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Xenoprotec: Antimicrobial Agent Derived from *Xenorhabdus indica*

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Abstract: The use of Xenoprotec in medical purposes as new antimicrobial agent with low production cost and affecting antibiotic resistant bacteria as *Staphylococcus aureus* and *Pseudomonas fluresence* had been carried out. Four bacterial strains were isolated (*Xenorhabdus indica*, *Xenorhabdus nematophila*, *Xenorhabdus* sp. and *Photorhabdus lumenscens laumondii*) from the four entomopathogenic nematodes species, *Steinernema abbasi, Steinernema carpocapsae* DD_{136}, *Steinernema carpocapsae all* strain and *Heterorhabditis bacteriophora* HP_{88}, respectively. The morphological and biochemical identification of the four bacterial isolates was confirming their genus characters and followed by genotyping identification using 16SrDNA technique with its specific primers. The antimicrobial activity was detected in the cell free supernatant (CFS) when screened against indicator pathogenic strains of both Gram +ve bacteria (*Staphylococcus aureus* and *Enterooccus fecalis*) and Gram –ve bacteria (*Pseudomonas flurecens, Vibrio anatum, Salmonella typhemurium* and *Aeromonus hydrophila*). We recorded that *X. indica* CFS gives the highest inhibitory reaction as it induced inhibition zone ranging from (9 to 35 mm) against all tested strains except *Vibrio anatum* and *Aeromonus hydrophila* strains which were not affected. The CFS of the selected strain (*X. indica*) was chemically fractionated into three main groups: protein fractions, lipid fractions and carbohydrate fractions. The three groups were tested against both Gram positive (*Staphylococcus aureus* and *Enterooccus fecalis*) and Gram negative (*Salmonella typhemurium* and *Pseudomonas flurecens*) indicator pathogenic microorganisms. The antimicrobial activity was estimated in the protein fractions only with inhibition zone 13, 16, 14 and 11 mm, respectively. The antibacterial activity was retained in the protein fractions having a molecular weight ranging from 100 to 300 kDa. Electrophoretic analysis of active protein fraction on 12% SDS-PAGE revealed that its molecular weight is 220 kDa (Xenoprotec) with recovery 1.5% from CFS total proteins. These results will open a fruitful scientific argue about the nature of the *Xenorhabdus spp*. antibiotics as it is the first time we recorded with persuasive evidences that the antibiotic activity was remained in the protein fraction.

Kew words: *Xenorhabdus* · *Photorhabdus* · Xenoprotic · Antimicrobial proteins · Entomopathogenic nematodes · *Staphylococcus aureus* · *Enterooccus fecalis* · *Salmonella typhemurium* and *Pseudomonas flurecens*

INTRODUCTION

Xenorhabdus nematophila is a Gram-negative symbiotic bacterium of an entomopathogenic nematode, *Steinernema carpocapsae* and is belonging to family *Enterobacteraceae* [1]. After entering the nematodes through insect natural openings they deliver the symbiotic bacteria from their intestine to the hemocoel of the insect hosts [2]. The bacteria will be logarithmically replicates in the insect rich haemolymph, then cause a depress to the host immune capacity against both the symbiotic nematodes and the bacteria themselves by inhibiting phospholipase A of the insect host [3,4]. At this immuno-compromised state, the infected insect can be susceptible to other saprophytic pathogens. To maintain monoxenic condition, *X. nematophila* synthesizes and secretes broad spectral antibiotics as well as narrow spectral bacteriocins [5,6], consequently inhibiting growth of other pathogens. The monoxenic state leads to lethal septicemia of the target insect, which is required for development of the symbiotic nematodes in the cadaver [7]. Various kinds of antibiotics

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against bacteria and fungi are synthesized and secreted during the bacterial cultures growth of two related entomopathogenic bacteria, *Xenorhabdus* and *Photorhabdus* [8]. Among them, indoles [9], dithiolopyrrolones [10] and xenocoumacins [11] are the antibacterial compounds synthesized by *Xenorhabdus* spp. The mechanism of antibacterial activity has not been elucidated, but primarily speculated as inhibition of RNA and protein synthesis by an accumulation of the regulatory nucleotide, guanosine-3 pyrophosphate (ppGpp) especially in indole compounds [12]. This antibiotic aspect of the entomopathogenic bacteria reflects the potential medical and agricultural importance of their metabolic products [13].

