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Antibacterial Activity of *Bacillus pumilus* Extract Against Some Gram-positive and Gram-negative Bacteria

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Abstract

In recent years there has been a growing interest in the marine environment as a promising source of new microorganisms with novel bioactive compounds against multi-drug resistant bacteria. The present study aimed to determine the antibacterial activity of marine *Bacillus pumilus* extract against Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis* as well as Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsilla pneumoniae*. These bacteria were isolated from field samples using routine isolation methods. A disc diffusion assay was performed to determine the antibacterial effect of *B. pumilus* extract against the selected pathogens. The extract exhibited a broad inhibitory spectrum against all tested bacteria with inhibitory diameter ranging from 19 to 30 mm. These pathogens were further screened against 10 different antimicrobial discs. The result of antimicrobial susceptibility demonstrated that the tested organisms were resistant to many of the used antimicrobials with MAR index ranging from 0.3-0.6. Moreover, the antibacterial effect of *B. pumilus* extract was tested using the transmission electron microscope that revealed significant damage in all treated compared to untreated bacteria. These results suggest an antibacterial potential of *B. pumilus* extract against some pathogenic bacteria comparable to that of commercial antimicrobials and could be possibly used as a promising antibacterial agent.

Keywords: *Bacillus pumilus*; Antimicrobial activity; TEM; Gram-positive; Gram-negative Bacteria

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1. Introduction

Antimicrobial resistance all over the world has been found in humans and animals **(Allen et al., 2010; Berendonk et al., 2015)**. Furthermore, it is one of the greatest health challenges of our time **(WHO, 2015)**. Food-borne pathogens continue to cause major public health problems throughout the world **(Heredia and Garcia, 2018).** *S. aureus* is a main cause of food poising associated human diseases, causing gastrointestinal disorders due to ingestion of food contaminated by *S. aureus* enterotoxins **(Le Loir et al., 2003; Kadariya et al., 2014)**. *S. aureus* causes as well serious illness with high mortality due to production of toxic shock syndrome toxin (TSST) **(Kong et al., 2016)**. Furthermore, it causes tissue damage and evades the immune system through production of coagulase, staphylokinase and protease enzyme **(Ballhausen et al., 2017)**. *S. aureus* infections in animals are reported as a cause of mastitis in dairy-producing animals, osteomyelitis in poultry, and skin abscesses, mastitis, and septicemia in farmed rabbits **(Fitzgerald, 2012)** and has been reported in companion animals (dogs, cats) and wildlife **(Heaton et al., 2020)**.

Enterococcus spp. are common members of the normal gastrointestinal (GI) flora of both livestock and humans **(Yost et al., 2011)**, with their concentrations in human and animal feces normally ranging from 10^3- 10^7 cells per gram **(Ashbolt et al., 2001; Ervin et al., 2013)**. *Enterococcus* spp. are resistant against some antimicrobials including cephalosporins and trimethoprim-sulfamethoxazole. *Enterococcus* isolates with multi-drug resistance to tetracyclines, macrolides, glycopeptides and streptogramins have been described **(Hammerum, 2012; Garrido et al., 2014)**.

Family of attaching and effacing (A/E) bacterial pathogens, which includes diarrhoeagenic enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC), remains a significant threat to human and animal health **(Yin et al., 2001)**. EPEC adheres to epithelial cells, colonizes the intestinal mucosa, and produces characteristic histopathological features known as attaching and effacing (A/E) lesion causing enteric infections in some animals especially rabbits **(Osek, 2003; Bhunia, 2018)**. Rabbit-origin EPEC causes substantial diarrhea-associated morbidity and has zoonotic potential **(Alton et al., 2013)**. Human and bovine origin EHEC isolates cause disease in infant pigs and rabbits in a similar manner, which is considered as a significant threat to human and animal health ensuring its zoonotic importance **(Smriti et al., 2011)**. EHEC has been associated with numerous outbreaks worldwide and constitutes a serious public health threat. EHEC produces a potent Shiga toxin (Stx) that causes hemorrhagic colitis and Hemolytic uremic syndrome **(Fernanda and Marcelo, 2015)**. Infection with *E. coli* that produce Shiga toxins may lead to diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome, which can cause acute kidney failure **(Aruna et al., 2010)**. *Pseudomonas aeruginosa* is the most critical species for public health considerations among pseudomonas species. *Pseudomonas aeruginosa* is more resistant to many antibiotics. In Europe, the current report of the European Centre for Disease Prevention and Control (ECDC) posted in 2016 confirmed that 33.9% of *P. aeruginosa* had been proof against at least one of the antimicrobial agents beneath surveillance (piperacillin, tazobactam, fluoroquinolones, aminoglycosides, and ceftazidime **(ECDC, 2018)**.

Marine microorganisms are widely distributed in oceans all over the earth and act as a great source of different natural products **(Olano et al., 2009** and **Hughes and Fenical, 2010)**. Wide varieties of diverse compounds were isolated from marine microorganisms for replacement of commercial antimicrobials **(Mayer et al., 2011; Villa and Gerwick, 2016)**. Further studies were developed to obtain therapeutic agents as antibacterial **(El-Gendy and Rateb, 2015; Biswas et al., 2016)**, antifungal **(El-Sheekh et al., 2015)**, antitumor **(Kumar and Rawat, 2011)**, anti-parasitic and insecticidal **(Tareq et al., 2014)**, antiviral **(Prieto et al., 2014)**, and anti-inflammatory **(Chopra et al., 2014)**.

