Screening of Natural β-lactamase Inhibitors (BLIPs) from Two Newly Isolated Egyptian Streptomyces strains

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ABSTRACT

β-lactam antibiotics are the most extensively used antibiotics to treat a variety of infections due to their safety, high selectivity, and efficacy. However, the main cause of antibiotic resistance is due to the misuse of antibiotics uptake which stimulated bacteria to produce β-lactamases. Therefore, there is an essential need for the development of novel β-lactam/β-lactamase inhibitor combinations. In this respect, 186 Actinomycetes isolates were screened for β-lactamase inhibitors (BLIs) using disc diffusion and modified Iodometric bioassays. Two Egyptian Streptomyces isolates (S.t-158 & S.t-6) were selected for their ability to exhibit BLIs. The Egyptian isolates S.t-158 and S.t-6 were molecularly identified by 16s rRNA gene sequencing with 100% and 98.9% nucleotide similarity to S. rochei and S. clavuligerus, respectively. β-Lactamase inhibitory protein (BLIP) gene (600 Bp) was detected in the Egyptian S. clavuligerus strain. In-silico docking of β-lactamase enzyme (KPC-2) against four sets of selected ligands was performed using Molecular Operating Environment to investigate possible binding interactions of selected bioactive compounds within the enzyme active site. Harmine, Borrelidin and SRB-2 showed high binding energy values of -5.699, -6.531 and -5.631, respectively. These compounds form hydrogen bonds with KPC-2 active site residue-Ser70 or -Ser130 and prevent it from attacking β-lactam antibiotics, thus inactivating it. The BLIs could be useful for synergic therapy with β-lactams antibiotics to overcome the β-lactamase resistance.

Key words: β-lactamases; *S. clavuligerus; S. rochei;* β-Lactamase inhibitory protein

1. INTRODUCTION

β-lactam antibiotics are the most widely used ones due to their activity spectra, mode of action, and safety profile (Tsivkovski and Lomovskaya 2020). They are characterized by a β-lactam ring, and are divided into four classes, these are penicillins, cephalosporins, monobactams, and carbapenems (King *et al*. 2017; Barale *et al*. 2022). They bind irreversibly to the peptidoglycan cross-linking enzymes essential for bacterial cell wall (Tooke *et al*. 2020). The persistent exposure of bacteria to β-lactam antibiotics select the resistant isolates within the bacterial population, where resistant bacteria produce β-lactamases (Bhat *et al*. 2021). β-lactamases

are produced by some Gram-negative and Gram-positive bacteria, which covalently bind to the carbonyl moiety of β-lactam ring and hydrolyze its amide bond (Tooke *et al*. 2020).

Two strategies have been employed to overcome β-lactamase-mediated resistance, these are (i) modification of the β-lactams structure (e.g. carbapenems and expanded-spectrum cephalosporins), and (ii) inhibition of β-lactamases by the development of selective β-lactamase inhibitors (BLIs) (Docquier and Mangani 2018). In 1970, BLIs were introduced in clinical medicine (Ives and Bushell 1997). The combination of BLIs with β-lactam antibiotics represent a powerful strategy to prevent the cleavage of β-lactams and restore their potency (Bushand and Jacoby 2010). BLIs are classified into two groups; traditional BLIs which include the three FDA-approved commercially available ones (clavulanate, tazobactam, and sulbactam) (Jalde and Choi 2020). The second group is a "non-β-lactam", they are three BLIs introduced into clinical practice namely: 1. avibactam in combination with ceftazidime (approved in 2015), 2. vaborbactam (approved in 2017) in combination with meropenem, and 3. relebactam (approved in 2019) in combination with imipenem (Tsivkovski and Lomovskaya 2020).

Unfortunately, the effectiveness of β-lactamases is further enhanced either by mutations that increase β-lactamase expression or via structural intragenic mutations, which led to the enhancement of their specificity and efficacy (Drawz and Bonomo 2010; Cheng *et al*. 2020). BLIs are potent candidates that control multidrug-resistant bacteria, however, there are some limitations because currently available BLIs only work against serine β-lactamases, whereas metallo-β-lactamases continue to spread, and evolve resistance to all β-lactams. Therefore, there is a critical need for discovering novel BLIs from different sources and the development of novel β-lactam/BLIs combinations to tackle the spreading resistance (Song *et al*. 2017; Mary *et al*. 2021; Zhuang *et al*. 2021).

