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Effect of bacteriocin nanoparticles in control of staphylococcosis in *Diplodus sargus* (white seabream) broodstock.

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Abstract

An infection was reported in *Diplodus sargus* (white seabream) broodstocks in an experimental research station characterized by 28% mortalities (14 died out of 50) and clinical signs represented as darkening and black discoloration of the skin with severe hemorrhagic ulcers, loss of scales, severe destruction and deformity in the fin rays, opacity and bilateral exophthalmia, necrosis on caudal peduncle area. *Staphylococcus aureus* could be isolated from freshly dead and clinically infected fish samples with percentages of 71.4 and 72.2 % respectively. Antibiogram profile of three selected isolates showing that they were multidrug resistant *S. aureus.* Inhibitory activity of bacteriocin and bacteriocin nanoparticles obtained from Lactic acid producing bacteria (LAB) isolated from the gastrointestinal tract (GIT) of *Pomatomus saltatrix* fish against isolated *S. aureus* was screened. The results showed that used bacteriocin nanoparticles could increase the growth inhibition for tested microorganisms.

Keywords: *Diplodus sargus, S. aureus; antibiogram; bacteriocin; probiotics; nanoparticles.*

Introduction

Diplodus sargus (white seabream) is considered a good candidate for aquaculture as it has a high commercial value and good acceptability by consumers **(Golomazou *et al*., 2006)**.

Bacterial diseases are the most dominant pathogens that are responsible for severe diseases and outbreaks in marine-cultured fish **(Pridgeon and Klesius, 2012)**. Most of the bacterial disease etiological agents are considered a part of the normal flora that is present in water, stress condition as bad water quality, inadequate diet, overcrowding, and frequent handling during artificial spawning are considered to be predisposing factors of lowering fish immunity and subsequently initiating disease conditions **(Aboyadak *et al*., 2016).**

Staphylococcosis caused by Staphylococcus spp. Staphylococcus bacteria are important opportunistic human pathogens and leading cause of a wide variety of diseases in humans and animals **(Edwards *et al*., 2012)**. For example, *S. aureus* is the aetiological agent responsible for a large extent of morbidity and mortality globally, in both hospital and community settings **(Shah and Tyagy, 1986).** In aquatic animals, three Staphylococcus species (*S. epidermidis*) **(Kusuda and Sugiyamam, 1981)**, *S. aureus* **(Baxa *et al*., 1985)** and *S. warneri* **(Gil *et al*., 2000)** have been reported as disease-causing agents.

S. aureus was reported to be the causative agent of an eye disease during 1982 and 1983 in India, causing mortalities in silver carp, Hypophthalmichthys molitrix **(Shah and Tyagy, 1986)**. The typical symptoms of staphylococcosis are exophthalmia and swollen lesion on

the tail **(Kusuda and Sugiyamam, 1981)**.

Excessive and incorrect use of antibiotics considered the major selective force for the development of resistance **(Levy, 2002).**
Antibiotic resistance in *S. aureus* is a major clinical problem, in particular in infections caused by methicillin-resistant *S. aureus* (MRSA) **(Edwards *et al*., 2012)**.
The overuse of antimicrobial drugs to prevent or treat infections in human and veterinary medicine contributes to the increased frequency and dissemination of AMR (Antimicrobial resistance) **(WHO, 2007),** thus necessities for searching and finding new sources of substances with antimicrobial properties **(Raphaël and Meimandipou, 2017).**

Lactic acid bacteria (LAB) are common microorganisms in foods and constitute the natural intestinal microbiota of humans and most animals **(Rojo-Bezares *et al*., 2006).** LAB produce a wide range of antimicrobial metabolites which include organic acids, diacetyl, hydrogen peroxide, antibiotics and bacteriocins.
Bacteriocins are ribosomal synthesized, extracellularly released bioactive peptides which have a bactericidal or bacteriostatic effect on other (usually closely related) species.
Probiotics have received increasing attention as an alternative to in-feed antibiotics and for the purpose of improving productivity in the poultry industry **(Shin *et al*., 2008).** LAB, particularly Lactobacillus spp., is one of the probiotic groups which make up a large group of microorganisms in the GIT of all humans and animals. They can be tolerant to acid and bile, adhere to the intestinal epithelium of the hosts; they show an antagonistic activity against pathogenic bacteria and keep their viability during processing and storage **(Musikasang *et al*., 2009).**

Bacteriocins are a group of polypeptides that are produced by a variety of Gram-negative and Gram-positive bacteria, and exhibit bactericidal or bacteriostatic activity, usually against species closely related to the producing strain **(El-Gendy *et al*., 2013).** Over the last decade, bacteriocins have gained considerable attention due to their potential applications in the food industry as natural biopreservatives, and more recently in the health industry as antimicrobial agents **(El-Gendy *et al*., 2013)**

Nanoscience is a new interdisciplinary subject that depends on the fundamental properties of nanosize objects. Nanoparticles possess wondrous optical, electronic, magnetic, and catalytic properties than the bulk material owing to their high surface area to volume ratio **(Poulose *et al.*, 2014).**

The current work was aimed to report an infection occurred in White Sea bream broad stocks (*Diplodus sargus*) kept at an experimental research station and to isolate and screen bacteriocin and silver nanoparticles producing probiotics bacteria that isolated from GIT of *Pomatomus saltatrix* fish with study the antagonistic effect of its bacteriocins on pathogenic *S. aureus* isolated from cultured sargus fish .