The antibiotics of the three *Xenorhabdus* strains (*X. nematophila*, *X. budapestensis* and *X. szentirmaii*) were powerful in bioassay against mastitic isolates of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* isolated from dairy cows but the sensitivity of the isolates differed from each other. So, *Xenorhabdus* antibiotics are effective against mastitic isolates and should be further evaluated for their potential in both prevention and control of mastitis [14].

This study has focused on the analysis of antibacterial spectrum of Xenoprotec against animal pathogenic bacteria, which have not been thoroughly evaluated as antibacterial targets of the three *Xenorhabdus* spp. (*X. indica*, *X. nematophila* and *X. nematophila all*) and one *Photorhabdus* spp. (*Photorhabdus lumenscens laumondii*) and has reported the identification of a new compound synthesized by them.

MATERIALS AND METHODS

1. Nematode propagation: *Steinernema abbasi, Steinernema carpocapsae* DD_{136}, *Steinernema carpocapsae* all strain and *Heterorhabdus bacteriophora* HP_{88}, nematode symbiotic for the bacteria, *X. indica*, *X. nematophila* and *X. nematophila* sp.) and one *Photorhabdus* sp. (*Photorhabdus lumenscens laumondii*), The nematodes were maintaining in the laboratory by passaging it through last-instars larvae *Galleria mellonella* according to Dutky *et al*., [15]. in the Department of Parasitology and Animal Diseases, National Research Centre, Dokki, Egypt.

2. Bacterial strains and growth conditions: Bacterial isolates were isolated from its symbiotic nematode by the method of Akhurst [1]. For each subculture, the phase status was determined by culturing on Brain heart agar, MacConky agar and NBTA (2.3% nutrient agar [Difco], 0.0025% bromothymol blue [Merck], 0.004% 2,3,5-triphenyltetrazolium "Merck") and measuring antibacterial activity against *Pseudomonas fluorescens* (indicator strain). Bacterial cells were grown in Brain heart infusion broth for liquid cultures and on Brain heart infusion agar for solid cultures.

3. Identification of bacterial isolate: Gram stain of the bacterial isolate was performed according to Cruickshank [16]. The activities of oxidase were examined by the procedure of Sneath [17]. Colony characters were examined on brain heart agar, MacConky agar and NBTA plates. The phenotypic identity of the bacterial isolates were confirmed using morphological, biochemical and physiological characteristics as defined by Thomas and Poinar [18,19] and Boemare and Akhurst [20].

4. Bacterial identification using 16SrDNA technique: Bacterial identification by 16SrDNA was carried out according to Jung *et al*. [21]. Total genomic DNA was extracted from the bacterial isolate [22] by using bacterial genomic DNA purification kit (Edge BioSystems) USA. The bacterial cells were lysed using Spheroplast buffer, lysis 1&2 solutions. All bacterial cell residues and proteins were adsorbed by Advamax beads. After extraction, the genomic DNA was precipitated with equal volume of isopropanol. Pellet DNA was washed 2x with 70% ethanol and dry overnight. The purified DNA was resuspended with deionized distilled water and used for PCR template.

