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Improvement of Bacteriocin Production by *Bacillus subtilis* NK16via Elicitation with Prokaryotic and Eukaryotic Microbial Cells

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Abstract: Since microorganisms normally exist with other species in nature, they have developed complex metabolic as a result of such interspecies interactions. Our strategy for the elicitation of *Bacillus subtilis*NK16 presented in this study is based on utilizing some aspects of these interactions by introducing microbial cells to *Bacillus subtilis* NK16 culture in order to enhance bacteriocin production. Elicitation experiments were carried out by introducing live and dead cells as well as culture supernatant of live and dead cells of *Staph. aureus*, *Bacillus sp* and *E. coli* as prokaryotic cells and *Saccharomyces cerevisiae* and *Aspergillus niger* as eukaryotic cells separately to the *Bacillus subtilis* NK16 culture at zero time. Based on the results obtained in this study, *Bacillus subtilis* NK16 increased its production of bacteriocin as a result of interaction with microbial elicitor cells. The maximum enhancement was achieved in the culture elicited with live cells of *Staph. aureus* at an inoculation level of 0.5% with an increase of approximately 8-folds.

Key words: *Bacillus subtilis*, bacteriocin, improvement,interspecies interaction, elicitation.

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Introduction

Among all, the molecules involved in microbial defense system, bacteriocins or antimicrobial peptides represent one of the best-studied microbial defense systems that serve as a good example for explaining evolution and ecological behavior of cells. Furthermore, potential bacteriocins can serve as a natural alternative to therapeutic antibiotics for treating bacterial infections and also can find application in food preservation. Bacteriocins properties make them typical as alternatives to antibiotics. They can inhibit the growth of bacteria of the same species (narrow spectrum) or other genera (broad spectrum) and their range of activity often depends on the mechanisms of action of each bacteriocin (1, 2). They are heat-stable, small peptides made of short chains of about 20-60 amino acid residues, however longer chains can also be found (2). Most bacteriocins are products of Gram-positive bacteria, as

reported in BACTIBASE dataset. Rare bacteriocins from Gram-negative bacteria have been described and even fewer from Archaea domain (3).

Microbial natural habitats are usually rich in different types of microbial species that can locally deplete nutrient resources leading to great competition. One of the strategies that used by microorganisms for the survival in the nature is to produce antimicrobial compounds that usually induced via interspecies interaction by signaling molecules or cell to cell contact (4). Therefore, the presence of a competitor in the fermentation culture of the antimicrobial compound producer is believed to be an important factor that may affects the production of these compounds such as antibiotics and bacteriocins (5,6). Indeed, elicitation is based on mimicking the presence of another competing species in the fermentation culture in order to stimulate the producer to switching on its secondary metabolism that may yields antimicrobial compounds. In this context, Barefoot *et al*., (6) reported that lactacin B production by *Lactobacillus acidophilus N2* was improved in the presence of the indicator strains *L. delbrueckii* ATCC 4797. In addition, Sip *et al*., (7) showed that divercin production by *Carnobacterium divergens* AS7 was affected as a result of presence of competitor strain *C. piscicola* NCDO 2765.The aim of the present work was to study the possibility of enhansment of bacteriocin production by *Bacillus subtilis* NK16 using microbial cells elicitors.

Materials and Methods

Microorganisms

Bacteriocin producing isolate of *Bacillus subtilis* NK16 was isolated from a soil sample which was subjected to regular biochemical tests in order to characterize the genus. The isolate was further characterized as *Bacillus subtilis* through using API 50 CHB and API 20 E for the identification of Gram-positive and endospore-forming bacteria according to online API database (bioMérieux, Inc).

Staphylococcus aureus, E. coli, Bacillus sp, *Saccharomyces cerevisiae* and *Aspergillus niger* isolates were used as elicitors in this study. All these isolates were provided by the Department of biotechnology/ College of Science at the University of Baghdad.

Preparation of Bacterial Inoculums

Inoculums of *Bacillus subtilis*NK16 and elicitors (*Staphylococcus aureus, E. coli, Bacillus* sp and *Saccharomyces cerevisiae*) were prepared as follows: a few loopfuls growth from an overnight culture on nutrient agar was inoculated into a 100 ml Erlenmeyer flask containing 20 ml of BHI broth. This culture was incubated for 24 hrs in an incubator at 37°C. After the incubation, a haemocytomtere was used to adjust the number of cells to be approximately 1×10^8 cells/ml for *Bacillus subtilis* NK16 and 1×10^7 cells/ml for elicitors. Inoculum of *Saccharomyces cerevisiae* was prepared by using sabouraud medium instead of nutrient medium. The inoculum of each elicitor was centrifuged at 10000 rpm for 10 min to get live elicitor cells and supernatant. If dead cells were required, the culture

flask was placed in boiling water for 30 min before separating by centrifugation. Than, the cells death was verified after each heat treatment by plating them on nutrient agar. Live and heat-killed cells of the elicitor were then washed and re-suspended in equal volume of phosphate buffer (0.1 M, pH 7.2) (17).

Erlenmeyer flask containing 50 ml of sabouraud broth and incubated at 30°C for 5 days. After the incubation, a known volume of the culture was centrifuged at 10000 rpm for 10 min to obtain live elicitor hyphae and culture supernatant. If dead hyphae were required, the culture flask was autoclaved before separating by centrifugation. Live and dead hyphae were then washed and re-suspendedin equal volume (culture volume before centrifugation) of sterile phosphate buffer (0.1 M, pH 7.2). In addition, a sterile cork borer was used to cut 5 mm growth agar plug from 7 days old culture of *Aspergillus niger* grown on sabouraud agar.