Bacillus is one of the most important antimicrobials producing genus, producing antibacterial, antifungal and a wide range of bioactive compounds that are active against various microorganisms

(Kim, 2003; Chopra et al., 2014; Bacon et al., 2015). Thousands of marine *Bacillus* are known to produce antimicrobial substances and less than 1% has been examined for their pharmaceutical activity, for example, *Bacillus silvestris, Bacillus cereus, Bacillus marinus, Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens* and *Bacillus pumilus* **(Eshwari and Kannahi, 2018; Lv et al., 2020)**. *B. amyloliquefaciens* AMM strain inhibited *Salmonella enterica* and a broad range of other pathogenic bacteria **(Zhang et al., 2020; Omar et al., 2021)**. Various reports confirmed the ability of *Bacillus* species to produce antimicrobial agents and compounds with potential biotechnological and pharmaceutical applications **(Hassan et al., 2014)**. *B. pumilus* was isolated from soil **(Padaria et al., 2014)**, sea, and marine sediments **(Liu et al., 2013)**. *Bacillus pumilus* contributes in a wide range of symbiotic relationships, having antifungal effect against *Gaeumannomyces graminis* that damage wheat crops **(Sari et al., 2007)**, antimicrobial activity inhibiting the growth of harmful bacteria **(He, 2011; Alvarez et al., 2012)** and as animal and human probiotics **(Prieto et al., 2014)**. *Bacillus pumilus* exhibited antimicrobial effect against *S. aureus* ATCC6538, *M. luteus* CMCC28001, Variant *S. gallinarum* CVCC79207, *S. enterica* ATCC13076 and *P. multocida* CVCC474 **(Chu et al., 2019)**. Further**,** *B. pumilus* MMM exhibited antimicrobial effect against *Aeromonas hydrophila* **(Malash et al., 2016)**. Therefore, here the antimicrobial activity and the action of *B. pumilus* MMM extract against some Gram-positive and Gram-negative bacteria was studied.

2. Materials and Methods

2.1. Samples

A total of 35 field samples were collected; 15 cloacal swab samples from diarrheagenic chicken, 10 milk samples from mastitic cattle, 10 nasal swabs from calves with respiratory manifestations **(Bailly and Scott, 1998; Waltman et al., 1998)**. Samples were cultured onto mannitol salt agar media and Baird-parker agar media for isolation of *S. aureus* **(Ahmed, 2015)**, cultured onto MacConkey's agar media and Eosin-Methylene Blue agar media for isolation of *E. coli* and *K. pneumoniae* and cultured onto nutrient agar, MacConkey's agar media and cetrimide agar media for isolation of *P. aeruginosa* **(Cheesbrough, 1985)**. The suspected colonies were identified based on colonial and microscopic characteristics using Gram's staining, motility test, biochemical tests including oxidase, catalase, triple sugar iron (TSI), hydrogen sulfide (H_2S), indole, methyl red (MR), Voges-Proskauer (VP), citrate utilization, urease, nitrate reduction, sugar fermentation according to **Murray et al (1984), Macfaddin (2000)**, and **Quinn et al (2002)**. *Bacillus subtilis* and *Enterococcus faecalis* used in the subsequent analysis were previously isolated and characterized by **Awwad et al (2018)**.

2.2. Bacterial extract

Ethyl acetate extract of *B. pumilus* MMM was previously prepared according to **Malash et al (2016)**.

2.3. Screening for antibacterial activity

The antibacterial activity of *B. pumilus* MMM extract against the test bacterial isolates was determined by the disk diffusion method **(Drago et al., 1999)**. It was performed with some modifications using an 18 hrs culture at 37°C in 10 ml of nutrient broth. The cultures were adjusted to approximately 10^5 CFU/ml with sterile PBS. Five hundred microliters of the suspensions were spread over the plate containing nutrient agar by using a sterile cotton swab to get a uniform microbial growth on both test and control plates. The bacterial extract was sterilized by filtration through a 0.45 µ membrane filter. Under aseptic conditions, sterilized Whatman paper discs **(Prabuseenivasan et al., 2006)** were soaked with 50 µl of the extract and placed onto the agar surface. Paper discs sodden with sterile saline solution was placed on the seeded plate as a negative control. The plates were left for 30 min at room temperature to allow the diffusion of the extract, then incubated at 37°C for 18 hrs. After the incubation period, the zone of inhibition was measured.

2.4. Antimicrobial susceptibility test

The bacterial isolates were tested for their antibiogram using disc diffusion method according to the guideline of the National Committee for Clinical Laboratory Standard institute **(CLSI, 2015)**. Ten commercial antimicrobial discs *(*Oxoid Limited, Basingstoke, Hampshire and UK*)* were selected: chloramphenicol (C, 30 µg), spiramycin (SP, 100 µg), levofloxacin (LEV, 5 µg), doxycycline (DO, 30 µg), ceftriaxone (CRO, 30 µg), streptomycin (S, 10 µg), spectinomycin (SPT, 10 µg), lincomycin (L, 2 µg), ciprofloxacin (CIP, 5 µg) and ampicillin (AM, 10 µg). All plates were incubated at 37°C for 18 hrs **(Khan et al., 2019)**. The results were estimated by measuring the diameter of the inhibition zone for each well and expressed in millimeters.

2.5. Determination of the effect of B. pumillus MMM extract on the bacterial isolates

The effect of *B. pumilus* MMM extract on different bacteria was detected using transmission electron microscope (TEM). One ml of each tested bacteria was incubated separately for 24 hrs with the *B. pumilus* MMM extract (1:1 vol/vol). The cells were harvested by centrifugation for 5 min at 5000 rpm and then examined using (TEM) (The Central lab, Faculty of science, Alexandria University) according to **Padmavathy and Vijayaraghavan (2008)**.

3. Results

3.1. Bacterial isolates

From 35 samples, *S. aureus* was detected in 6 out of 10 milk samples, *E. coli* was detected in 12 out of 15 samples, *P. aeruginosa* was detected in 2 out of 10 milk samples, and *K. pneumoniae* was detected in 6 out of 10 nasal samples (**Table 1**).