Interestingly, the Egyptian soil is considered a precious source for Actinomycetes which exhibited an uncommon capacity for possible novel, clinically valuable secondary metabolites, such as, antibacterial, antivirals, and enzymes, etc (Elnadi *et al*. 2022). Due to the aforementioned reasons, the present study aims to isolate Actinomycetes from Egyptian soil to explore novel BLIs.

2. Materials and methods

2.1. Materials

Standard antibiotics used in this study were purchased from Sigma-Aldrich (USA). Hi-media antibiotic discs were obtained from (Hi-media, India). All other reagents and chemicals were of analytical grade.

2.2. Isolation of actinomycetes

Soil samples were collected in sterile plastic bags from different governorates in Egypt (Alexandria, Matruh, Damietta, Ismailia, Minufyia, El-Beheira, and Minya). Ten grams' samples were suspended in 90 mL of saline solution (0.9% NaCl, w/v), and serially diluted. One mL aliquot was spread on sterile agar plates containing Glucose Yeast Malt (GYM) Streptomyces medium and onto an inorganic salt starch medium (ISP Medium No. 4), incubated for 5 days at 30oC, Nystatin and Cyclohexamide (50 µg/mL) were added to prevent any fungal growth (Goodfellow and Williams 1983). Colonies that were initially identified as Actinomycetes were selected, purified by conventional-streak technique and maintained on slants at 4oC.

2.3. Bacterial strains and inoculum

Clinical isolates including, Escherichia coli (-12, BL-12), Klebsiella pneumonia (-10, -M.S1, -M.S2, -M.S3), and

Acinetobacter baumannii were obtained from hospitalized patients in El-Mabaret Educational Hospital, Alexandria and El-Salam International Hospital, Cairo in Egypt (Elnadi *et al*. 2022). Strains were examined for their ability to produce β-lactamases by Iodometric test (Livermore and Brown, 2001) and agar disc diffusion assay with different β-lactam antibiotics according to Clinical Laboratory Standards Institute guidelines (CLSI 2010). Bacterial inoculums were prepared by cultivating test strains onto nutrient agar (NA) plates supplemented with (Ampicillin 10 µg/mL or Ceftriaxone 30 µg/mL) as an inducer for β-lactamases production, plates were incubated at 37°C for 18-24 h. 3-5 identical colonies from each plate were picked, transferred into 5 mL of 0.9% sterile saline solution and adjusted to 0.5 McFarland standard (1-2×10-8 CFU/mL).

2.4. Preparation of Actinomycetes fermentation broth

All Actinomycetes isolates were streaked on Glucose Yeast malt (GYM) medium (Glucose: 4 g/L, Yeast extract: 4 g/L, Malt extract: 10 g/L, CaCO3: 2 g/L). Pure colonies were obtained, picked and inoculated into an inorganic salt starch broth medium (ISP No.4) containing (g/L): Soluble Starch 10, Dipotassium hydrogen orthophosphate 1, Magnesium sulphate.7H2O 1, Sodium chloride 1, Ammonium sulphate 2, Calcium carbonate 2, Ferrous sulphate.7H2O 0.001, Manganese chloride.7H2O 0.001, and Zinc sulphate.7H2O 0.001 (Waksman and Lechevalier 1962) and incubated in rotary shaker incubator (HYSC, Korea) at 150 rpm at 28°C for 7 days. Cell-free supernatant was prepared by centrifugation at 13,000 rpm at 4°C for 10 min.