Cultivation Methods and Media

Brain Heart Infusion (BHI) broth was used for the cultivation of *Bacillus subtilis* NK16 inoculated at a level of 2% (v/v) and then incubated in an orbital shaker at 30°C and 200 rpm for 48hrs. After the incubation, samples were taken for the analyses of bacteriocin. For more reliability, each run was conducted either in triplicate or duplicate and the results were represented as the arithmetic average.

Elicitation Experiments

Elicitation with *S. aureus*, *E. coli*, *Bacillus* sp, *S. cerevisiae* and *A. niger* were achieved using live or heat-killed cell and culture supernatant. Six different inoculum levels of live elicitor cells or hyphae (0.2, 0.5, 0.75, 1, 2, 3) % and three inoculum levels of heat-killed elicitor cells or hyphae (1, 2, 3) % were added separately, to the *Bacillus subtilis* NK16 fermentation culture at zero time. In addition, culture supernatant was added to the *Bacillus subtilis* NK16 fermentation culture at zero time in three levels (1, 2, 3) %. In addition, growth agar plug (1, 2, 3) of 7 days old culture of *A. niger* was also added to the *Bacillus* fermentation culture at zero time. The same fermentation conditions were used for all elicitation experiments. For more reliability, all experiments were accompanied with pure culture of *Bacillus* which will be referred to as the control.

Inoculums of *Aspergillus niger* were prepared as follows: a few loopfuls of *Aspergillus niger* growth on sabouraud agar was inoculated into a 100 ml

Determination of Growth

The growth of *Bacillus subtilis* NK16isolate was measured as the dry weight of cell material. A known volume of the culture was filtered with vacuum through pre-dried and pre-weighed filter paper (0.2μm, Whatman). The filter paper was thereafter placed in an oven at 60°C for 24 hrs and then weighted. The difference in weights represented the mass of cells in the samples of the culture. This method was used with pure culture of *Bacillus subtilis* NK16 while in the elicited cultures this method was not followed because of the difficulty of separating two microorganisms which are growing in the same fermentative liquid.

Determination of Bacteriocin Activity

Bacteriocin activity was determined using the critical dilution assay which is similar to the minimum inhibitory concentration technique (MIC) for antibiotic assessment (8). This method involved preparation of a twofold dilution series of the *Bacillus subtilis* NK16 culture to be tested and then bacteriocin activity was determined against *Staphylococcus aureus* in each dilution using agar well diffusion assay. Two handred µl of an overnight growth culture of *Staph. aureus* containing approximately 1×10^7 cells /ml was mixed with 25 mL of a sterile Muller Hinton agar kept at 45-50°C in a water bath. The mixtures were kept at the same temperature until poured into sterile plastic Petri dishes and allowed to solidify. Circular wells of 5mm in diameter were cut using a sterile cork borer and then low melting temperature Muller Hinton agar was used to seal the bottom of the wells. 100 µl aliquots of the filtered cells free supernatant (CFS) were dispensed in the wells and then plates were incubated for 24 hrs at 37°C. Following the incubation, the growth inhibition zone around each well was examined. The highest dilution generating an inhibition zone indicated the strength of bacteriocin activity. Thus, the bacteriocin activity is a proportional to the reciprocal of the highest dilution factor producing a detectable inhibition zone (DF). The bacteriocin activity which is known as arbitrary unites (AU) was calculated using the following equation:

$$\mathrm{AU/ml} = \frac{1}{DF}\frac{1000}{volums\ spotted\ in\ \mu l}$$

Results and Discussion

Different microorganisms (*Staph. aureus*, *E. coli*, *Bacillus* sp, *S. cerevisiae* and *A. niger*) were chosen as the likely competitors for *Bacillus subtilis* NK16. These microorganisms were selected because they are familiar and save in the microbial labs and can be easily cultured which makes them widely used as a model in the bacteriological studies. In addition, they can be found in different environments such as soil, water, air and decomposing plant matter. Although, the natural habitat of *E. coli* is the intestinal tract of human and *Staph. aureus* is the skin as normal flora, nevertheless it can also be naturalized in the soil and water. Consequently, evolutionary interaction mechanisms may exist between *Bacillus subtilis* NK16 and those microorganisms.

The motivation for our work was to enhance the production of bacteriocin without effecting the growth of *Bacillus subtilis NK16.* Thus, the concentration of the elicitor cells was fixed to be at level necessary for elicitation but without overcoming the growth of *Bacillus subtilis* NK16. Moreover, in order to avoid such problem, the eliciting capability of the dead cells of elicitors was examined in comparison with live cells. If the dead cells show an eliciting effect, then any influence on the growth of *Bacillus subtilis* NK16 by the competition stress can be avoided. Based on results, the heating treatment was effective and the cells were already killed as no growth was observed. In addition, the counting of heat killed cells via counting chamber was performed before the process of killing in order to prevent any possible damage that might happen in cells due to the

boiling which can affect the cells counting. In all elicitation experiments, bacteriocin production and activity in pure and elicited cultures were investigated.