Universal PCR primers [23] were used to amplify the 16SrDNA of the bacterial isolate. Forward and reverse primers were '5-AGG GYT ACC TTA CGA CTT-3' and '5-GTT TGA TCC TGG CTC AG-3', respectively. The PCR reaction was done using Go Taq PCR Core system "Promiga" USA Cat. # A9281. All PCR tubes were kept on ice and the follwing PCR mixture was added to each tube: 5µl of 10x PCR buffer, 0.8µl of dNTP mix (12.5 mM each), 2µl of 25 pmol forward primer, 2µl of 25 pmol reverse primer, 0.25µl of Taq DNA polymerase (5 units/µl), 2µl $MgCl_2$ 25 mM solution, 2 µl of template DNA and 34.95µl f sterile distilled water. The negative controls contained all components for the PCR except the template DNA. Conditions were, preheating at 95°C for 2 min, 30 cycles of 95°C for 30 sec, 50°C for 30 sec, 72°C for 1 min and final extension was carried out at 72°C for 5 min.

Total bacterial genomic DNA and the PCR product were separated in a 0.7% agarose gel containing 0.5µg of Ethidium bromide/ml where 50V was applied for 45 min and then visualized under UV light with an imager (the imager, software version 2.03; Appligene Inc.). Set of 100bp DNA ladder with stain "SibEnzyme Ltd., Russia" was used.

5. Fermentation properties and growth curve of the isolated strain: Measurement of growth was carried out according to Wang *et al*., [24]. Cellular growth was measured by optical density of culture at 600 nm, culture pH, Colony Forming Units (CFU) and biomass concentration (g/dry weight) were determined using calibration curve.

6. Antibiotic Purification and characterization of its nature: The bacterial isolates were grown on brain heart infusion broth at 23°C for 48h. The Cell Free Supernatant (CFS) was collected by centrifugation at 10,000 xg for 30 min at 4°C the CFS was sterilized by filtration using 0.22µm Amicon filter under aseptic conditions. The CFS was fractionated according to their chemical nature by dissolving the lipid in equal volume of organic solvent (chloroform) at room temperature for 60 min with contentious shaking. The dissolved lipid layer was separated by careful aspiration. The protein content of CFS was separated by precipitation using ammonium sulfate as mentioned by Carbral *et al*. [25]. Ammonium sulfate was gradually added with stirring to the CFS to 90% saturation. The precipitate was collected by centrifugation at 7,000 xg and then dissolved in 50mM sodium phosphate buffer, pH (7.2). The ammonium sulfate precipitation was dialyzed against distilled water overnight. The remaining water soluble carbohydrates was concentrated by sucrose over night followed by dialyses against distilled water overnight. The antimicrobial activities in lipid, protein and carbohydrate fractions were tested as mentioned by Cherif *et al*. [26].

7. Characterization of protein fractions owns the antimicrobial properties: The protein fractions posies the antimicrobial activity was monitored by 7.5 native PAGE to identify the active protein fractions and number of protein fractions. The 100kDa Vivaspine concentrator maintained the active antimicrobial protein fraction which is one major band seen on 12% SDS-PAG (Fig. 4). The antimicrobial activity was tested against two main sensitive organisms (*Staphylococcus aureus* and *Salmonella typhemurium*) both of them showed a significant sensitivity for the group own more than 100 kDa molecular weight.

8. Assay of antibacterial activity: The antibacterial activity of the tested materials was performed using antibacterial spot technique as mentioned by Cherif *et al*. [26]. In a clean sterile 9 cm Petri-dish 6 ml of sterile Miller Hinton agar was poured. Ten microliter of tested sample were mixed with equal volume of chloroform, spotted on the agar and dried for 30 min at 37°C. Another 6 ml of Miller Hinton agar contain bacterial suspension of tested bacterial pathogens was mixed and poured at 40-45°C. The clear zone were measured in right axis from the boundaries [21]. The tested pathogens of animal origin were: *Pseudomonas flourecence, Salmonella typhemurium*, *Staphylococcus aureus* and *Enterooccus fecalis*. A collection of antibiotic disks were used as a control in a comparative study including: Vancomycin (VA), Norfloxacin (NOR) and Novobiocin (NV).

9. Recovery process: The protein concentration was monitored during the process of purification according Bradford [27]. The protein recovery and the specific activity were recorded as follow:

The specific activity (S.A) was calculated as

S.A. = Unites / total proteins in microgram

one unite: Is the unite that can induce 1 mm inhibition zoon on Miller Hinton agar inoculated with *Pseudomonas fluorescence* after 24h of incubation at 37°C.