Materials and Methods

Collection of samples

In April 2019, white sea bream (*Diplodus sargus*) broad stocks of 210-500 gm weight kept in an experimental fish research station in Alexandria governorate facing an infection resulted in 28% mortalities (14 died out of 50) and clinically characterized by darkening of the skin, fin rot, severe skin ulceration leading to exposing of muscular tissue, exophthalmia and skin hemorrhage. Seven Freshly dead and 18 clinically infected live fish were collected and transported in polyethylene plastic bags 2/3 water with 1/3 atmospheric air to the laboratory for bacteriological examination.

Clinical and post mortem examination:

The fish were examined for any external abnormalities; interior of the body was exposed and examined for changes according to **Noga (2000)**.

Bacteriological examination:

Isolation of bacteria:

Fish opened under aseptic condition, a loopful was taken from the internal organs (kidney, liver, spleen), gills and muscle inoculated into tryptone soya broth (TSB) and incubated aerobically at 37ºc for 24 hrs., then

streaked on Tryptone soya agar (TSA) supplemented with 3% Nacl and incubated at 37ºc for 24-48hrs. The isolated colonies were picked up, purified and streaked onto slope agar as stock culture for identification; and onto semi-solid agar media for preservation and motility.

Identification of bacteria:
Morphological identification of the golden yellow colonies grew on (TSA) was carried out: colonial characterizations by inoculating the colonies onto Mannitol salt agar and microscopically by staining of the isolated colonies with Gram stain. Biochemical identification was carried out including Catalase, Oxidase, Urease, Mannitol fermentation, Growth on high salt containing media (nutrient broth and nutrient agar supplemented with 3% Nacl) , Haemolysis on 5% sheep blood agar, Slide coagulase, Tube coagulase test and motility **(CruickShank *et al*.,1980).**

Antibigram profile of the isolates:
Antimicrobial susceptibility test was carried out for 3 selected *S. aureus* isolates by disk diffusion method according to **Baeur *et al*. (1966).** The antimicrobial discs used were obtained from **Himedia**. The diameter of inhibition zone of each antimicrobial was measured in mm and interpreted according to **CLSI (2006)**.

Preparation of bacteriocin nanoparticles:
Isolation and identifications of LAB isolates (Probiotics)
Pomatomus saltatrix dead fish samples (two samples) were collected from markets in Alexandria province. The ventral surface of the fish was opened with sterile scissors after washing the fish skin with 70% ethanol. One gram of the intestinal tract content of each fish was removed under aseptic condition and placed into previously weighed flasks containing storage medium **(Bucio Galindo *et al*., 2006).** Intestinal content was homogenized in a storage medium using a vortex mixer. One milliliter was transferred to reduced neutralized bacterial peptone (NBP, **Oxoid**) 0.5 g/L, NaCl 8g/L, cysteine 0.5 g/L, pH adjusted to 6.7 **(Hartemink and Rombouts, 1999).** Afterwards serial dilutions were spread on plates of MRS (MRS, **Oxoid**) and incubated anaerobically at 30°C for 72 h.
Identification of the isolates depends on cell morphology, biochemical tests and confirmed by API system.

Probiotics properties
Biochemical tests to determine Probiotic Characteristics
1. pH Tolerance
Acid tolerance of the selected isolates was investigated at different pH. First, MRS broths with different pH including 3, 4, 5 and 6 were prepared using HCl 1% and NaOH 1 N and divided in universal bottles according to **Kim and Austin (2008)**. The broths media along with control bottles were autoclaved and then inoculated with overnight culture of the selected strain in MRS broth and Nutrient broth and incubated at 37°C.

2. Bile Salt Tolerance
Bile salt tolerance was further tested in MRS broth and Nutrient broth which included 0.0, 0.15 and 0.3% (w/v) Ox gall bile salt (**Oxoid**). Duplicate bottles of MRS broth and Nutrient broth containing filtered different concentrations of bile salt were inoculated by 30 µl of cultured strain and incubated at 37°C **(Kim and Austin, 2008).**

3. Antibiogram profile
Antibiotic sensitivity test was carried out for selected strain on the most common antibiotics in aquaculture by disc diffusion technique **(Akinjogunla *et al*., 2010).** They included Gentamycin (GM, 30 µg), Tetracycline (TE, 30µg) and Meropenem (M, 30µg). 50µl of the 24 h broth culture of the strains was spread on MRS agar, Nutrient agar and, antibiotic Biodiscs were subsequently placed on plates. Finally, the plates were incubated at 37°C for 24 to 48 h to observe and measure the inhibition zone **(Kim and Austin, 2008).**