The growth of *Bacillus subtilis* NK16 isolate in BHI broth was investigated. The typical time course of growth (dry weight) and bacteriocin production by *Bacillus subtilis* NK16 is presented in Figure (1). The exponential phase was started where biomass concentration noticeably increased from 0.16 to 2.96 mg/ml after 31hrs of incubation which marks the start of the stationary phase. Then, *Bacillus subtilis* NK16 population was increased slightly to 3.3 mg/ml after 41hrs of cultivation. Thereafter, population growth was ceased, and the growth curve becomes horizontal. The production of bacteriocin was started during the exponential phase after 9 hrs of incubation. At the end of the exponential phase (at 24 hrs. of incubation), the activity of bacteriocin was approximately 80 AU/ml which is the maximum production obtained under the conditions used in this experiment. No further increased was observed in the production of bacteriocin during the stationary phase suggesting that bacteriocin produced by *Bacillus subtilis* NK16 is a primary metabolite and associated with growth. In this context, several studies have mentioned that numerous bacteriocins are produced during the active growth phase (9, 10).

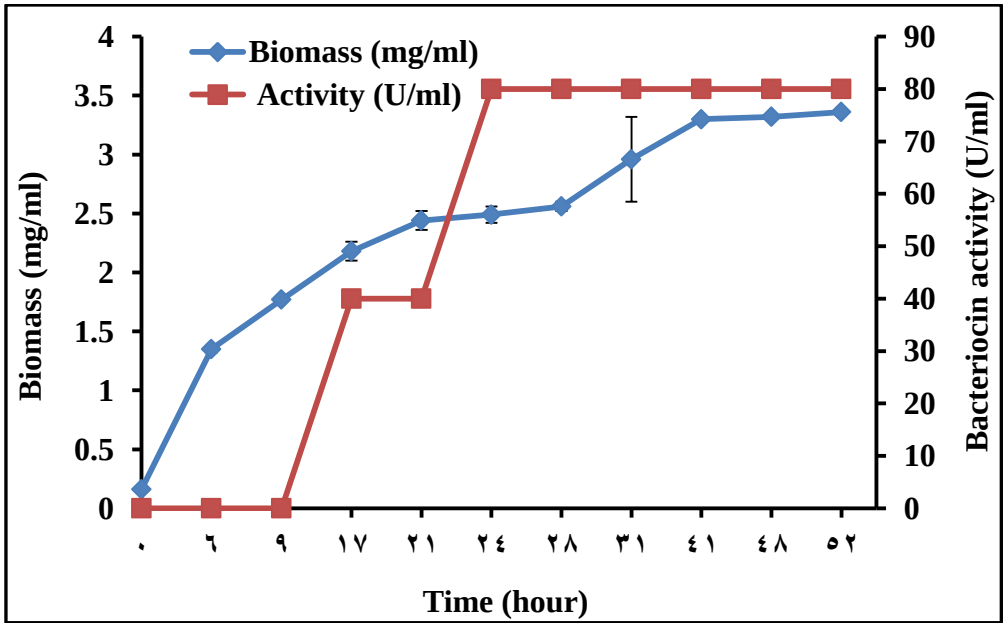

Figure 1: Time course of cell growth and bacteriocin production by *Bacillus subtilis* NK16in a shaker incubator at 30 °C and 150 rpm

Elicitation with *Staph. aureus*

As can be seen in Figure (2a), bacteriocin production was significantly enhanced when live cells of *Staph. aureus* were added to the *Bacillus subtilis* NK16 fermentation medium. Maximum production of bacteriocin was obtained in the culture elicited with 0.5% level of *Staph. aureus* inoculum (640 AU/ml) compared with 160 and 320 AU/ml attained in cultures elicited with 0.1 and 0.75 % level, respectively. Production of bacteriocin in the control culture was 80 AU/ml after 48 hrs of incubation. Elicitation with live cells of *Staph. aureus*, corresponds to an increase of 2, 4 and 8 folds in cultures elicited with 0.1, 0.5 and 0.75 % inoculation level respectively, compared with the control culture. In addition, bacteriocin production was significantly decreased in cultures supplemented with 2 and 3% inoculum level of the elicitor because the level of *Staph. aureus* used was sufficient to overcome the growth of *Bacillus subtilis* NK16.