3.2. Antibacterial activity of B. pumilus MMM extract

Disc diffusion assay was performed to determine the antibacterial activity of *B. pumilus* MMM extract against six bacterial isolate, three Gram positive (*S. aureus, B. subtilis* and *E. faecalis*) and three Gram negative (*E. coli, P. aeruginosa, and K. pneumoniae*). The results revealed that the extract exhibited inhibitory effect against the tested bacteria with variable potency. The extract showed high activity against *B. subtilis* (30 mm), *E. faecalis* (30 mm) and *E. coli* (25 mm). However, moderate activity was observed against *S. aureus* (20 mm), *K. pneumoniae* (20 mm) and *P. aeruginosa* (19 mm) as illustrated in **Figure 1** and **Figure 2**.

3.3. Antimicrobial susceptibility

Antimicrobial susceptibility testing was carried out using 10 commercial antimicrobial discs. Most of the tested organisms were resistant to spectinomycin and lincomycin, while, sensitive to levofloxacin. The susceptibility, MAR index values and resistance patterns are shown in **Table 2** and **Table 3**.

3.4. The effect of B. pumilus MMM extract on the bacterial isolates

The effect of the extract of *B. pumilus* MMM on *S. aureus, B. subtilis, E. faecalis, E. coli, P. aeruginosa* and *K. pneumoniae* was evaluated using TEM. The untreated (control) bacterial cells showed continuous smooth cell wall, cell membrane, even distribution of cytoplasm and nuclear material. However, after incubation with *B. pumilus* MMM extract, the bacterial cells became irregular, decreased in size, shriveled, leakage of the contents of the cells occurred and the cells were found to be closer together. The cell wall was degenerated, with lost smoothness and uniformity.

The cytoplasm has lost its even distribution with clumping of cytoplasmic material, granulation inside the bacterial cell and massive destruction as shown in **Figures 3**, **4**, **5**, **6**, and **7**.

Table 1. Number of samples and number of isolated bacteria

Samples	Total No. of exmined samples	No. of +ve isolates
Cloacal swabs	15	12 *E. coli*
Milk samples	10	6 *S. aureus* 2 *P. aeruginosa*
Nasal swabs	10	6 *K. pneumoniae*
Total	35	26

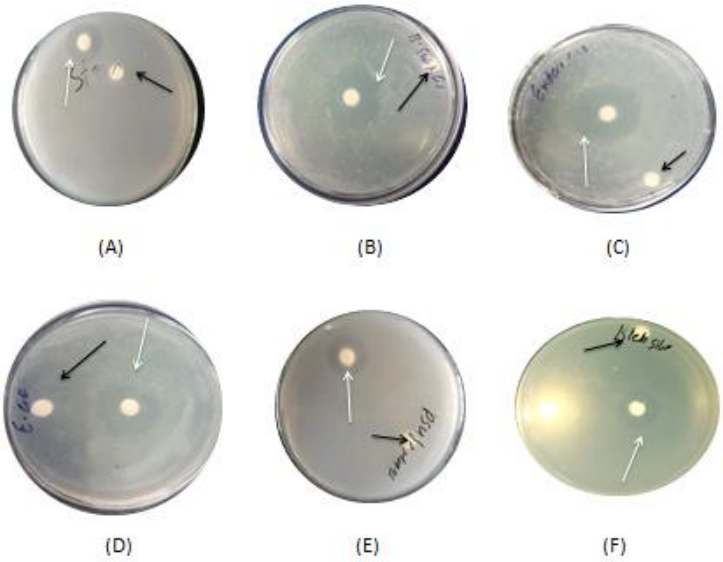

Figure 1. Antibacterial activity of *Bacillus pumilus* MMM extract against A) *S. aureus* (20 mm), **B)** *B. subtillis* (30 mm), **C)** *E. faecalis* (30 mm), **D)** *E. coli* (25 mm), **E)** *P. aeruginosa* (19 mm), **F)** *K. pneumoniae* (20 mm) using the disc diffusion method. White arrow indicates reaction zone and black arrow indicates the negative control.

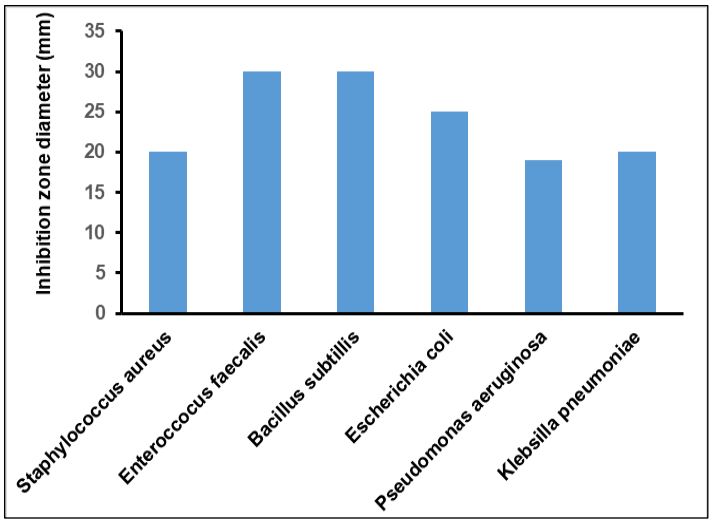

Figure 2. Antibacterial activity of *Bacillus pumilus* MMM extract against tested bacteria, expressed as inhibition zone (mm) using the disc diffusion method