2.5. Disc diffusion bioassay and minimal inhibitory concentration (MIC)

0.1 mL of bacterial test strains were added to 15 mL of molten Mueller-Hinton Agar (Oxoid, UK), mixed well, poured into sterile Petri-dishes and allowed to stand for 10 min. Standard antibiotic discs containing 100 µg/disc piperacillin, 30 µg/disc ceftriaxone (3rd generation cephalosporin), and 30 µg/disc ceftazidime (3rd generation cephalosporin), were separately impregnated with 30 µL of Streptomyces broth. Loaded discs were aseptically placed onto the surface of the inoculated medium. Negative controls were standard antibiotic discs of piperacillin (100 µg/disc), ceftriaxone (30 µg/disc), and ceftazidime (30 µg/disc). Standard discs containing amoxicillin/clavulanic acid (20/10 µg/disc) were used as a positive control for BLIs. Loaded discs were allowed to diffuse by keeping the inoculated plates at 4ºC for 1 h, then plates were incubated at 37ºC for 18-24 h. The inhibition zone diameters (ZOI) were measured in mm. The probability of the existence of BLIs activity is detected by the enlargement of the size of ZOI around combination discs of β-lactam antibiotic and Actinomycetes broth. MIC was determined using the conventional agar plate dilution method as described by CLSI guidelines (CLSI 2010). Plates were inoculated with 5 µL of each test strain suspension (5×10-4 CFU) and aerobically incubated for 18-24 h at 37°C. MIC was defined as the lowest concentration from Actinomycetes broth showing no visible bacterial growth. Standard antibiotic discs alone were used as a negative control. The synergistic effect was evaluated by comparing the ZOI resulting from the combination discs to those resulting from β-lactam antibiotic discs alone. All assessments were done in triplicate.

2.6. Iodometric assay

The test was carried out with some modifications based on Livermore and Brown (2001) method. Penicillin hydrolysis by β-lactamase producers yields penicilloic acid, which reduces iodine, and decolorize starch-iodine complex. Pure cultures of test strains were streaked on NA plates

containing 0.2% soluble starch. The plates were incubated overnight, then flooded with 3 mL of the fermentation broth of actinomycetes isolates and incubated at 37ºC for 30 min. Excess broth was discarded, and plates were flooded with 3 mL of phosphate buffer (pH 6.4) containing iodine:potassium iodide (1:5) and Penicillin-G (50 mg/mL). β-lactamase inhibitory activity is detected by the appearance of persistent deep purple or blue color around colonies for >10 min.

2.7. Molecular identification, multi-alignment, and phylogenetic analysis

Genomic DNA of selected actinomycetes isolates was extracted using DNA-Extraction kit (Intron-Biotechnology, Korea) based on Kim *et al*. (2011) method. PCR amplification of 16S rDNA was performed using universal forward and reverse primers; 27F-primer 5′AGA GTT TGA TCC TGG CTC AG 3′ and 1492R-primer 5′CTA CGG CTA CCT TGT TAC GA 3′. DNA banding of 16S gene amplicons were visualized using UV-transilluminator (Thermo-Fisher, USA) after PCR amplification. PCR products were purified using gene JET™ DNA-purification kit (Intron-Biotechnology, Korea), and sequenced using ABI 3730xl DNA sequences. Nucleotides were compared with sequences retrieved from GenBank database at National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/GenBank/index.html) using Basic Local Alignment Search Tool (BLAST) available at (http://www.ncbi. nlm.gov/BLAST/). Sequences phylogenetic analysis was generated using MEGA-integrated software for Molecular Evolutionary Analysis (http://www.megasoftware.net/). Multiple alignments (http://multalin.toulouse.inra.fr/multalin/) were used to determine the difference in nucleotide sequence between studied samples (Stecher *et al*. 2020).

2.8. β-Lactamase inhibitory protein (BLIP) gene detection

The presence of BLIP gene in the genomic DNA of S. clavuligerus isolate S.t-6 was detected using one set of specific primers for BLIP gene from S. clavuligerus. PCR amplification was performed forward primer-ATG AGG ACA GTG GGG ATC GG and reverse primer-TTA TAC AAG GTC CCA CTG CC using PCR (Thermo-Fisher, USA). Agarose gel (1%) electrophoresis was used for the visualization of the PCR product at 80 V for 40 min in Bio-Rad submarine (8x12 cm).