4. Antibacterial effect of probiotic LAB against *S.aureus* isolates
Two of the probiotics LAB strains isolated from fish: *Lactobacillus brevis* 1; and *Lactobacillus sakei* 2 were further tested for their antibacterial activity with used *Lactobacillus casei*

ATCC 7469 as positive control against three *S.aureus* F1;F2&F3 isolated from cultured sargus fish using a standard agar disc diffusion test **(Ghanbari *et al*.,2013).**

5. Bacteriocins production (according to the method described by Elayerja *et al.,* 2014).

6. Antibacterial activity of bacteriocin

Antibacterial activity was determined by agar well-diffusion assay against target organism as report in the previous work of **Mahesh and Satish (2008).** Plates were prepared by adding 1 ml (~10^6 CFU/ml) from an overnight culture of *Lactobacillus casei* ATCC 7469 as positive control and the probiotics isolates against three isolates of *S. aureus* (F1, F2, F3) isolated from cultured *Diplodus sargus* fish obtained during the study to 200 ml of plate count agar medium (PCA, **Oxoid**) held at 37°C. The agar was then immediately dispensed into round sterile 8.5 cm diameter Petri dishes and after solidification; wells (3 mm diameter) were made by removing the agar by a sterile metal borer. Subsequently, 30 µL of neutralized and filter-sterilized supernatants of culture obtained from overnight cultures of the probiotic strains grown in MRS broth at 37°C, were dispensed in individual wells. The plates were incubated for 2 h at 4°C and subsequently overnight at 37°C after which the diameter of the inhibition zones was measured.

7. Biosynthesis of silver nanoparticles (AgNPs)

For biosynthesis of AgNPs, 50 ml of cell filtrate of bacteriocin was mixed with 0.3M $AgNO_3$ solution (Sigma-Aldrich) and reaction mixture without $AgNO_3$ was used as control. The prepared solutions were incubated at 28 ° C for 24 h. All solutions were kept in dark to avoid any photochemical reactions during the experiment. The AgNPs were purified by centrifugation at 10,000 rpm for 10 min twice, and collected for further characterization (**Sagar and Ashok, 2012).**

Silver nanoparticles (AgNPs) generally in the size range of 10.32- 25.42 nm upon addition of 0.3 mM silver nitrate.

Statistical Analysis

Data are presented as the mean ± standard deviation, and n represents the number of tested strains and the control.

Results

Clinical signs

The naturally infected fish showed, darkening and black discoloration of the skin with severe hemorrhagic ulcers, loss of scales, severe destruction and deformity in the fin rays, opacity and bilateral exophthalmia, necrosis on caudal peduncle area.

P.M. lesions

Necropsy findings showed ascetic fluid in the abdominal cavity, liver was congested in some fishes while pale in others, congested kidney, congested gills, haemorrhage in the underlying musculature and congested spleen.

Isolation and identification of bacteria

S. aureus could be isolated from freshly dead (5 out of 7), clinically infected fish (13 out of 18) and total examined samples (18 out of 25) with percentages of 71.4, 72.2 and 72 % respectively as in table (1). It was phenotypically characterized by golden yellow colonies on TSA and Mannitol salt agar, β haemolysis on 5% sheep blood agar, non motile on semisolid media; microscopical examination of Gram stained isolate showing Gram positive cocci arranged in a grape like clusters.

By biochemical examination, it was catalase positive, oxidase negative, ferment mannitol, coagulase positive.

Table (1). *S. aureus* isolated from examined fish samples

Sample	No.	+ ve	%
Freshly dead	7	5	71.4
Clinically infected	18	13	72.2
Total	25	18	72

Table (2). Antibigram profile of 3 selected *S. aureus* isolates

Antimicrobial	Interpretation of inhibitory zone diameter			
	S		R	
	No.	%	No.	%
Amoxyclav (AMC)(30 µg)	-	0	3	100
Ampicillin (AM)(10µg)	-	0	3	100
Cefotaxime (CTX)(30µg)	-	0	3	100
Ciprofloxacin (CIP)(µg)	3	100	-	0
Erythromycin (S)(10µg)	-	0	3	100
Gentamycin (CN)(10µg)	3	100	-	0
Imipenem (IPM) (10 µg)	3	100	-	100
Penicillin G (P)(10 units)	-	0	3	100
Streptomycin (S)(10µg)	-	0	3	100
Tetracycline (TE)(30µg)	3	100	-	0
Trimethoprimsulphamethoxazole(COT)(25µg)	3	100	-	0
Vancomycin (Va)(30µg)	3	100	-	0

S= Susceptible R= Resistant

Table (2) showing that *S. aureus* isolates are resistant to more than 2 classes of antibiotics means that they are multi-drug resistant (MDR).

