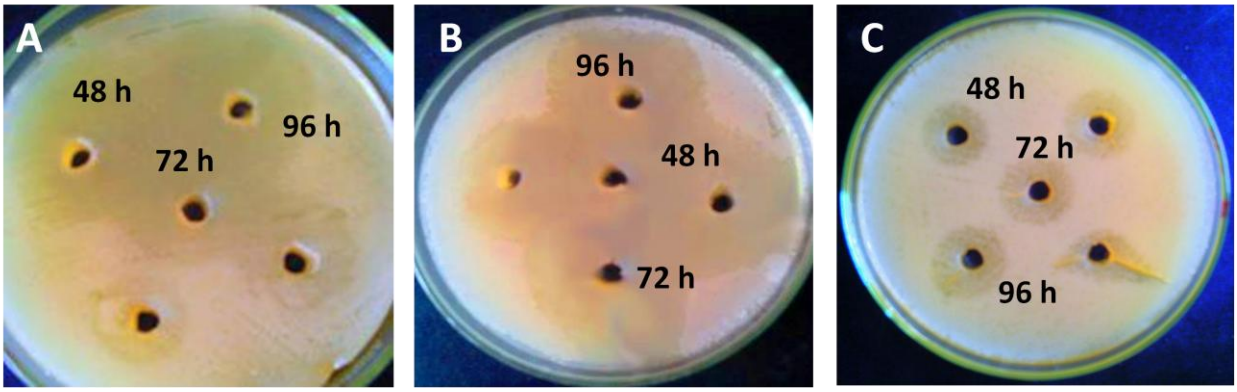


Figure 5. Antibacterial activity of crude enzyme preparations against pathogenic bacterial strains.



A. Antibacterial activity of crude enzyme preparations of *Bacillus sp.* MTZ-1 against VRE (Vancomycin-resistant *Enterococcus*). B. Antibacterial activity of crude enzyme preparations of *Bacillus sp.* MTZ-1 against *S.paratyphi A.* C. Antibacterial activity of crude enzyme of *Bacillus sp.* MTZ-1 against *B.subtilis.*

Figure 6: Antibacterial activity of crude enzyme preparations at different time intervals.

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