2.9. Molecular docking, receptor, ligands, and analysis

Molecular docking was performed using Molecular Operating Environment (MOE) for windows, Chemical Computing group, Canada (Ye *et al*. 2020). Selected molecules are placed in an appropriate configuration to interact with a receptor, to understand the mode of interaction between the enzyme and the inhibitor. 3-D crystal structure of β-lactamase enzyme (KPC-2) complexed with vaborbactam PDB ID: 6TD0 (Rehman et al. 2019; Tooke *et al*. 2020) was downloaded from freely accessible Protein Data Bank (PDB) and loaded into MOE. Downloaded enzyme was optimized, protonated, and energy minimized using default parameters of MOE: Temperature at 300°K, pH of 7, salt concentration of 0.1, and the geometry was performed using the field strengths in the MMFF94x force field implanted into MOE software. All downloaded files were loaded into MOE, subjected to energy minimization in the MMFF94X force field to an RMS gradient of 0.05, partial charges were automatically calculated. Databases were constructed for each group of ligands within MOE and saved as mdb files. The site finder tool within MOE software was used to search for the active site within the receptor "enzyme" and creating dummy atoms at alpha sphere centers. Docking was done using MOE software, the parameters

setting for molecular docking were as follows: (i) dummy atoms were used as the active site, triangle matcher was used as the replacement methodology to find the best 20 poses of molecule docked, the retain of both the first and second scoring was set to 20, London dG as a scoring methodology (rescoring:1), force field as refinement (default values used), GBVI/WSA dG (rescoring:2) (ii) mdb files of the ligand databases to be docked was loaded for dock calculations. The conformation with the lowest docking score was chosen to study the binding orientations of the ligands. The images of 2-D interaction plot were captured using MOE ligand binding interaction and 3-D images were taken using UCSF Chimera 1.11 software.

3. Results and discussion

3.1. Screening of BLIs production

The production of β-lactamases by bacteria remains one of the most significant threats to the efficacy of this life-saving class of antibiotics. Therefore, research for new BLIs has become an urgent need to overcome the drawbacks of classical BLIs. A total of 186 actinomycetes were isolated from different regions in Egypt (Fig. 1). Most isolates belong to Streptomyces genus, with its typical morphological characteristics. Colonies were leathery, tough, earthy odor, and pigmented, with substrate and aerial mycelia (Jüttner and Watson 2007). Actinomycetes, especially Streptomyces spp. are known for their capability to produce bioactive secondary metabolites such as antimicrobial, anti-tumoral, enzymes, and enzyme inhibitors (Ahmad et al. 2017). Screening for BLIs has been carried out extensively in actinomycetes bacteria, and has resulted in the discovery of clavulanic acid and BLIPs (Tooke *et al*. 2020). The fermentation broth of the two isolates (S.t-158 and S.t-6) showed possible BLIs activity. The fermentation broth of isolate S.t-158 or S.t-6 alone didn't show any inhibition zone against all tested isolates, but when S.t-158 was combined with ceftriaxone, it inhibited all test strains. However, the combination between it and piperacillin showed inhibition against test strains E. coli-12, K. pneumonia-M.S2, and K. pneumonia-M.S3. Also, isolate S.t-6 alone didn't show any antibacterial activity against all test strains, but when combined with ceftriaxone it showed inhibition against E. coli-12, E. coli-BL21, K. pneumonia-10 and K. pneumonia-M.S1 (Table 1). This effect may be attributed to that the BLIs produced from S.t-158 and S.t-6 isolates are not the same type, since they inhibit different test strains that produce different types of β-lactamases (Tooke *et al*. 2020).