Table (3). Biochemical tests of probiotics strains

No.	Gram staining	Catalase	NH3 from arginine	Ribose	Mannitol	CO_2 from glucose	API System
1	+	+	+	+	+	+	*Lactobacillus brevis 99.7%*
2	+	+	-	+	-	-	*Lactobacillus sakei 98.5%*
3	+	+	-	-	+	-	*Lactobacillus plantarum 98.9%*

Three of the LAB strains isolated from *Pomatomus saltatrix* fish: *Lactobacillus brevis* 1; and *Lactobacillus sakei* 2 and *Lactobacillus plantarum* 3.

Table (4). pH Tolerance of Probiotics strains

Strain No.	pH-3	pH-4	pH-5	pH-6
1	Survive	Grow	Grow	Grow
2	Survive	Grow	Grow	Grow
3	No growth	Survive	Grow	Grow

Table (5). Bile Tolerance of probiotics strains

Strain No.	0 Bile salts	0.15 % concentration	0.3% concentration
1	Grow	Survive	Survive
2	Grow	Survive	Survive
3	Grow	No growth	No growth

Table (6). Antibiogram profile of probiotics strains

Antibiotics	Strains No. 1	2	3
Tetracyclin	Susceptible	Resistant	Susceptible
Gentamycin	Resistant	Susceptible	Susceptible
Ampicillin	Resistant	Resistant	Resistant

Table (7). Antibacterial effect of probiotic LAB against *S.aureus* F1;F2&F3 isolates

Probiotics / *S.aureus*	Diameter of the inhibition-zone (mm) F1	F2	F3
ATCC	1.8±0.1	1.0±0.3	2.0±0.2
1	1.5±0.01	0.9±0.1	1.8±0.2
2	1.0±0.2	-	0.8±0.3

No. of *S. aureus* isolates = 3
Data are presented as mean ± SD

Table (8). Antibacterial effect of bacteriocin with silver nanoparticles (AgNPs) against *S.aureus* isolates

Probiotics / *S.aureus*	Diameter of the inhibition-zone (mm) F1	F2	F3
ATCC	3.2±	2.1±	2.4±
1	2.6±	1.7±	2.2±
2	1.6±	0.5±	1.4±

No. of *S. aureus* isolates = 3
Data are presented as mean ± SD

Photo. (1): *Diplodus sargus* fish shows severe hemorrhagic ulcer with hemorrhagic patches in the underling musculature and severe destruction of the fin rays of the dorsal and tail fin .

Photo. (2): *Diplodus sargus* fish shows blackish discoloration, exophthalmia and severe destruction of the fin rays of the dorsal and tail fin.

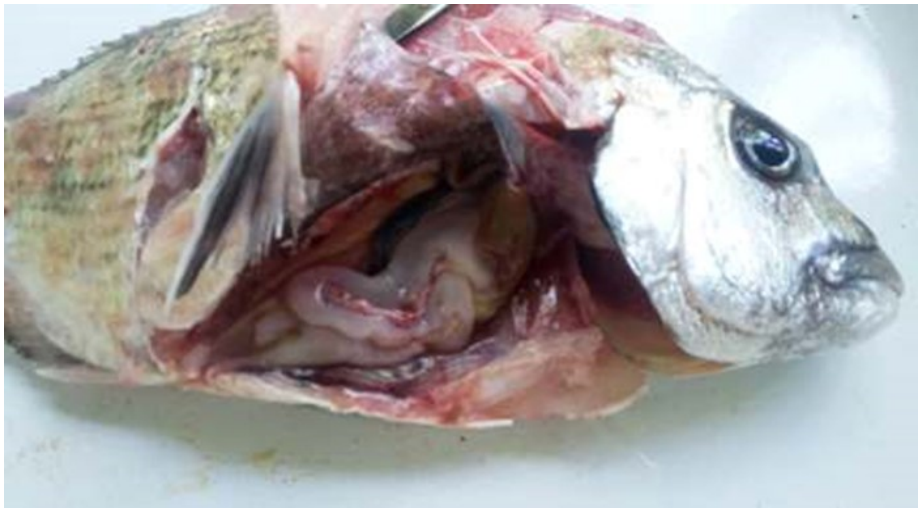

Photo. (3): *Diplodus sargus* fish shows ascetic fluid in the abdominal cavity, congested liver, congested spleen, haemorrhages on the visceral organs and in the underlying musculature.