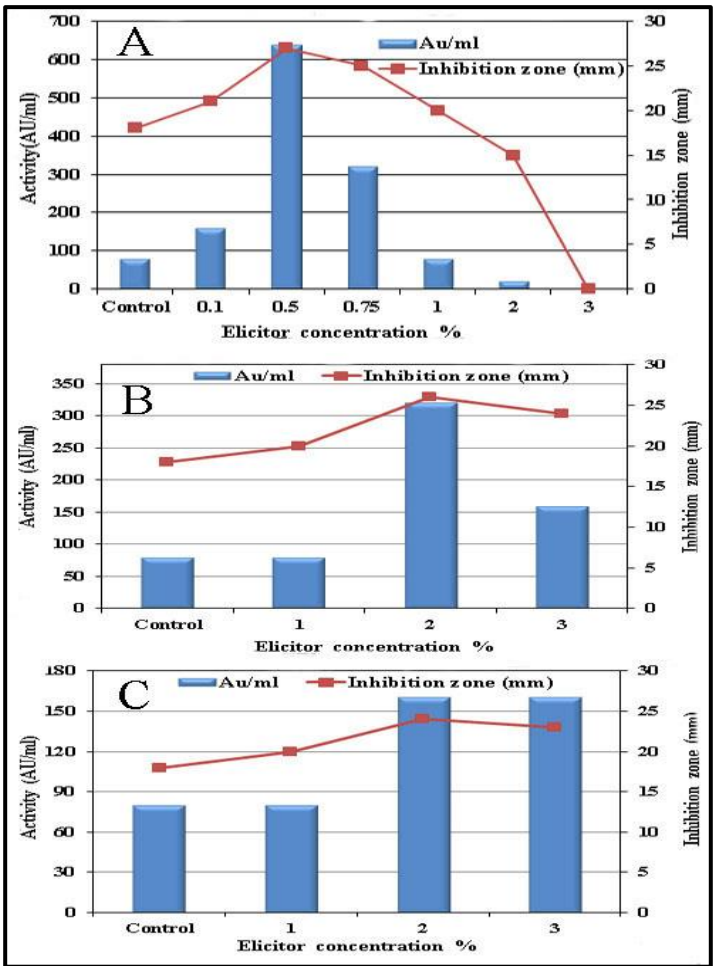

Figure 2: Production of bacteriocin by *Bacillus subtilis NK16* in cultures elicited with live cells (A), heat killed cells (B) and culture supernatant (C) of *Staph. aureus* in BHI broth at 30 C^{o} and 150 rpm.

Furthermore, bacteriocin production by *Bacillus subtilis* NK16 was also enhanced when heat-killed cells of *Staph. aureus* were added at 2 and 3% inoculum level compared with control culture (Figure 2b). Production of bacteriocin in the pure culture reached its maximum activity of 80 AU/ml at 48hrs of incubation. Production of bacteriocin in cultures elicited with 2 and 3% level of heat-killed cells of *Staph. aureus* were 320 and 160 AU/ml respectively, attained after 48 hrs of incubation. No increased was found in the culture supplemented with 1% of heat-killed cells of *Staph. aureus*. Elicitation with heat killed cells of *Staph. aureus* therefore, corresponds to an increase of 2 and 4.5 folds in the maximum bacteriocin concentration compared with the control culture.

Chang *et al*., (11) reported that, maximum enhancement of Kimchicin GJ7 production by *Leuconostoc citreum* GH7 was obtained in the presence of heat-killed cells of *Leuconostoc plantarum* KFRI 464. In that study, cell debris fraction of *Leuconostoc plantarum* KFRI 464 showed much higher inducing activity than the intracellular fraction. Moreover, bacteriocin production was significantly enhanced when culture supernatant of *Staph. aureus* was added to the *Bacillus subtilis* NK16 fermentation medium. Maximum production of bacteriocin was obtained in the culture elicited with 2 and 3% (v/v) level of supernatant (160 AU/ml) compared with 80 AU/ml attained in culture elicited with 1 % level which was similar to that achieved in the control culture (Figure 2c).

Elicitation with *E. coli*

As can be seen in Figure (3a), bacteriocin production was significantly enhanced when live cells of *E. coli* were added to the *Bacillus subtilis* NK16 fermentation medium. Maximum bacteriocin production was obtained in the culture elicited with 0.2 % level of *E. coli* inoculums (320 AU/ml) compared with 160 AU/ml attained in culture elicited with 0.5 % level of *E. coli* inoculum. Elicitation with 0.2 and 0.5 % levels of live *E. coli* cells therefore, corresponds to an increase of 2 and 4 folds in the maximum bacteriocin concentration respectively, compared with the control culture. In addition, Figure (3a) shows no considerable effect on bacteriocin production in culture elicited with 0.75% levels of live *E. coli* cells inoculum. Whereas, production of bacteriocin was obviously suppressed in cultures elicited with 1, 2 and 3 % levels due to the effect on *Bacillus subtilis* NK16 growth that happened as result of presence of *E. coli* cells at levels sufficient to overcome the growth of *Bacillus subtilis*NK16.

In addition, results presented in Figure (3b) showed that heat-killed cells of *E. coli* had no considerable effect on bacteriocin production by *Bacillus subtilis* NK16. The production pattern of bacteriocin in the control culture and that elicited with heat killed cells of *E. coli* were approximately similar. Similarly, culture supernatant of *E. coli* had no significant effect on bacteriocin production by *Bacillus subtilis* NK16 (Figure 3c). The production pattern of bacteriocin in the control culture and that supplemented with culture

supernatant of *E. coli* were approximately similar.

Elicitation with *Bacillus* sp

Live cells of *Bacillus* sp had an obvious suppressed effect on bacteriocin production when added to the *Bacillus subtilis* NK16 fermentation medium. As can be seen in Figure (4a), bacteriocin production was notably decreased when live cells of *Bacillus* were added at 0.75, 1, 2, 3 % inoculation levels, whereas no effect was found when live cells of *Bacillus* were added at 0.2 and 0.5 % level. This may due to the high competition between the twomicroorganisms that effect on *Bacillus subtilis* NK16 growth leading to suppress the production of bacteriocin.