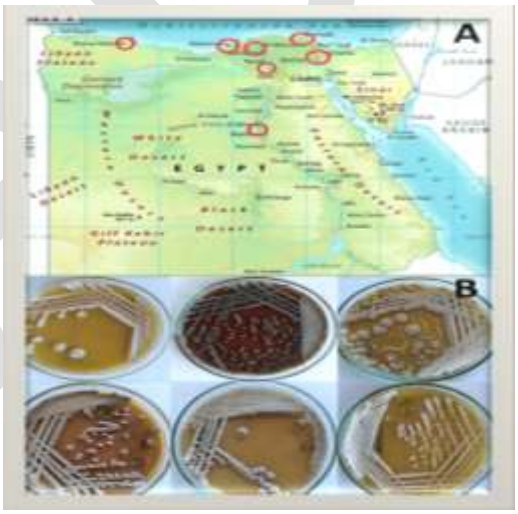

Fig 1: Soil samples collection sites from different Egyptian governorates (A). Actinomycetes isolates with different colors of aerial and substrate mycelia

Table 1. Effect of fermentation broth of Egyptian Isolate S.t-158 and S.t-6 on some clinical test strains.

Test Strains	S.t-158	S.t-6	PIR 100	CRO 30	CAZ 30	AMC 30	S.t-158 +PIR100	S.t-158 +CRO30	S.t-158 +CAZ30	S.t-6 +CRO30	S.t-6 +PIR100	S.t-6 +CAZ30
***E. coli*-12**	ND	ND	ND	ND	ND	8	9	9	ND	10	10	ND
***E. coli*-BL21**	ND	ND	ND	ND	ND	7	ND	10	ND	9	9	ND
***K. pneumonia*-10**	ND	ND	ND	ND	ND	15	ND	10	ND	10	8	ND
***K. pneumonia*-M.S1**	ND	ND	ND	ND	ND	10	ND	8	ND	11	11	ND
***A. baumannii*-1**	ND	ND	ND	ND	ND	10	ND	10	ND	ND	ND	ND
***K. pneumonia*-M.S2**	ND	ND	ND	ND	15	10	10	9	15	ND	ND	15
***K. pneumonia*-M.S3**	ND	ND	ND	ND	16	10	ND	10	18	ND	ND	16

S.t-158: Fermentation broth of S.t-158 isolate, **S.t-6:** Fermentation broth of S.t-6 isolate**, CRO30:** ceftriaxone (30 µg/disc), **PIR100:** piperacillin (100 µg/disc), **CAZ30:** ceftazidime (30 µg/disc), **S.t-158+CRO30:** isolate S.t-158 combined with ceftriaxone, **S.t-158+PIR100:** isolate S.t-158 combined with piperacillin, **CAZ30+S.t-158:** Ceftazidime combined with isolate S.t-158 **AMC30:** amoxicillin/clavulanic acid (20/10 µg/disc), **ND:** Not detected.

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3.2. Iodometric assay

Possible BLI activity can be identified with Iodometric assay. The Iodometric test is based on the detection of hydrolysis of β-Lactam molecule forming penicilloic acid that bind to iodine, making it unavailable to starch to form a purple iodine-starch complex (Lee and Komarmy 1981). Inoculated plates with β-Lactamase-producing bacteria, flooded with Iodine solution and Penicillin-G (50 mg/mL) showed a de-colorization zone around all tested strains within 5 min (Fig. 2A) indicating the presence of β-Lactamase activity of the tested strains against Penicillin-G. In another set of experiments, inoculated plates were flooded with 3 mL of fermentation broth of Streptomyces isolates, and incubated at 37ºC for 1 h before adding the Iodine solution and Penicillin-G. A purple to blue zone was developed within 5 min around test strains indicating the failure of β-Lactamases produced by these strains to hydrolyze Penicillin-G, and thus the formation of starch-iodine complexes (Fig 2B). Obtained results support the existence of BLIs in the fermentation broth of the two Egyptian Streptomyces isolates.