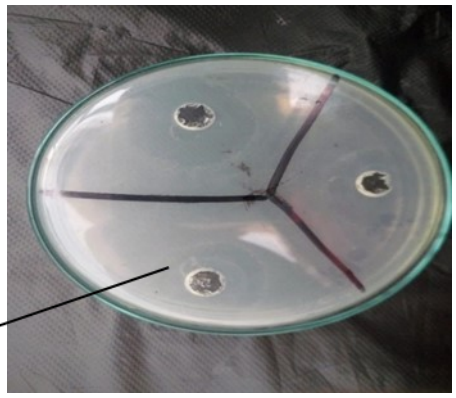

Photo (4). The inhibitory zone produced by *Lactobacillus brevis1* bacteriocin against *S. aureus*

Discussion

Fish usually succumb to opportunistic bacterial infection due to physiological imbalance. Stress factors such as nutritional deficiencies, poor quality water, and overstocking are predisposing factors in the development of fish infections **(Gufe *et al*., 2019).**

In aquatic animals, three Staphylococcus species (*S. epidermidis*) **(Kusuda and Sugiyamam, 1981)**, *S. aureus* **(Baxa *et al*., 1985)** and *S. warneri* **(Gil *et al*., 2000)** have been reported as disease-causing agents.

Table (1) illustrated that *S. aureus* could be isolated from freshly dead, clinically infected fish and total examined samples with percentages of 71.4, 72.2 and 72 % respectively. *Staphylococcus aureus* isolated from fish were previously reported by **Soliman *et al*. (2014)** with in a percentage of 17%, **Samia and Hala (2017)** with in a percentage of 42% and **Gufe et al. (2019)** with in a percentage of 19%.

The most important phenotypical features used in the identification of *S. aureus* its ability to produce coagulase, an enzyme that causes clotting of the blood plasma **(Qing *et al*., 2012).**

Coagulase production is an important phenotypic determinant of *S. aureus* which is associated with virulence as it resists phagocytosis and helps bacteria in virulence **(Bhanderi *et al*., 2009)**

Staphylococcus aureus is well known for its ability to acquire antibiotic resistance, both historically in relation to penicillin, erythromycin and tetracycline and more recently methicillin and vancomycin resistance) (**Gaze *et al*., 2008)**

The antibiogram profiles of the tested *S. aureus* isolates were clarified in table (2) showing that they were susceptible to ciprofloxacin, gentamycin, imipenem, tetracycline, trimethoprimsulphamethoxazole and vancomycin while they were resistant to amoxyclav, ampicillin, Cefotaxime, erythromycin, penicillin G and streptomycin so, the isolated *S. aureus* are considered as multidrug resistant.

Multidrug resistant *S. aureus* isolated from fish was previously reported by **(Najiah *et al*., 2012), Soliman *et al*. (2014)** and **Gufe *et al*. (2019).**

The number of infections caused by antibiotic-resistant bacteria is rising worldwide **(Levy and Marshall, 2004).** The resistance of the strains to antibiotics could be explained by the possibility of the heavy use of these compounds in aquaculture, several of which are non-biodegradable, thus increasing antibiotic selective pressure in water, facilitating the transfer of antibiotic-resistant determinants between aquatic bacteria, including fish and human pathogens, and allowing the presence of residual antibiotics in commercialized fish and shellfish products **(Alanis, 2005, Seiler and Berendonk, 2012).**

Clinical signs and P.M. lesions

Clinical signs and P.M. lesions (Photo1, 2, 3) were similarly reported by **Shah and Tyagy (1986)** in silver carp and by **Soliman *et al*. (2014)** in tilapia.

Clinical signs and PM lesions may be due to many toxins secreted by *S. aureus* . like toxic shock syndrome toxin-1 (TSST-1), the Staphylococcal enterotoxins (SEs), the exfoliative toxins (ETs) and leukocidin **(Doškař *et al*.,**

2010).

Fish could be a potential source of bacteriocin-producing (bacteriocinogenic) bacteria and extensive screening of gut associated microorganisms may be taken up to avoid the use of antibacterial drugs in aquaculture **(Sahoo *et al*., 2016).** Reports indicated that the LAB isolated from diverse fish species, other aquatic organisms, culture water and sediments possess antagonistic activity against the fish pathogens **(Shahid *et al*., 2017).** Hence, the potential use of bacteriocinogenic LAB as probiotics and bio-protective agents has received growing attention during the last decade **(Heo *et al*., 2012).** According to **Elayaraja *et al*. (2014),** genera Lactobacillus, Lactococcus, Streptococcus, Pediococcus, Oenococcus, Enterococcus, Leuconostoc, and Carnobacterium produce a variety of bacteriocins. Numerous investigations on isolation and characterization of bacteriocins and bacteriocinogenic LAB from different sources are available, however, lesser research has been done on bacteriocins of LAB from fish **(Gómez-Sala *et al*., 2015).**

Table (4) showed the pH tolerance test indicates the minimum tolerance of the bacteria at high acidic medium and tolerance was increased as the pH rises especially for *Lactobacillus brevis* 1; and *Lactobacillus sakei* 2.

A similar bile tolerance (table 5) is observed in the case of *Lactobacillus brevis* 1; and *Lactobacillus sakei* 2when used in different concentration.