RESULTS

Isolation and identification of bacterial isolates: The direct microscopical examination and growth on MacConky agar reveled that the isolated four strains are belonging to Gram negative Enterobactereacae group. They also belonging to Genus *Xenorhabdus* and Genus *Photorhabdus* which confirmed later by biochemical characters. The resulted colonies on Brain Heart agar were cream white colonies with regular round edges and translucent appearance with convex surface (Fig. 1a) and those grown on MacConky agar were capable to adsorb the neutral red stain (Fig. 1b) while those grown on NBTA were capable to adsorb bromothymole blue and triphenyltetrazolium stain giving dark blue- green colonies in alkaline pH (Fig. 1c). The biochemical characters of the isolated four strains are recorded in Table 1 and all of the four strains were the same.

Table 1: Biochemical identification of the isolated strain

Nematod Strain	*Heterorhabdus bacteriophora* Subgroup Hp_{88}	*Steinernema carpocapsae* Dd_{136}	*Steinernema carpocapsae*	*Stinernema abbasi*
Microorganism associated	*Photorhabdus lumenscens laumondii*	*X. nematophila all*	*X. nematophila*	*Xenorhabdus indica*
Gram stain	Gram –ve short bacilli (or coccobacilli)			
Catalase	-ve	-ve	-ve	-ve
Oxidase	+ve	+ve	+ve	+ve
Urease	-ve	-ve	-ve	-ve
Glucose fermentation	-ve	-ve	-ve	-ve
Maltose fermentation	+ve	+ve	+ve	+ve
Succrose fermentation	+ve	+ve	+ve	+ve
Manitol fermentation	+ve	+ve	+ve	+ve
Lactose fermentation	+ve	+ve	+ve	+ve
Blood agar haemolysis	-ve	-ve	-ve	-ve
Motility	-ve	-ve	-ve	-ve
Pigment production on NA	+ve (Red pigments)	+ve (Brown pigments)	+ve (Brown pigments)	+ve (Brown pigments)
L-Cystien hydrolysis	-ve	-ve	-ve	-ve

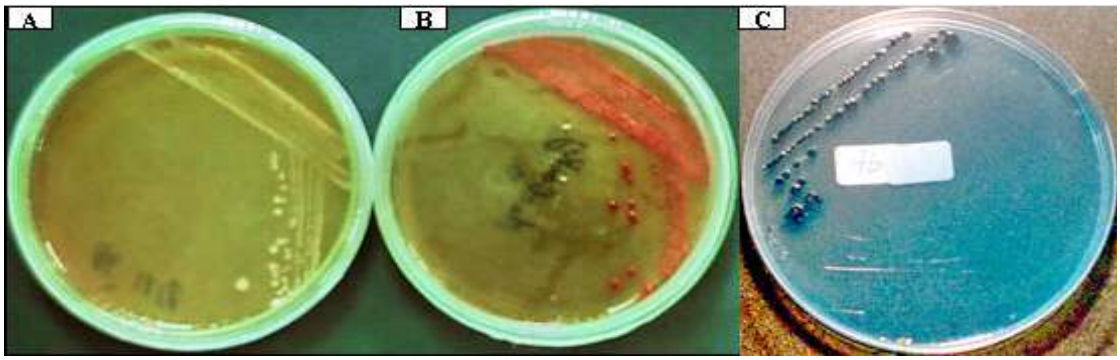

Fig. 1: *Xenorhabdus* spp. *indica* grown on Brain Heart Agar (A), MacConky agar (B) and NBTA (C)