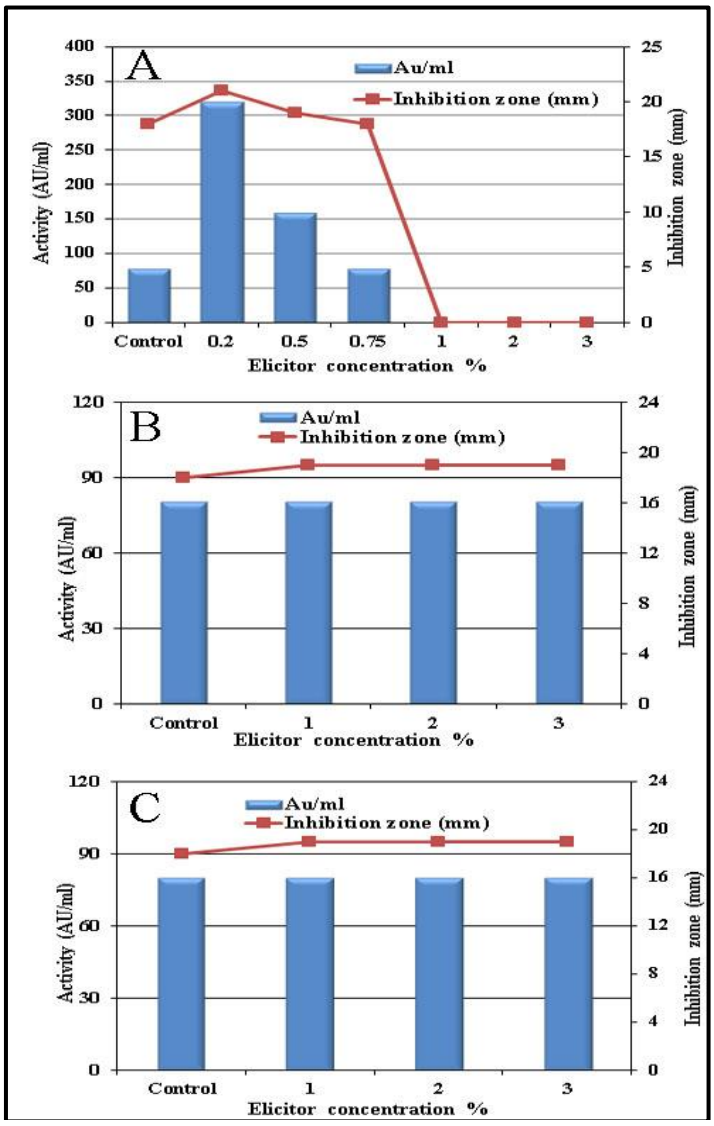

Figure 3: Production of bacteriocin by *Bacillus subtilis NK16* in cultures elicited with live cells (A), heat killed cells (B) and culture supernatant (C) of *E. coli* in BHI broth at 30 C^{o} and 150 rpm.

As shown in Figure (4b), production of bacteriocin was only enhanced in the culture elicited with 3% inoculation level of heat killed cells of *Bacillus* sp. The activity of bacteriocin produced in this culture was160 AU/ml attained after 48 hrs of incubation, and it corresponds to an increase of 2 folds compared with the control culture. Moreover, no change was observed in the production of bacteriocin in cultures elicited with 1 and 2% inoculation level of heat killed cells of *Bacillus***.** Culture supernatant of *Bacillus* sp had the same role as the culture supernatant of *Staph. aureus* in terms of eliciting *Bacillus subtilis* NK16. Production of bacteriocin was stimulated and increased notably by 2 folds in cultures supplemented with 2 and 3 % levels of *Bacillus* culture supernatant compared with the control culture (Figure 4c). In addition, no effect observed in the production of bacteriocin in the culture supplemented with 1% level of *Bacillus* culture supernatant.

Previous studies have reported the usage of *E. coli*, *Staphy. aureus* and *Bacillus* sp as elicitors for enhancing antibiotic production. In this context, Ge *et al* ., (12) used live cells and supernatant of *Bacillus Subtilis*, *Escherichia coli, Lactabacillus plantarum* and *Lactococcus lactis* to enhance the production of paracin by *Lactobacillus paracasei HD1-7*. In that study, they found that *L. paracasei HD1-7* changed its antimicrobial peptide production as a result of competition with the elicitor bacteria such that paracin production was significantly enhanced. In addition, Benitez *et al.* (13) used live and thermally inactivated cells of *E. coli*, *Staph. aureus* and *Bacillus cereus* to observe their effect in the production of antimicrobial from *Bacillus amyloliquefaciens.* They found that the antimicrobial activity was increased when cultivated in the presence of dead cells of *E. coli* and live cells of *Staphyl. aureus*. Luti and Mavituna (14) used live and dead cells of *Bacillus subtilis*, *E. coli* and *Staphylococcus aureus* to enhance the production of undecylprodigiosin by *Streptomyces coelicolor.* The pure culture of *S. coelicolor* in a defined medium produce higher concentration of actinorhodin compared with undecylprodigiosin which is more important due to its antitumor activities. In that study, they found that *S. coelicolor* changed its antibiotic production pattern as a result of challenge with elicitor bacteria such that undecylprodigiosin production was significantly enhanced and actinorhodin decreased. Moreover, Maldonado *et al.* (15) reported that Plantaricin production in *Lactobacillus plantarum* NC8 was induced by quorum-sensing mechanism through co-culture with Gram-Positive Bacteria.