From table (6) the result of the antibiotic susceptibility test of the probiotics strains indicates the strains were sensitive to ampicillin and there were variations to other antibiotics. On the other hand, from the tables 4;5&6 it has been found that *Lactobacillus brevis* 1; and *Lactobacillus sakei* 2 were pH tolerance, bile tolerance and antibiotic good.
From the probiotic properties results the two promising strains *Lactobacillus brevis* ; *Lactobacillus sakei* were selected for isolation of their bacteriocins for confirmed their antibacterial effect.

Table 7 showed that *Lactobacillus brevis* 1 has inhibitory effect higher than *Lactobacillus sakei* 2 especially against FI and F3. Bacteriocins, are ribosomal-synthesized antimicrobial peptides, and LAB are the most common producers **(Silva *et al*., 2018).** They are small cationic molecules of 30–60 amino acids, form amphiphilic helices and are stable at 100°C for 10 min. During the last decade probiotic LAB with antimicrobial potential has achieved interest in aquaculture **(Muñoz-Atienza *et al*., 2013),** and the use of bacteriocins as supplements or adjuncts could be an eco-friendly approach to alleviate antibiotic overuse and resistance **(Lagha *et al*., 2017).**

The bacteriocin-like substances produced by *Lactobacillus brevis* 1, and *Lactobacillus sakei* 2 were further tested and revealed that the substances were stable after treatment for transfer it to nanoparticles table (8). In the food industry, bacteriocins have been represented to be potent antimicrobials in hurdle technology, which involves the use of combined treatment to increase the efficacy of food preservation **(Cotter *et al*., 2005).** The integration of nanotechnology and biotechnology may be considered as a most recent example of this hurdle technology. Research in the field of nanotechnology in the past two decades has opened doors to unlimited opportunities for solving several problems associated with a wide range of biological products. In the food sector, the interaction between nanoparticles and bacteriocins holds high potential to be beneficial in increasing the antimicrobial spectrum of the latter. The interaction may also lead to a reduction in the requirement of high bacteriocin dosage, and an extension in the shelf life of food.
Compared with the positive control, the diameters of inhibition zones increased for all the three *S. aureus* F1;F&F3 (Table 8). Photo 4 showed the inhibition zone that produced by *Lactobacillus brevis* 1 against *S.aureus* F1. The AgNPs produced could inhibit about two times increased compared with natural bacteriocins (table 7). Thus, AgNPs could be evaluate as mean broad- spectrum antibacterial agents. Which were extreme important pathogenic. Since the biosynthesized AgNPs appeared considerable antimicrobial activity, it could be po-

tential to use widely in food and feed applications.

Conclusion and recommendations

From the previously mentioned results, it can be concluded that multidrug resistant *S. aureus* could infect *Diplodus sargus* broadstocks causing high mortalities and bacteriocin nanoparticles has an inhibitory effect on it. In spite of viability of probiotic strains, their ability to adhere and colonize the digestive tract and their subsequent ability to modulate the intestinal microbiota are of high importance to investigate, no information is available on these topics in fish. These issues should be considered in future studies on administration of probiotics in aquaculture.

Another paper will be established to apply bacteriocin nanoparticles in experimentally infected marine fish with multidrug resistant *S. aueus* and evaluate its antibacterial effect *in vivo.*

References

Aboyadak, I.M. Sabry, N.M.; Ali, N.G. and El-Sayed, H.S. (2016). Isolation of Staphylococcus epidermidis, Bacillus cereus and Pseudomonas stutzeri from diseased European sea bass (Dicentrarchus labrax) for the first time in Egypt. Egypt. J. Aquat. Biol. Fish., 20(4): 103-114.

Akinjogunla, O.J.; Inyang, C.U. and Akinjogunla, V.F. (2010). Bacterial species associated with anatomical parts of fresh and smoked Bonga Fish (Ethmalosa fimbriata): Prevalence and Susceptibility to Cephalosporins. J. Microbiol, 6: 87-97.

Alanis, A.J. (2005). Resistance to antibiotics: are we in the postantibiotic era?. Archiv. Med. Res., 36, 6, 697–705.

Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C. and Truck, M. (1966). Antibiotic Susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45, 493-496.

Baxa, D.V.; Kawai, K.; Ando, H. and Kusuda, R. (1985). Edwardsiella tarda and Staphylococcus aureus isolated from cultured red sea bream. Reports of the USA Marine Biological Institute Kochi University, 7, 1–8.

Bhanderi, B.B.; Roy, A.; Yadav M.M.; and Joshi, C.G. (2009). PCR based detection of virulence associated genes of Staphylococcus aureus from clinical and subclinical bovine mastitis. Royal Vet. J. India. No 5, Vol I and II: 20-26 Jan. and July 2009.

Bucio Galindo, A.; Hartemink, R.; Schrama, J.W.; Verreth, J.A.J. and Rombouts, F.M. (2006). Presence of lactobacilli in the intestinal content of freshwater fish from a river and from a farm with a recirculation system. Food Microbiol., 23: 476-482.