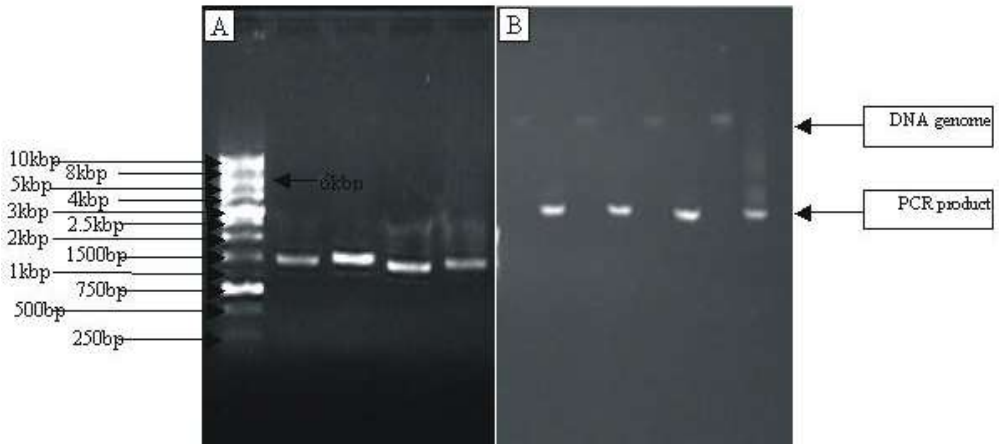

Fig. 2: (A)PCR products screening on 0.7% agarose gel stained with Ethedium Bromide and examined under UV. 10 kbp ladder Lane (1), *Xenorhabdus indica* Lane (2), *X. nematophila* lane (3), *X. nematophila all* Lane (4) and *Photorhabdus lumenscens laumondii* Lane(5). (B) both extracted total bacterial genome and PCR product for each strain subsequently as follow: *Xenorhabdus indica* Lane (1&2), *X. nematophila* Lane (3&4), *X. nematophila all* Lane (5&6) and *Photorhabdus lumenscens laumondii* Lane(7&8)

Table 2: The fermentation process results after 24 and 48h illustrated the O.D. 600 and CFU versus pH for the four isolated strains

Bacterial strain	Time of incubation	O.D. 600 nm CFUx 10^9/ml	O.D. at 660 nm CFUx 10^9/ml	pH
X. indica	24	0.185	0.140	7.2
	48	3.331	3.075	8.4
X. nematophila	24	2.486	2.301	8.0
	48	3.106	2.880	8.5
X. nematophila all	24	2.652	2.444	8.5
	48	3.244	2.963	9.5
P.lumenscens lumenscens laumondii	24	2.430	2.222	8.0
	48	3.131	2.931	8.5

Table 3: Antimicrobial inhibitory effect of CFS (10µl) of the four isolated strains on indicator pathogenic strains: Gram +ve (*Staphylococcus aureus* and *Enterococcus faecalis)* and Gram -ve *(Pseudomonas flurecens, Vibrio anatum, Salmonella typhemurium* and *Aeromonus hydrophila*) comparing with the inhibitory effect of three standard antibiotic discs

Tested bacterial strains	*P. lumencence lumindii*	*X. nematophila*	*X. nematophila all*	*X. indica*	VA	NV	NR
S. aureus	-ve	-ve	-ve	+ve(35 mm)	+ve(21 mm)	+ve(38 mm)	+ve(26 mm)
Entero. fecalis	-ve	-ve	-ve	+ve(15 mm)	+ve(24 mm)	+ve (18 mm)	+ve(16 mm)
P. flurecens	+ve(11 mm)	+ve(15 mm)	+ve(16 mm)	+ve(15 mm)	-ve	- ve	+ve(38 mm)
V. anatum	-ve	-ve	-ve	-ve	-ve	-ve	+ve(25 mm)
Salmonella typhymurium	-ve	-ve	-ve	+ve(9 mm)	-ve	+ve (7 mm)	+ve(18 mm)
A. hydrophila	-ve	-ve	-ve	-ve	+ve(10 mm)	+ve(24 mm)	+ve(30 mm)

VA: Vancomycine, NV: Novobiocine, NOR: Norfloxacine

Bacterial identification by 16SrDNA: The identification of the isolated strains were confirmed by using specific PCR probe using specific primers where both the extracted bacterial DNA genome (Fig. 2b) and the resulted PCR product (1.4 kbp) were screened using 0.7 agarose gel electrophoresis (Fig. 2 a&b). the resulted PCR products were 1.4 kbp as what determined through plotting the ladder base pair versus the relative mobility (*Rf*) of the PCR products.