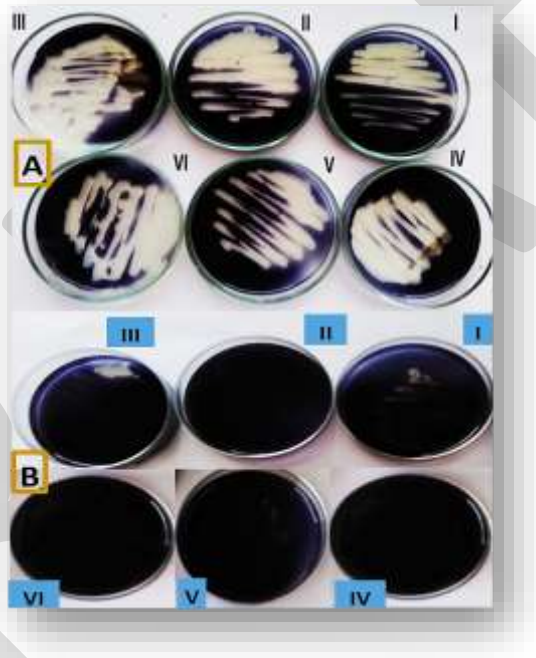

Fig.2. Iodometric assay of β-Lactamase producing-isolates before (A) and after treatment with fermentation broth of *Streptomyces* Egyptian S.t-6 or S.t-158 isolates, I: *E. coli*-12, II: *E. coli*-BL21, III: *K. pneumonia*-10, IV: *K. pneumonia* -MS.1, V: *A. baumannii* -1, VI: *K. pneumonia* -MS.2.

3.3. Molecular identification, multi-alignment, and phylogenetic analysis

Sequencing of highly conserved 16S rRNA gene facilitate differential identification among *Streptomyces* spp. (Song *et al*. 2004). PCR amplification of DNA generated a PCR product at 1500 bp (Fig. 3). According to NCBI GenBank, 16S rRNA gene sequencing of S.t-158 (862 bp) and S.t-6 (954 bp) isolates revealed a 100% and 98.96% nucleotides similarity (E-value 0.0) with *S. rochei*-T178.2 and *S. clavuligerus*-BS33 strains, respectively. Results were deposited in GenBank under

accession number MT071504 and MT071505, respectively. 16S rRNA sequences of the two Egyptian S.t-158 and S.t-6 isolates and that of *S. rochei* T178.2 and *S. clavuligerus* BS33 strains were studied through multi-alignment analysis. Ten nucleotide variations were found between 16S rRNA gene sequence of isolate S.t-6 and 16S rRNA gene partial sequence of *S. clavuligerus* BS33 strain at positions 45, 109, 265, 490, 497, 515, 665, 733, 754, and 817. The sequences of 1st fifty-hits were retrieved from the database and used to construct phylogenetic tree by Neighbor-Joining method using Molecular Evolutionary Genetics Analysis (MEGA-X) software (Stecher *et al*. 2020). Based on phylogenetic analysis the sequences can be classified in two clades where, 16s rRNA sequence of S.t-158 isolate was grouped in the same clade with *S. rochei* strain T178.2 with 100% nucleotide similarity. While, 16s rRNA sequence of S.t-6 isolate was grouped in the same clade with *S. clavuligerus* strains BS33, CSSP040, ATCC 27064, JCM 4710, and NBRC 13307 (Fig. 3).

3.4. BLIP gene detection

Naturally occurring proteinaceous inhibitors of β-lactamases, such as BLIP, BLIP-I, and BLIP-II have been detected in the fermentation broth of *Streptomyces* spp. BLIP was first isolated from *S. clavuligerus* in 1990, BLIPs have been found to be potent, competitive inhibitors of class A β-lactamases (Eiamphungporn et al. 2018). BLIP is a low molecular weight protein (17.5-kDa), it binds to and inhibits class A β-lactamases from *S. aureus*, *B. licheniformis*, and *B. cereus*, but does not efficiently bind to class B, C, or D β-lactamases (Brown and Palzkill 2010). BLIP mechanism of inhibition depends on the non-covalent competitive binding to β-lactamases, inserting β-hairpin loops into β-lactamase active site, masking it from binding and hydrolyzing β-lactam antibiotics (Law et al. 2018). Molecular detection of BLIP gene confirmed the ability of the Egyptian S.t-6 isolate to produce BLIP. PCR amplification has generated fragment of ~600 bp (Fig. 3D) which confirm the ability of *S. clavuligerus* Egyptian S.t-6 isolate to produce one of the BLIPs. Same result was obtained by Doran *et al*. (1990) who detected BLIP gene length with 606 bp from *S. clavuligerus* and its fermentation broth inhibited *B. cereus* penicillinase.