Table 2. Antimicrobial susceptibility of tested bacteria

Bacteria	**Antimicrobials**									
	C	**Sp**	**LEV**	**DO**	**CRO**	**S**	**SPT**	**L**	**CIP**	**AM**
S. aureus	20 (S)	15 (I)	21 (S)	21 (S)	21 (S)	21 (S)	- (R)	- (R)	20 (I)	5 (R)
B. subtillis	25 (S)	19 (I)	20 (S)	- (R)	10 (R)	- (R)	- (R)	- (R)	18 (I)	10 (I)
E. faecalis	- (R)	21 (S)	21 (S)	- (R)	- (R)	- (R)	- (R)	- (R)	18 (M)	16 (I)
E. coli	15 (I)	16 (I)	22 (S)	15 (S)	35 (S)	16 (S)	- (R)	- (R)	18 (I)	- (R)
P. aeruginosa	15 (I)	21 (S)	19 (I)	25 (S)	- (R)	- (R)	21(S)	- (R)	15 (I)	- (R)
K. pneumonia	21 (S)	19 (I)	20 (S)	15 (S)	- (R)	22 (S)	- (R)	- (R)	20 (I)	- (R)

* The number indicates the diameter of the inhibition zone (mm), (-) indicates no inhibition, S; sensitive, I; intermediate, R; resistant. Chloramphenicol (C; 30 µg), spiramycin (SP; 100 µg), levofloxacin (LEV; 5 µg), doxycycline (DO; 30 µg), ceftriaxone (CRO 30 µg), streptomycin (S; 10 µg), spectinomycin (SPT; 10 µg), lincomycin (L; 2 µg), ciprofloxacin (CIP; 5 µg) and ampicillin (AM; 10 µg).

Table 3. Antimicrobial susceptibility patterns and MAR index of the bacterial isolates

Bacteria	**Antimicrobial resistance pattern**	**MAR index**
S. aureus	SPT, L, AM	0.3
B. subtillis	DO, CRO, S, SPT, L	0.5
E. faecalis	C, DO, CRO, S, SPT, L	0.6
E. coli	SPT, L, AM	0.3
K. pneumonia	CRO, SPT, L, AM	0.4
P. aeruginosa	CRO, S, L, AM	0.4

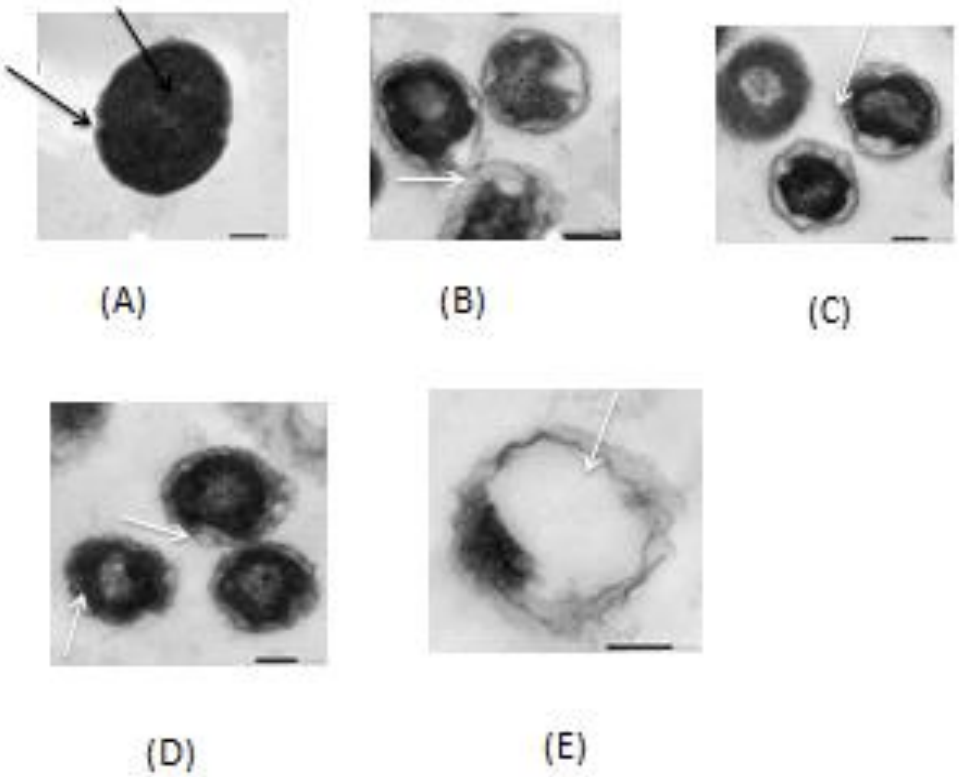

Figure 3. TEM micrographs of bacterial isolates treated with *B. pumilus* MMM showing untreated *S. aureus* cells (A) and treated *S. aureus* cells (B), (C), (D) and (E). Untreated cells showed continuous smooth cell wall, cell membrane, even distribution of cytoplasm and nuclear material (A) as indicated by black arrow. Treated cells became irregular, decreased size with degenerated cell wall (B, C, D) with leaking of cytoplasmic content (E). As indicated by the white arrow.