CLSI (2006). Clinical and Laboratory Standards Institute: Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved Guideline M45-A. CLSI, Wayne, PA, USA.

Cotter, P.D.; Hill, C. and Ross, R.P. (2005). Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. 3, 777–788.

CruickShank, R.; Duguid, R.; Mormion, B.P. and Swain, R.H.A. (1980). Medical Microbiology 12th Ed., Vol. II Reprinted, Churchil Livingstone Edinburgh London, New York.

Doškař, J.; Pantůček, R.; Růžičková, V. and Sedláček, I. (2010). Molecular Diagnostics of Staphylococcus aureus. In, Detection of Bacteria, Viruses, Parasites and Fungi. Springer, pp. 139- 184.

Edwards, A.M.; Massey, R.C. and Clarke, S.R. (2012). Molecular mechanisms of Staphylococcus aureus nasopharyngeal colonization. Molecular Oral Microbiol., 27:1–10.

Elayaraja, S.; Annamalai, N.; Mayavu, P.; and Balasubramanian, T. (2014). Production, purification and characterization of bacteriocin from Lactobacillus murinus AU06 and its broad antibacterial spectrum. Asian Pac. J. Trop. Biomed. 4, S305–S311. doi: 10.12980/APJTB.4.2014C537.

El-Gendy, A.O.; Essam, T.M.; Amin, M.A.; Ahmed, S.H. and Nes, I.F. (2013). Clinical screening for bacteriocinogenic Enterococcus faecalis isolated from intensive care unit inpatient in Egypt. J. Microb. Biochem. Technol. 4, 161–167. doi: 10. 4172/1948-5948. 1000089.

Gaze, W. O'Neill, C.; Wellington, E. and Hakey (2008). Antibiotic resistance in the environment, with particular reference to

MRSA. Adv. Appl. Microbiol., 63,249–280

Ghanbari, M.; Jami, M.; Kneifel, W. and Domig, K.J. (2013). Antimicro-bial activity and partial characterization of bacteriocins pro-duced by lactobacilli isolated from sturgeon fish. Food Contro. l32: 379–385.

Ghanbari, M.; Rezaei, M.; Jami, M. and Nazari, R.M. (2009). Isolation and characterization of Lactobacillus species from intestinal con-tents of beluga (Huso huso) and Persian sturgeon (Acipenserpersicus). Iranian Journal of Veterinary Research, Shiraz University., 10: 152–157.

Gil, P.; Vivas, J.; Gallardo, C.S. and Rodriguez, L.A. (2000). First isolation of Staphylococcus warneri, from diseased rainbow trout, Oncorhynchus mykiss (Walbaum), in northwest Spain. J. Fish Dis., 23: 295–8.

Golomazou, E.; Athanassopoulou, F.; Vagianou, S.; Sabatakou, O.; Tsantilas, H.; Rigos, G. and Kokkokiis, L. (2006). Diseases of White Sea bream (Diplodus sargus L.) Reared in experimental and commercial conditions in Greece. Turk. J. Vet. Anim. Sci., 30(1): 389-396.

Gómez-Sala, B.; Munoz-Atienza, E.; Sánchez, J.; Basanta, A.; Herranz, C.; Hernández, P.E. and Cintas, L.M. (2015). Bacteriocin production by lactic acid bacteria isolated from fish, seafood and fish products. Eur. Food Res. Technol. 241, 341–356.

Gufe, C.; Hodobo, T.C.; Mbonjani, B.; Majonga, O.; Marumure,J.; Musari, S.; Jongi, G.; Makaya, P.V. and Jairus Machakwa, J. (2019). Antimicrobial Profiling of Bacteria Isolated from Fish Sold at Informal Market in Mufakose, Zimbabwe. Int. J. Microbiol., 2019, 7 pages

Hartemink, R. and Rombouts, F.M. (1999). Comparison of media for the detection of bifidobacteria, lactobacilli and total anaerobes from faecal samples. J. Microbiol. Methods. 36: 181-192.

Heo, W.S.; Kim, E.Y.; Kim, Y.R.; Hossain, M.T. and Kong, I.S. (2012). Salt effect of nisin Z isolated from a marine fish on the growth inhibition of Streptococcus iniae, a pathogen of streptococcosis. Biotechnol. Lett. 34, 315–320.

Kim, D.H. and Austin, B. (2008). Characterization of probiotic carnobacteria isolated from rainbow trout (Oncorhynchus mykiss) intestine. Lett. Appl. Microbiol. 47(3): 141-147.

Kusuda, R. and Sugiyama, A. (1981). Studies on the characters of Staphylococcus epidermidis isolated from diseased fish. I. Morphological, biological and biochemical properties. Fish Pathology, 16,15–24.

Lagha, A.B.; Haas, B.; Gottschalk, M. and Grenier, D. (2017). Antimicrobial potential of bacteriocins in poultry and swine production. Vet Res. 48:22. doi: 10.1186/s13567-017-0425-6.