Measurement of growth: In this study we compared the growth curve via monitoring the optical density at 600 nm wave length of the fermentation media in correlation with the developed pH versus the colony forming unite in order to determine the growth phase and it is obvious from Table 2 that there is a direct relationship between the optical density, pH and CFU. By simple observation for the results we can notice that the perfect time for harvesting the antimicrobial agent is after 48h of fermentation where the pH ranging from 8.5 to 9.

Assay of antibacterial activity: Regarding to Table 3 it is noticeable that the CFS induced the highest inhibitory effect on *P. flurecens* and *S. aureus* while the remaining three isolates only affect *P. flurecens* in variable inhibition zone ranging from 11 to 16 mm in width (Table 3).

Antimicrobial agent nature: Through testing the antimicrobial activity of protein fractions, lipid fractions and carbohydrate fractions against Gram negative and Gram positive pathogens it was obvious that the antimicrobial activity was detained in the protein fraction only. *Staphylococcus aureus* was the most sensitive Gram positive tested microorganism while *Salmonella typhemurium* was the most sensitive Gram negative microorganism (Table 4).

The active protein fractions were passed through a molecular weight sieve of different molecular weight cut-off (10, 30, 50, 100 and 300 kDa). The resulted protein fractions were assayed for their antibacterial activities, where the antibacterial activity was remained in the protein fractions having a molecular weight ranging from 100 to 300 kDa. Native electrophoretic analysis of the total protein fractions extracted from *X. indica* CFS cleared that it consist of ten protein fractions Fig. 4A. However by using molecular weight sieve of different molecular weight cut-off (10, 30, 50, 100 and 300 kDa.) the antimicrobial activity remained in the protein fraction of molecular weight ranging from 100 to 300 kDa Fig. 5A&B. Electrophoretic analysis of active protein fraction on 12% SDS-PAGE revealed that its molecular weight is about 220 kDa Fig. 4B. These results were confirmed via *in situ* bio-assaying of the active protein fraction on gel against *Pseudomonas flourecenc* Fig. 5C.

Table 4: Screening the antimicrobial activity and inhibitory zone (in mm) in the protein, lipid and carbohydrate fractions against pathogenic Gram positive and Gram negative microorganisms

Tested bacterial strains	Bacterial Strains	*X. indica CFS*	*X. indica protein fractions*	*X. indica CHO fractions*	*X. indica Lipid fractions*	NR
Gram +ve micro-organisms	*Enterococcus fecalis*	+ ve(15±1 mm)	+ve (13±0.5 mm)	-ve	-ve	+ve(26 mm)
	Staphylococcus aureus	+ ve(20±0.5 mm)	+ve(16±0.5 mm)	-ve	-ve	+ve(16 mm)
Gram -ve micro-organisms	*E. coli*	-ve	-ve	-ve	-ve	+ve(19 mm)
	Salmonella typhemurium	+ve(13±0.1 mm)	+ve(14±1 mm)	-ve	-ve	+ve(38 mm)
	Pseudomonas fluorescens	+ve(12±1 mm)	+ve(11±1 mm)	-ve	-ve	+ve(25 mm)
	Aeromonus hydrophila	-ve	-ve	-ve	-ve	+ve(18 mm)

Table 5: The recovery % of active antimicrobial protein during the process of fractionation and purification in correlation with active units/ml and the specific activity

Recovery process	Sample volume in ml	Protein concentration in µg/ml	Total protein in µg	Recovery %	Units/ml	Specific activity
Filtrated supernatant	100.00	10.52	1000.52	100.0	2000	0.0199
Ammonium sulfate ppt	35.00	4.10	143.50	14.3	-------	---------
Vivaspine concentrated sample	1.83	8.52	15.59	1.5	1600	1.0280