Generally, *S. clavuligerus* is known to produce clavulanic acid, which is a potent BLI when applied in low levels or when culture filtrate was added to β-lactamase-labile antibiotics, widening the antibiotic spectrum to include many resistant β-lactamase-producing microbes (Reading and Cole 1977). Likewise, Awad and El-Shahed (2013) reported that the fermentation broth of *Streptomyces rochei* NRC-77 showed BLI activity against penicillin-resistant *E. coli* strain and was identified as clavulanic acid.

3.5. Molecular docking studies

In-silico docking of β-lactamase enzyme (KPC-2) against four sets of selected ligands was performed using MOE to investigate possible binding mode of interactions of ligands within the enzyme active site. β-lactamases in this study are classified as class-A serine β-lactamases, they are key antibiotic resistance in Gram-negative, they hydrolyse β-lactam antibiotics through the attack of β-lactam ring by the nucleophilic serine to form an acyl-enzyme complex (Fig. 4) followed by deacylation to release the inactive hydrolyzed product (Tooke et al. 2020). Among *S. rochei* secondary metabolites that have been studied is **Borrelidin** (**PubChem CID**:6436801) an 18-membered polyketide macrolide that showed the highest binding energy value of -6.531 (Kcal/mol), the nitrogen atom (no. 7) of the compound forms hydrogen bonds with residues Ser70(A) at a distance of 2.66 Å and Ser70(B) 2.84 Å within KPC-2 active site.

Also, the carbonyl oxygen (no. 5) of **SRB-2** (**PubChem CID**:132570971) forms hydrogen bond with residues Ser70(A) at distance of 2.59 Å and Ser70(B) at distance of 2.75 Å with a binding energy value of -5.631 (Kcal/mol). **Harmine** (**PubChem CID**:5280953) a hallucinogenic alkaloid showed the highest binding energy value of -5.699 (Kcal/mol), the nitrogen atom (no. 2) of the compound forms hydrogen bonds with residues Ser70(A) at distance of 3.03 Å and Ser70(B) at distance of 3.09 Å ring (no. 6) forms arene-H bonds with residues Asn132 and Thr237, respectively, also ring (no. 5) forms arene-H bond with residues Thr237 **(**Fig. 4). Results suggest that these bioactive compounds represent possible inhibitors for KPC-2, all compounds form hydrogen bonds with the enzyme active site residue Ser70 or Ser130 and prevent it from attacking the β-lactam antibiotics (Table 2) (Tooke *et al*. 2020).

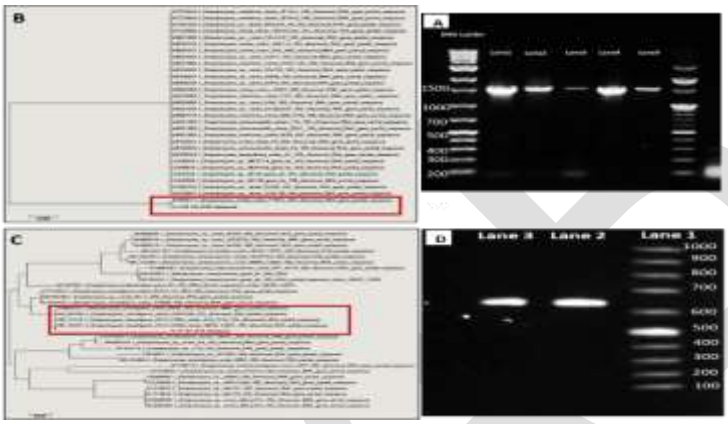

Fig. 3. PCR amplification of genomic DNA (A) of the selected isolates with 16S rRNA universal primers 27F-1492R, Lanes contain PCR products from DNA: Lane 1 & 4: Isolate-S.t.-158, Lane 2, 3 & 5: Isolate S.t.-6. Neighbor-joining tree to illustrate the taxonomical topology of isolate S.t-158 (B) and S.t-6 (C). Amplified fragment of BLIP gene detected in genomic DNA of *S. clavuligerus* Egyptian isolate S.t-6, Lane 1: DNA Marker, Lane 2 and 3: *S. clavuligerus* isolate S.t-6 (D)