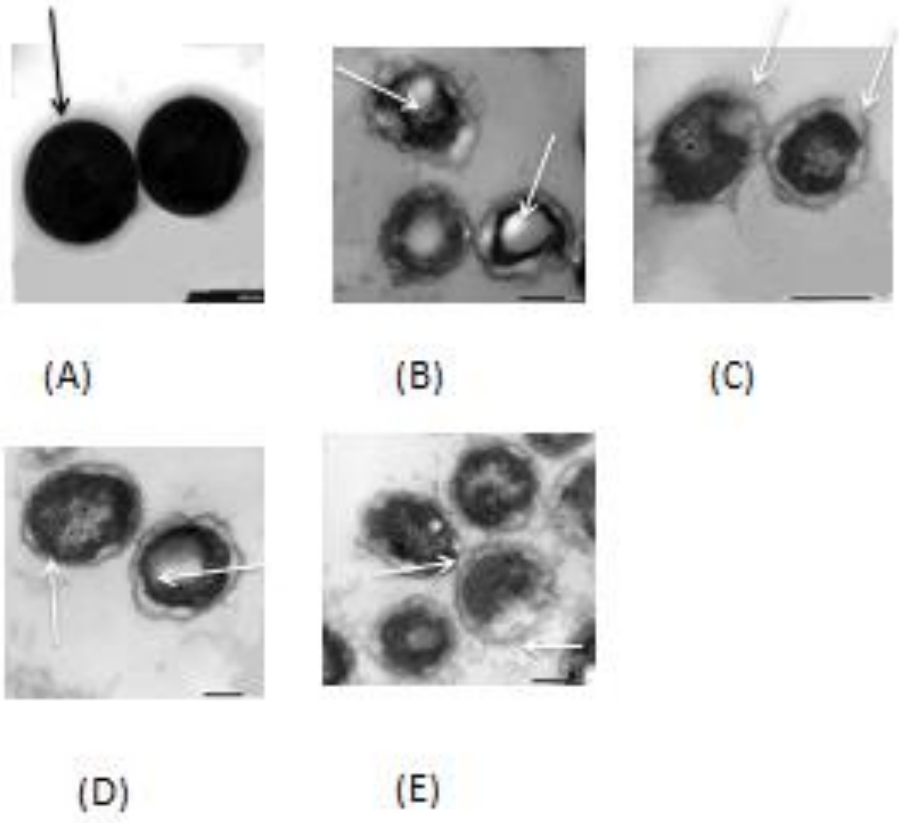

Figure 4. TEM micrographs of bacterial isolates treated with *B. pumilus* MMM showing untreated *E. faecalis* cells (A) and treated *E. faecalis* cells (B), (C), (D) and (E). Untreated cells showed continuous smooth cell wall, cell membrane, even distribution of cytoplasm and nuclear material (A) as indicated by black arrow. Treated cells were found to be closer together (C and E), degenerative changes in cell wall (B, C, D, and E), cytoplasm has lost its even distribution showing clamping of cytoplasmic material and granulation inside the bacterial cell (B and D) as indicated by white arrow.

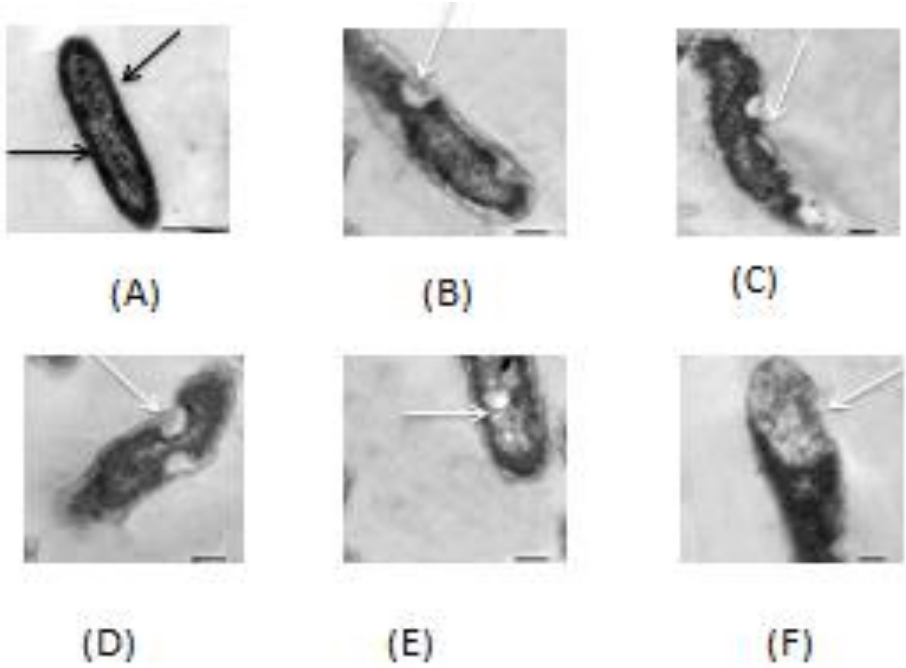

Figure 5. TEM micrographs of bacterial isolates treated with *B. pumilus* MMM showing untreated *E. coli* cells (A) and treated *E. coli* cells (B), (C), (D) and (E). Untreated cells showed continuous smooth cell wall, cell membrane, even distribution of cytoplasm and nuclear material (A) as indicated by black arrow. Treated cells became irregular with degenerative changes in cell wall (C and D) and budding scar (F). Cytoplasm has lost its even distribution showing clamping of cytoplasmic material and granulation inside the bacterial cell (B, C, D and E) as indicated by white arrow.