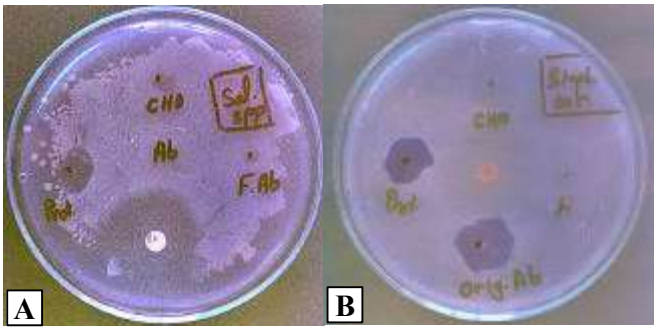

Fig. 3: Inhibitory effect of the protein, lipid and carbohydrate fractions of *X .indica* CFS on the growth of *Salmonella typhymurium* (A) and *Staphylococcus aureus* (B)

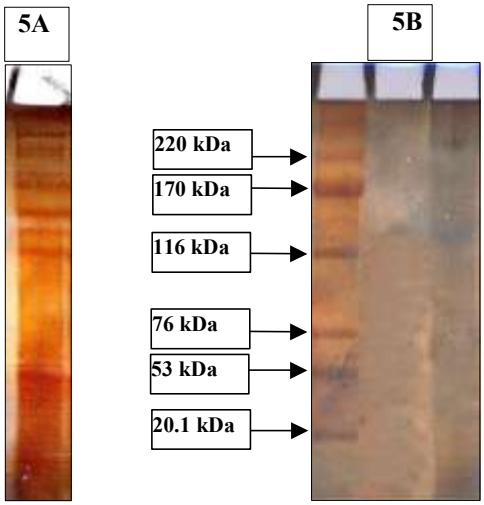

Fig. 4: Electrophoretic analysis of *X. indica* total protein fractions by 7.5% native-PAGE. (5B): SDS-PAGE analysis of the active protein fraction (220 kDa) lane 2 versus molecular high weight markers [Myosin 220 kDa, α2-Macroglobulin 170 kDa, β-Galactosidase 116 kDa, Transferrin 76 kDa, Glutamic dehydrogenase 53 kDa and Trypsin inhibitor 20.1 kDa]

Fig. 5: The antimicrobial activity of *X. indica* CFS protein fractions >10 kDa and >100 kDa against *Salmonella typhemurium* (A) and against *Staphylococcus aureus* (B). In situ inhibitory effect of protein fraction in plyacrylamide gel lane against *Pseudomonas flourecence* (C)

Recovery process: The antimicrobial protein fraction recovered from total extracted proteins in the *X. indica* CFS represented only 1.5% approximately and yield 1600 active unit/ml while the crude protein extract yielded 2000 unit/ml (Table 5). However the specific activity was remarkably increased from 0.0199 to 1.028 (i.e. increased about 51 times).

DISCUSSION

The developing microbial resistance to the newly discovered antibiotic and the adverse side effects of synthetic and semi-synthetic antibiotics inspire us to find out a new antibiotic derived from microbial source through natural antagonism programme developed in the Ecological system. One of the most interesting microorganisms was the symbiotic bacteria developed in the entemopathogenic nematodes which maintain the monoxenic status in the infected insects by secreting broad spectral antibiotics as well as narrow spectral bacteriocins and prevents other putrefactive bacteria from growth. The bacteria of interest in this study is *X. indica* which isolates from entomopathogenic nematode *Steinernema abbasi*.

The previous literatures [28] indicated that the antibacterial proteins needed 6-8h to be synthesis and continue to be produced for 72h and we agree with that however these antimicrobial protein dose not inhibit or destruct symbiotic bacteria (*X. indica*) but the accumulation of flhDC-dependent cytotoxin, a 10,790-Da peptide called á-Xenorhabdolysin in the growth media targeted the plasma membrane of insect hemocytes [28] and this is obvious from the growth curve where the pH elevated to 9.5 after 48h.