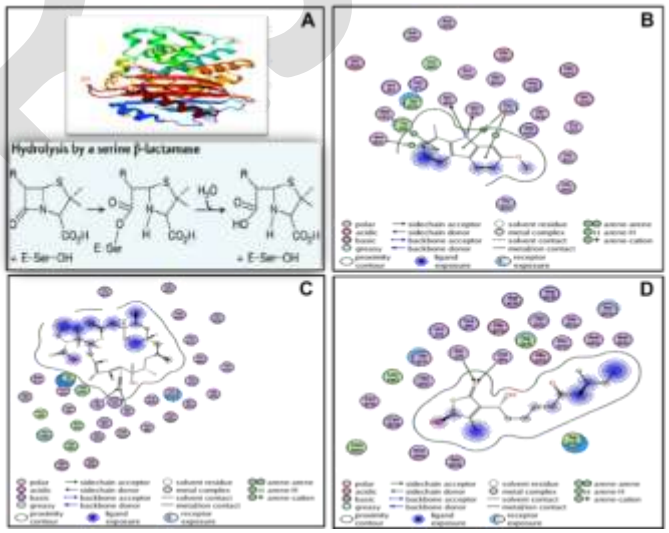

Fig. 4. The crystal structure of target enzyme KPC-2, (Tooke et al. 2020) and β-lactam hydrolysis by serine β-lactamases (A). Interaction diagram of the complex between Harmine (B), Borrelidin (D), and SRB-2 (E) with the active site of β-lactamase enzyme (KPC-2) using molecular docking analysis.

Table 2. Summarized ligands interaction within the active site of β-lactamase enzyme KPC-2.

Compound	Score	RMSD	Ligand	Receptor	Interaction	Distance	E (Kcal/mol)
Harmine	-5.699	0.5942	N-2	Ser70(A)	HBD	3.03	-0.6
			N-2	Ser70(B)	HBD	3.09	-1.8
Borrelidin	-6.531	1.118	N-7	Ser70(A)	HBA	2.66	-1.7
			N-7	Ser70(B)	HBA	2.84	-2.5
SRB-2	-5.631	1.07	O-5	Ser70(A)	HBA	2.59	-1.4
			O-5	Ser70(B)	HBA	2.75	-1.9

RSMD: Root-mean-square deviation of atomic positions, **HBA**: Hydrogen bond acceptor, **HBD**: Hydrogen bond donor.

4. Conclusions

This study demonstrated natural BLIs activity produced by two Egyptian *Streptomyces* isolates (S.t-158 & S.t-6). The two Egyptian *Streptomyces* isolates were molecularly identified as *S. rochei* and *S. clavuligerus*. Both strains exhibited clavulanic acid, while *S. clavuligerus* S.t-6 exhibited one of the BLIP produced by ~600 bp detected BLIP-gene. The whole results could enhance the activities of β-lactam antibiotics against multi-drug resistant bacteria. Studies on enzyme kinetics, and molecular docking indicated the efficacy potential of selected bioactive compounds in the selected candidates. However, synergistic efficacy studies with many β-lactam drugs should be the subject of future studies. By employing a series of dynamics simulation and molecular docking, it is clear that the binding interactions between selected compounds and catalytic amino residues -Ser70 or -Ser130 of KPC-2 explain the strong affection between the selected bioactive molecules in the enzyme active site. The binding energy obtained from molecular docking simulation strongly suggests that selected natural bioactive compounds possess strong capability being BLIs, where they change the conformational state of KPC-2, thus inhibiting it.

Contributions

Conceptualization and supervision: Hanafy Hamaza and Rateb Abbas.
Laboratory work, and data analysis: Borhan Eltabey, Rateb Abbas, and Noha Sorour.
Writing and editing: Noha Sorour, Borhan Eltabey, and Rateb Abbas.
All authors read and approved the final manuscript.

Supplementary data are available upon request.

Conflict of Interest Statement

All authors declare that there are no financial/commercial conflicts of interest.

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