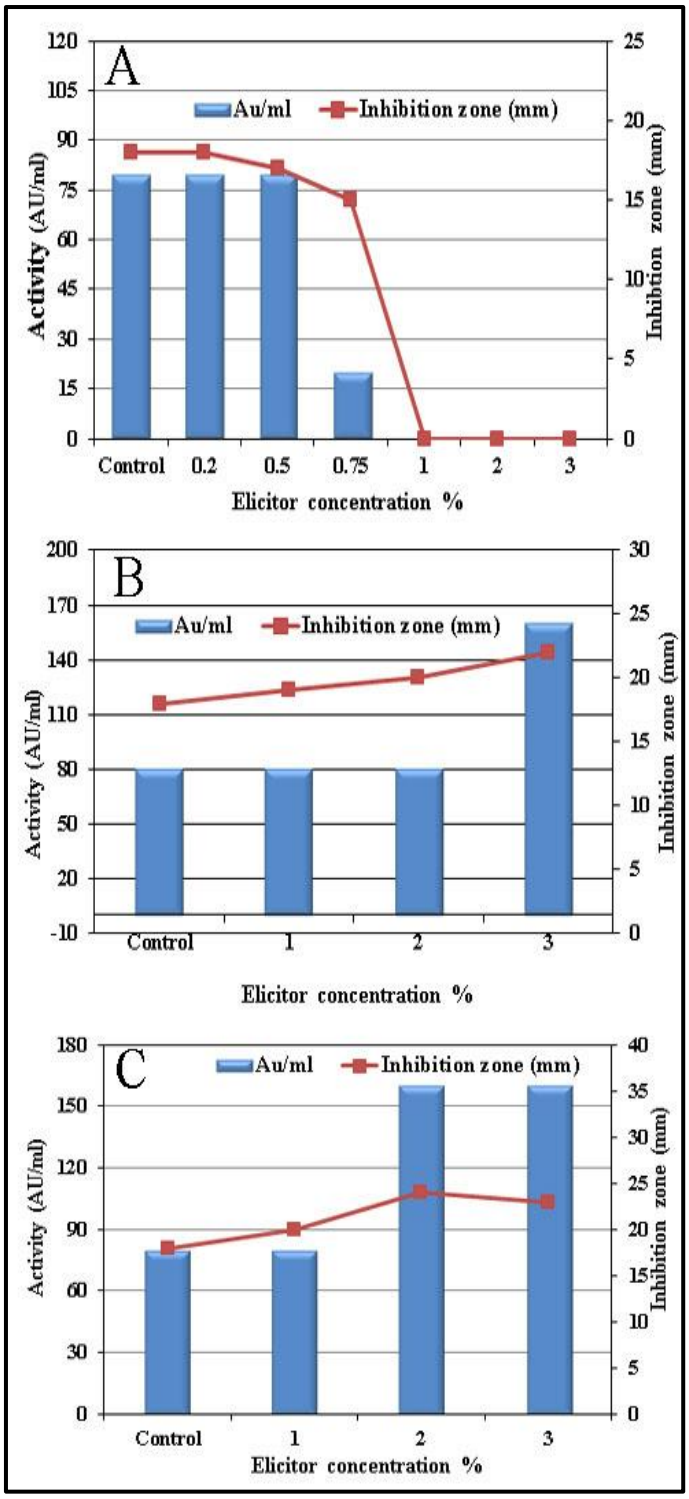

Figure 4: Production of bacteriocin by *Bacillus subtilis NK16* in cultures elicited with live cells (A), heat killed cells (B) and culture supernatant (C) of *Bacillus* sp in BHI broth at 30 C^{o} and 150 rpm.