In early time Maxwell *et al*. [29] mentioned the production of antimicrobial proteins from *X. nematophila* in the media and this is may be a preliminary discovery of these antimicrobial proteins. However we disagree with him about the specificity of these antimicrobial protein because these proteins have the selective ability to distinguish between two bacterial tribe within the same family as we can see from the results it affect *Salmonells* spp. and could not affect on *E. coli* (Table 4). Also we can notice the great correlation between F. *Pseudomonadacae* and F. *Aeromonadacae* however only F. *Pseomonadacae* are affected, So these antimicrobial proteins had a high specificity.

Maxwell *et al*. [29] recorded that antibacterial activity of some *X. nematophil*a isolates was greatest against bacteria of the family *Bacillaceae*, moderate against those of the family *Pseudomonadaceae* and least against the family *Enterobacteriaceae*. However in this study we recorded that the antimicrobial protein (Xenoprotec) produced from *X. indica* had a great effect on Genus *Staphylococcus* and Genus *Enterococcus faecalis* than G. *Salmonella* than G. *Pseudomonas* while G. *Escherichia* and family *Aeromonadacae* did not show any effect. Antibacterial proteins affected *Staphylococcus aureus* [Penicillin Resistant Strains (PRS) and Methicillin Resistant Strains(MRS)] and *Pseudomonas flourecence* (PRS) [30, 31] which indicate that these antibacterial proteins are related to a new antibiotic generation.

Xenoprotec is antimicrobial protein of high molecular weight 220 kDa. The known antimicrobial proteins are belonging to either bacteriocins or antimicrobial peptides. Bacteriocins are proteinaceous compounds lethal to bacteria other than the producing strain. As a group, bacteriocins are heterogeneous and they are classified largely based on their molecular weight differences [32]. Some bacteriocins are peptides consisting of only 19 to 37 amino acids, whereas others are large peptides with molecular weights of up to 90,000 Da. Some small

bacteriocins contain unusual amino acids originating from modifications of conventional amino acids after translation. The activity spectrum of bacteriocins can be narrow and confined to inhibition of closely related species, or it can be relatively broad and include many different bacterial species [33]. While Antimicrobial peptides are typically relatively short (12 to 100 aminoacids), are positively charged (net charge of 2 to 9), are amphiphilic and have been isolated from single-celled microorganisms, insects and other invertebrates, plants, amphibians, birds, fish and mammals, including humans [34, 35].

The effect of Xenoprotec on both Gram +ve and -ve resistant microorganisms raise the inquire about its mode of action. The previous authors [9, 10, 11, 12] revealed the antimicrobial activity induced from *Xenorhabdus* bacteria to many chemical compounds like indoles, dithiolopyrrolones and xenocoumacins and non of them was protein in nature. The mechanism of antibacterial activity was related to their ability to inhibit RNA and protein synthesis by an accumulation of the regulatory nucleotide, guanosine-3 pyrophosphate (ppGpp) especially in indole compounds but because the nature of Xenoprotec differ from these compounds we assumed that the mode of action will be different. Xenoprotec needs further investigation to study its mode of action and formulation process via testing it *in vivo* challenge. Also the high molecular weight of Xenoprotect diminished the possibility of membrane-permeabilizing mechanisms depending upon interaction with the bacterial cell membrane in several prominent models called variously (the barrel-stave, carpet, detergent, toroidal pore and aggregate models) have been proposed [36], while the inhibitory effect of β-lactamase resistant microorganisms increase the possibility of cell non-membrane-permeabilizing mechanism through translocation across the membrane and into the cytoplasm without causing major membrane disruption.

In sum the obtained results paved the way for use of Xenoprotec in medical purposes as new antimicrobial agent with low production cost and affecting antibiotic resistant bacteria (Penicillin Resistant Strains and Methicillin Resistant Strains) as *Staphylococcus aureus* and *Pseudomonas fluresence*.

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