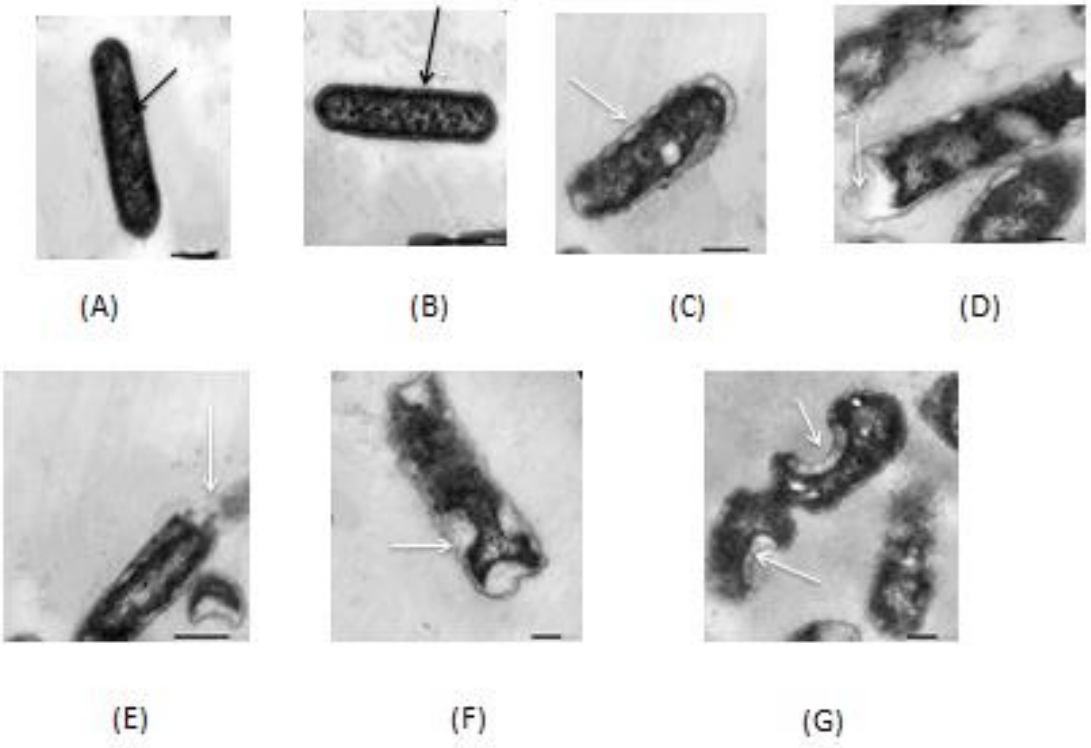

Figure 6. TEM micrographs of bacterial isolates treated with *B. pumilus* MMM showing untreated *P. aeruginosa* cells (A and B) and treated *P. aeruginosa* cells (C), (D), (E), (F) and (G). Untreated cells showed continuous smooth cell wall, cell membrane, even distribution of cytoplasm and nuclear material (A and B) as indicated by black arrow. Treated cells became irregular with degenerative changes in cell wall (D, E, F, and G), cytoplasm has lost its even distribution showing clamping of cytoplasmic material and granulation inside the bacterial cell (C, D, F, and G) as indicated by white arrow.

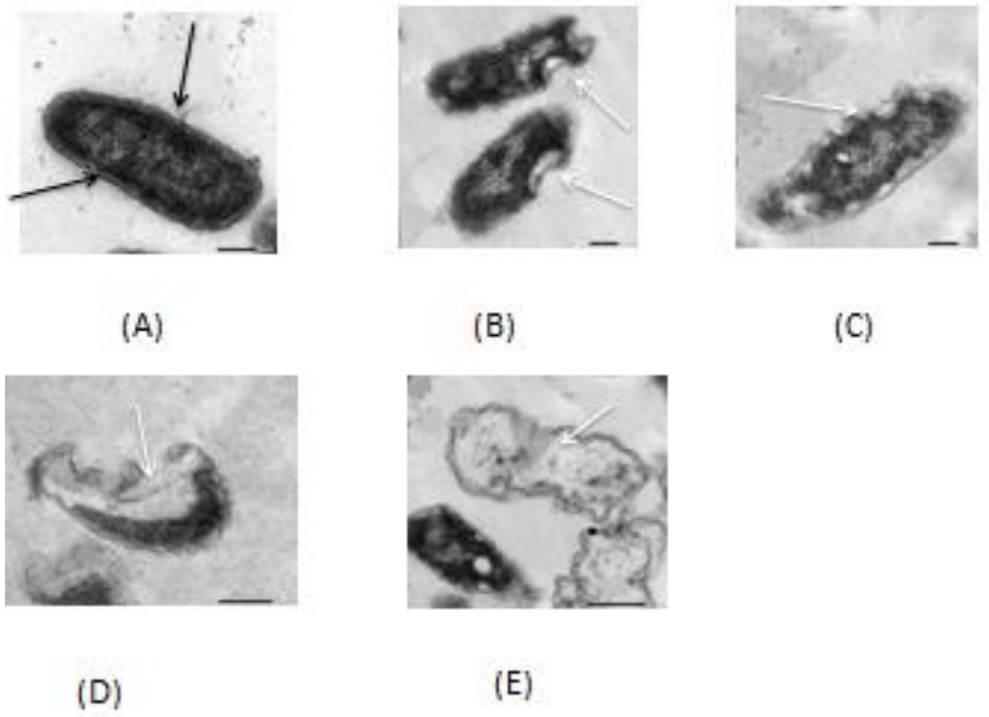

Figure 7. TEM micrographs of bacterial isolates treated with *B. pumilus* MMM showing untreated *K. pneumonia*e cells (A) and treated *K. pneumonia*e cells (B), (C), (D) and (E). Untreated cells showed continuous smooth cell wall, cell membrane, even distribution of cytoplasm and nuclear material (A) as indicated by black arrow. Treated cells became irregular with degenerative changes in cell wall (B and D), cytoplasm has lost its even distribution showing clamping of cytoplasmic material (C) and leaking of cytoplasmic content (E) as indicated by white arrow.

4. Discussion

The rapid emergence of resistance to current antimicrobials has raised public health concerns worldwide **(Overbye and Barrett, 2005; Tenover, 2006)**. Many infectious pathogens, especially Gram-negative bacteria have developed resistance to conventional antimicrobials **(Alba et al., 2012)**. Antimicrobial products isolated from the *Bacillus* species could overcome the resistance of current antimicrobials **(Hassan et al., 2012)**. The *Bacillus* genus produces a huge variety of peptide antibiotics with specific simple chemical structures **(Baindara et al., 2013)**. Many of these peptides are appropriate for numerous applications **(Abriouel et al., 2011)**.