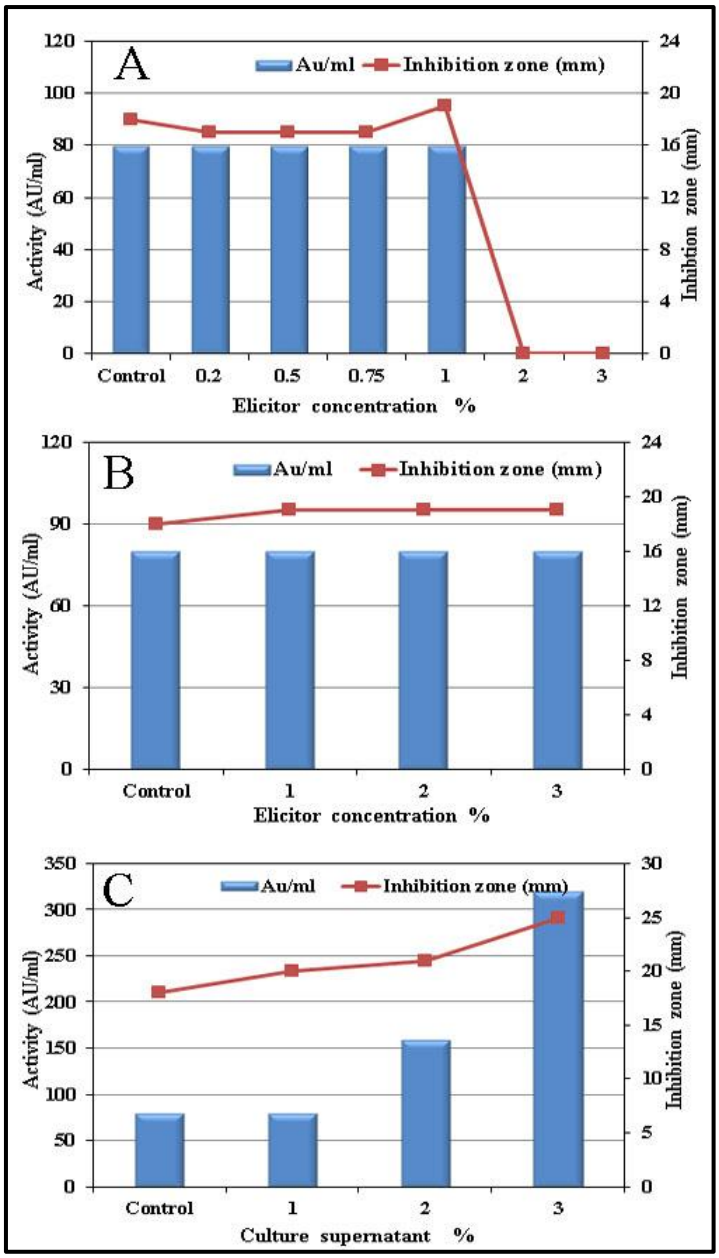

Figure 5: Production of bacteriocin by *Bacillus subtilis NK16* in cultures elicited with live cells (A), heat killed cells (B) and culture supernatant (C) of *S. cerevisiae* in BHI broth at 30 C° and 150 rpm.

Elicitation with *A. niger*

Several studies have examined the effect of interspecies interactions on antibiotic production by bacteria in a co-culture via using bacterial cells as elicitors (13, 14,19). However, far too little attention has been paid for using molds to elicit antibiotic production from bacteria. For this reason, the present study was designed to investigate the effect of live and dead

hyphae as well as growth agar plug of *A. niger* on bacteriocin production by *Bacillus subtilis* NK 16.

As can be seen in Figure (6a), bacteriocin production by *Bacillus subtilis* NK 16 was significantly enhanced in the culture elicited with 0.5, 0.75 and 1% of live hyphae of *A. niger*. The maximum activity of bacteriocin was obtained in culture elicited with 0.75 % (320 AU/ml) with approximately 4 folds increase compared with the control culture. Production of bacteriocin in the control culture was 80 AU/ml achieved after 48 hrs of incubation. Bacteriocin production in cultures elicited with 0.5 and 1% was also induced by 2 folds increase, whereas no effect was observed in cultures elicited with 0.2, 2, 3% of live hyphae of *A. niger*. In addition, dead hyphae of *A. niger* had no effect on bacteriocin production from *Bacillus subtilis* NK 16. Results presented in Figure (6b) showed that the production pattern of bacteriocin in the control culture and that elicited with dead hyphae of *A. niger* were approximately similar.

Figure (6c) illustrate that bacteriocin production was significantly enhanced when amounts of culture supernatant of *A. niger* were added to the *Bacillus subtilis* NK 16 fermentation medium. Activity of bacteriocin obtained in cultures elicited with 2 and 3 % level of *A. niger* culture supernatant was 320 AU/ml compared with 80 AU/ml attained in control culture. Elicitation with culture supernatant of *A. niger* therefore, corresponds to an increase of 4 folds in the maximum bacteriocin activity compared with the control culture. Moreover, production of bacteriocin was also stimulated as a result of adding growth agar plug of *A. niger* to the *Bacillus subtilis* NK 16 culture (Figure 6D). Activity of bacteriocin achieved in the culture elicited with 1 and 2 plugs of *A. niger* was 160 AU/ml obtained after 48 hrs of incubation. Comparing this value with the maximum production achieved in the control culture, the increase in the production of bacteriocin was therefore 2 folds.

It is apparent from the results obtained in this study, bacteriocin production was significantly enhanced in elicitation experiments except for the elicitation with heat-killed cells of *E. coli, S. cerevisiae, A. niger*, live cells of *Bacillus* sp and *S. cerevisiae* and supernatant of *E. coli* in which no important increased was observed. Maximum enhancement in the production of bacteriocin was observed in culture elicited with live cells of *Staph. aureus* with approximately 8-fold increase compared with the control culture. Therefore, it can be said that elicitation strategy based on exploiting inter-species interaction was useful to enhance bacteriocin production by *Bacillus subtilis* NK16 which is a valuable compound due to its antimicrobial properties.

One of the interesting findings in this study is that the supernatant and dead cells of elicitors had the same elicitation role as the related live cells in terms of increasing bacteriocin production. This is an important aspect as the addition of supernatant or dead cells to the fermentation medium could not affect the growth of the antibiotic producer which certainly, strengthens the case of using such an approach in the industrial applications.