In the present study, *B. pumilus* MMM extract was screened against six bacterial strains using disc-diffusion method to determine its inhibitory activity. The extract showed antibacterial effect against the tested bacterial strains and the effect varied between strains. The potent effect was observed against *B. subtilis* (30 mm), *E. faecalis* (30 mm) and *E. coli* (25 mm). However, moderate activity was observed against *S. aureus* (20 mm), *K. pneumoniae* (20 mm) and *P. aeruginosa* (19 mm). These results suggest that ethyl acetate extract of *B. pumilus* MMM contains antibacterial components, which exhibited an inhibitory effect against both Gram-positive and Gram-negative bacteria. The use of the agar diffusion technique indicated that the antibacterial compounds produced *B. pumilus* MMM were water-soluble as they readily diffused through the agar medium **(Chen et al., 2016)**.

By comparing the inhibition zone of *B. pumilus* MMM extract with other products reported in previous studies for screening of the antimicrobial activity against pathogenic bacteria, the *B. pumilus* MMM extract gave larger inhibition zone ranging from 19 to 30 mm. **Aboul-Ela et al (2019)** evaluated *Bacillus pumilus* extract for its antibacterial activity. They showed strong activity against *E. coli* and multi-drug resistant strain (MDR) *Pseudomonas sp*, moderate activity against *S. aureus*, and weak activity against *Klebsiella Pneumoniae* with inhibition zones of >10 mm zone, >5 mm zone, <5 mm, respectively. *B. pumilus* MMM extract gave an inhibition zone of 30 mm against *Aeromonas hydrophila* **(Malash et al., 2016)**. **Latorre et al (2016)** screened *B. subtilis* against some bacterial strains, the inhibition zones against *S. Enteritidis, E. coli,* and *C. difficile* were 5.7±0.58, 8.7± 1.76, 16± 2.08, respectively. Further, *B. amyloliquefaciens* against same bacterial strains and had inhibition zones of 8 ± 1.15, 10 ± 2, 22 ± 2, respectively**.**

Bacillus strains isolated from human gut microbiota (*B. amyloliquefaciens, B. siamensis, B. velezensis, B. nematocida, B. cereus, and B. pacificus*) were found to be antagonistic against Gram-positive *Bacillus cereus, Bacillus circulans, Staphylococcus aureus, and Streptococcus pyogenes* with inhibition zone of 10–17 mm. Further, against Gram-negative food-borne pathogenic bacteria as *Serratia marcescens, Escherichia coli, Salmonella*, and *Klebsiella pneumoniae* with growth inhibition zone of 10–20 mm **(Sánchez et al., 2021)**. *Bacillus subtilis* URID12.1 showed significant antibacterial activity against methicillin-resistant isolates of *Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes* and *Enterococcus faecalis* with inhibition zones of 22, 27, 16, 17, respectively, with no effect against *E. coli* and *K. peumoniae* **(Chalasani et al., 2015)**. The antibacterial activity of ethanol extracts of *P. radiata* molluscs was evaluated against five strains of bacteria. The results showed considerable activity towards all tested bacteria; *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. pneumoniae* with inhibition zones 16.12 ± 0.76 mm, 11.00 ± 1.00 mm, 18.00 ± 1.73 mm, 14.83 ± 0.70 mm, 0.80 ± 0.26 mm, respectively **(Sake et al., 2020)**. When ethyl acetate extracts from marine algae *P. gymnospora* was screened against bacterial strains, the inhibition zones for *S. aureus, B. cereus, E. coli, Salmonella, P. aeruginosa* and *E. faecalis* were 17.8±0.8 mm, 11.3±0.3 mm, 11.3±0.8 mm, 9.5±1.2 mm 10±1.2 mm, 9±0.5 mm, respectively **(Salem et al., 2011)**. Furthermore, antibacterial potential of methanol extract of *Thais savignyi* was evaluated against 5 clinical isolates by the agar well diffusion. The tested isolates showed inhibiting zones for *Bacillus cereus, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* of 16 mm, 8 mm, 11 mm, 9 mm, and 9 mm, respectively **(Ameri et al., 2017)**. On the other hand, the antibacterial activity of cinnamon essential oil (EOs) against different microbial strains (*E. coli, E. faecalis, S. aureus, P. aeruginosa, K. pneumoniae*) showed that all tested bacteria were sensitive to cinnamon essential oil with inhibition zones of 26.67±1.15, 36±0, 37.67±1.53, 22.67±1.15, and 26.33±1.53, respectively **(Oulkheir et al., 2019)**. When *Xestospongia testudinaria* sponge extract was screened against Gram-positive and Gram-negative bacteria, moderate activity with various inhibition zones were detected for *S. aureus, E. coli, K. pneumonia, S. typhi*, MDR *P. aeruginosa* and methicillin resistant *S. aureus* (MRSA) of 20.10 ± 0.26 mm, 9.5 ± 0.12 mm, 15.25 ± 0.34 mm, 15.25 ± 0.26 mm, 15.25 ± 0.45 mm, and 17.50 ± 0.23 mm, respectively **(Cita et al., 2017)**.