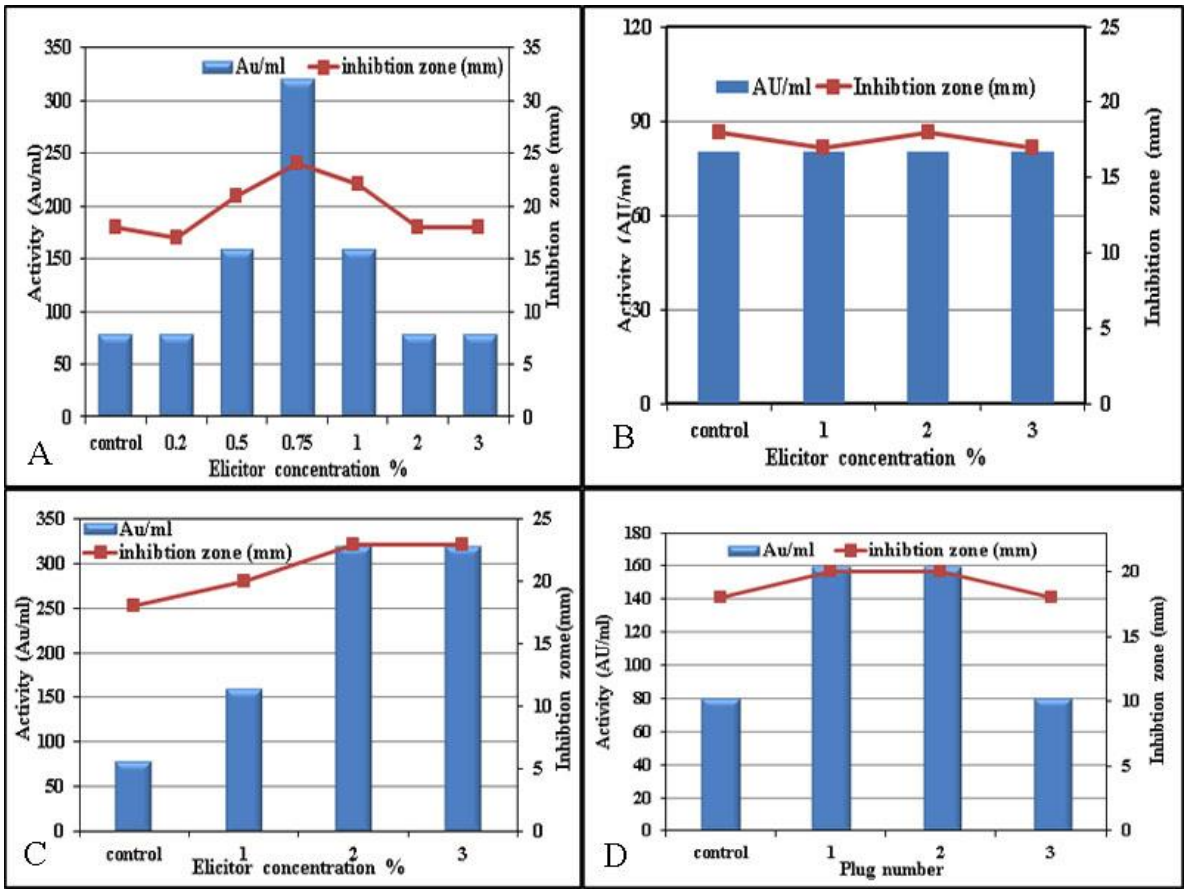

Figure 6: Production of bacteriocin by *Bacillus subtilis NK16* in cultures elicited with live cells (A), heat killed cells (B), culture supernatant (C) and growth agar plug (D) of *A. niger* in BHI broth at 30 C^{o} and 150 rpm.

Bacteriocin biosynthesis commonly appears in the log phase and extended during the stationary phase of the microbial growth culture.Stressful environmental conditions such as pH, temperature and the essential nutrients usually lead to induce bacteriocin production. In addition, the presences of some inducing compounds may affect the production of antibiotics. Based on results, dead cells and culture supernatant of some elicitors showed an elicitation effect. Obviously, the presence of dead elicitor cells or culture supernatant in the *Bacillus subtilis* NK16 fermentation medium could not cause any nutritional stress. Therefore, it can be suggested that the increase in bacteriocin production as a result of elicitation may not due to nutritional deficiency stress. In addition, culture conditions such as pH, temperature and aeration in both control and elicited cultures were similar, leading to conclude that the environmental conditions may not have been the reason for the change observed in bacteriocin production by *Bacillus subtilis* NK16 in the elicited cultures.

In fact, the mechanism of elicitation in the microbial cultures that created as a result of interspecies interactions and its

role in the induction of antibiotics production is not completely understood yet. However, in this context, most researches mentioned that signaling molecules of low molecular weight perhaps mediated the interspecies interactions process which can affect the production of antibiotics or bacteriocins (20, 21).Therefore, *Bacillus subtilis* NK16 may recognize the presence of the elicitor cells in the culture via some signaling molecules produced by the living elicitor cells or it may already found in the culture supernatant of the elicitor. Thus, as a result, *Bacillus subtilis* NK16 increased its production of bacteriocin as a strategy for protecting itself against a prospective competitor. Although the elicitors,in some cases, were used as dead cells, *Bacillus subtilis* NK16 may recognized some proteins or receptors on the surface of these cells which may not affected by the heat and act as signaling molecules. Certainly, further research and investigations need to be achieved before such speculation is established and the mechanism of elicitation is completely understood. Barefoot *et al*. (6) reported that lactacin B produced by *Lactobacillus acidophilus* N2 was increased as a result of adding dead, living, or washing cells of *Lactobacillus delbrueckii* ATCC 4797 to the fermentation culture, suggesting that the inducer element was cell associated that could be positioned on the cell envelope.

Conclusion

Antimicrobial compounds play an important defensive role against the invasion of other species by giving the producer an advantage over other surrounding invasive microorganisms. Therefore, the possible effects of exploiting the interspecies interactions in the production of antimicrobial compounds can be the induction of unexpressed biosynthetic pathways for novel bioactive compounds or the enhancement required in the productivity of antimicrobial compounds -producing strains as obtained in this study.Based on the results obtained in this study, *Bacillus subtilis* NK16 increased its production of bacteriocin by approximately 8-folds as a result of interaction with microbial cells which means that the elicitation strategy followed in this study is successful and useful.Therefore, future research should focus on testing this strategy of exploiting interspecies interactions with other types of bacteriocins particularly those which known as commercially important bacteriocins such as Nisins.

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