These results together with ours indicate that many microorganisms have high ability of producing natural products as *B. pumilus* MMM extract that are effective against a wide range of Gram-positive and Gram-negative bacteria and could be of great possible therapeutic or prophylactic potential. Antimicrobial susceptibility of bacterial isolates was investigated against 10 commonly used antimicrobials. It was observed that most of the bacterial isolates showed multiple resistance. By comparing *B. pumilus* MMM extract to commercial antimicrobials, all tested bacterial isolates were more sensitive to *B. pumilus* MMM extract in contrast to variable susceptibilities to commercial antimicrobials. As noticed with *B. subtillis* and *E. faecalis*, multiple antimicrobial resistance was detected with MAR index of 0.5 and 0.6, respectively, while *B. pumilus* MMM extract showed high activity against those isolates. This may indicate a more potent effect of the extract; however, *in vivo* effects are still under investigation to confirm such conclusion. When the inhibition zone of *B. pumilus* MMM extract was compared to that of commercial antimicrobials, the inhibition zone of the extract was equal to or higher than that of the antimicrobial. These results indicate the efficacy of *B. pumilus* MMM extract, however, they would need a strong purification process to be used in efficient concentrations as commercial antimicrobials and requires *in vivo* studies to elucidate such effects. Although it has been observed that strains of *Bacillus* spp produce substances with wide antibacterial activities, some of these products on the other hand showed narrow spectrum affecting a narrow range of bacteria as *B. amyloliquefaciens* M1 strain that had antibacterial activity against multidrug-resistant *Vibrio* species. *B. subtilis* (BSAP-254) extract exhibited narrow spectrum against *B. cereus, Bacillus anthracis* and *B. thuringiensis* **(Yeo et al., 2012; Torres et al., 2013)**. On the contrary, the *B. pumilus* MMM extract showed a high broad spectrum against both Gram-positive and Gram -negative bacteria.

To determine changes that occurred in the bacterial cells upon treatment wih *B. pumilus* MMM extract, TEM analysis was performed. Some reports have used TEM to evaluate bacterial cell structure changes **(Rattanachuay et al., 2010)**. Here, TEM demonstrated that the extract had severe destructive effects on the tested bacterial cells. The cell wall showed degenerative changes with severe rupture and decreased cell size. The results of our study agree with several others showing morphological changes in treated bacterial cells. *Aeromonas hydrophila* when treated with *B. pumilus* MMM extract showed lysis in cell wall, intracellular granulation, and damage in the nucleus **(Malash et al., 2016)**. *E. coli* when exposed to inhibitory concentrations of the Eugenia zeyheri and Syzygium legatii extracts showed morphological and ultrastructural damage as characterized by deformation in the cell shape, severe rupture of cell wall and the membrane integrity of the bacterial cells was affected **(Famuyide et al., 2020)**.

L. monocytogenes cells when treated with the Mentha Longifolia L. Essential Oil, showed changes of shape and morphology of the bacterial cells, removal of cellular contents and damaged cell wall **(Mahmoudi et al., 2016)**. The methanol extracts of *Ocimum Basilicum L* showed antimicrobial activity against *P. aeruginosa, Shigella sp., L. monocytogenes, S. aureus* and *E. coli* in the form of degradation of the cell walls **(Kaya et al., 2008)**. Furthermore, cell membrane disruption and lack of cytoplasm was evident at an early stage of treatment of bacterial cells with the *B. pumilus* MMM extract. The cytoplasm lost its even distribution and showed clumping of cytoplasmic materials. The cells also exhibited lack of cytoplasm as a result of the decrease of the cell membrane functionality as a barrier resulting in efflux of essential cytoplasmic components **(Gui et al., 2014)**. *E. coli* cells treated with 2.88 mg/mL of bacteriocin from *B. subtilis* GAS101 showed clear disruption of the cell membranes and the SEM images showed that the pore formation could be one of the possible mechanisms for its bactericidal action **(Sharma et al., 2018)**. Electron microscope investigation of *B. cereus* treated with propolis showed structural damage at the cellular level and irreversible cell membrane rupture at several locations with apparent leakage of intracellular contents **(Kim et al., 2011)**.

Salmonella typhi and *Listeria monocytogenes* treated with antimicrobial peptide produced by *Bacillus paralicheniformis*, showed by Electron microscope Cell bursting and leakage of the cytoplasmic contents **(Choyam et al., 2021)**. *S. aureus* treated with oil extract of tea tree, showed lack of cytoplasmic material **(Carson et al., 2002)**. *S. aureus* treated with *C. versicolor* extract showed by SEM malformed cells and obvious leakage of cytoplasmic materials **(Matijaševi et al., 2016)**. *Salmonella Typhi* and *E. coli* treated with Eugenol, showed by Electron microscope shrinkage and rough surface of treated cells indicating damage of the cell membrane **(Devi et al., 2010; Hartmann et al., 2010)**.

These results collectively indicate the loss of permeability and damage of the cytoplasmic membrane of treated cells. Furthermore, the bacterial cells aggregation probably occurred as a result of the draining out of intracellular material through the damaged cell wall. *S. Enteritidis* treated with *Porella arboris-vitae* extracts that acted by interfering with the outer cell walls of the bacterial cells, showed cell aggregation by TEM **(Tyagi et al., 2013)**.

The normal shape of tested bacterial cells was strongly affected by the *B. pumilus* MMM extract. The extract affected the different bacterial cells by destroying their membrane or the whole cell. This possible mode of action could reduce the chance of development of drug resistance in treated microbes and could offer an effective alternative to the treatment of multidrug-resistant infectious agents **(Chen et al., 2016)**. The broad-spectrum antimicrobial activities, even against many multidrug-resistant strains make the *Bacillus pumilus* MMM extract a possible attractive alternative to conventional antimicrobials. *Bacillus pumilus* MMM could produce soluble extracellular antibacterial substances responsible for inhibiting the growth of some Gram-positive and Gram-negative bacteria causing bacterial cell lysis. However, extensive studies are still required to confirm such effects *in vivo* and against other bacterial strains. These results suggest an important antibacterial potential of *B. pumilus* extract that could be used as a promising antibacterial agent as alternative to conventional antimicrobials to overcome the risk of antimicrobial resistance.

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