Levy, S.B. and Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses," Nature Medicine, 10, S12, S122–S129.

Levy, S.B. (2002). The antibiotic paradox:how the misuse of antibiotic destroys their curative powers.2Ed. Cambridge: Perseus Publishing, 353p.

Mahesh, B. and Satish, S. (2008). Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. Worl. J. Agri. Sci. 4, 839-843.

Muñoz-Atienza, E.; Gómez-Sala, B.; Araújo, C.; Campanero, C.; del Campo, R.; Hernández, P.E.; Herranz, C. and Cintas, L.M. (2013). Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC Microbiol. 13, 15-36.

Musikasang, H.; Tani, A.; H-kittikun A. and Maneerat, S. (2009). Probiotic potential of lactic acid bacteria isolated from chicken gastrointestinal digestive tract. Wor. J. Microbiol. Biotechnolo., 25, 1337–1345.

Najiah, M.; Aqilah, N.; Lee, K.; Khairulbariyyah, Z.; Mithun, S.; Chowdhury, A.J.K.; ShaharomHarrison, F. and Nadirah, M. (2012). Massive mortality associated with Streptococcus agalactiae Infection in Cage-cultured Red Hybrid Tilapia Oreochromis niloticus in Como River, Kenyir Lake, Malaysia. J. Biolo. Sci., 12, 438-442.

Noga, E. J. (2010). Fish Disease: Diagnosis and Treatment. Hoboken: John Wiley & Sons, Inc.

Poulose, S.; Panda, T.; Nair, P.P. and Théodore, T. (2014). Biosynthesis of silver nanoparticles, J. Nanosci. Nanotechnolo., 14, 2,

2038–2049.

Pridgeon, J.W. and Klesius, P.H. (2012). Major bacterial diseases in aquaculture and their vaccine development. CAB Rev., 7(48): 1-16.

Qing, W.U., M.D.; Yan Li, M.D.; Huixia Hu, M.S.; Ming Wang, M.D.; Zegang Wu, M.S. and Wanzhou Xu, M.S. (2012). Rapid Identification of Staph. aureus: FISH Versus PCR Methods. Lab Medicine 43, 6, 276-280.

Raphaël, K.J. and Meimandipou, A. (2017): Antimicrobial Activity of Chitosan Film Forming Solution Enriched with Essential Oils; an *in Vitro* AssayIran J Biotechnol. 152, 111–119.

Rojo-Bezares, B.; Sáenz, Y.; Poeta P.; Zarazaga, M.; RuizLarrea, F. and Torres, C. (2006). Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. Int. J. Food Microbiol., 111, 234–240.

Sagar, G. and Ashok, B. (2012). Green Synthesis of Silver Nanoparticles Using Aspergillus niger and Its Efficacy Against Human Pathogens. Euro. J. Exp. Bio., 2, 1654-1658.

Sahoo, T.K.; Jena, P.K.; Patel, A.K. and Seshadri, S. (2016). Bacteriocins and their applications for the treatment of bacterial diseases in aquaculture: a review. Aquacult. Res. 47, 1013–1027.

Samia, I.A. and Hala, A.E. (2017). Bacteriological studies on some virulence factors in Staphylococcus aureus isolated from chicken and Nile tilapia. Egypt. J. Chem. Environ. Health, 3, 1, 02-36.

Seiler, C. and T. Berendonk, T. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Frontiers in Microbiol. 3, 399.

Shah, K.L. and Tyagy, B.C. (1986). An eye disease in silver carp, Hypophthalmichthys molitrix, held in tropical ponds, associated with the bacterium Staphylococcus aureus. Aquaculture, 55, 1–4.

Shahid, M.; Hussain, B.; Riaz, D.; Khurshid, M.; Ismail, M. and Tariq, M. (2017). Identification and partial characterization of potential probiotic lactic acid bacteria in freshwater Labeorohita and Cirrhinusmrigala. Aquacult. Res. 48, 1688–1698.

Shin, M.S.; Han, S.K.; Ji, A.R.; Kim, K.S. and Lee, W.K. (2008). Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. Journal of Applied Microbiology, 105, 2203–2212.

Silva, C.C.G.; Silva, S.P.M. and Riberio, S.C. (2018). Application of bacteriocins and protective cultures in dairy food preservation. Front. Microbiol. 9, 594.

Soliman, M.K.; Ellakany, H.F.; Gaafar, A.Y.; Elbialy, A.K.; Zaki, M.S. and Younes, A.M. (2014). Epidemiology and antimicrobial activity of methicillin-resistant Staphylococcus aureus (MRSA) isolated from Nile tilapia (Oreochromis niloticus) during an outbreak in Egypt. Life Sci. J., 11, 10, 1245-1252.

World Health Organization (WHO) (2007). Critically Important Antimicrobials for Human Medicine: Categorization for the Development of Risk Management Strategies to Contain Antimicrobial Resistance Due to NonHuman Antimicrobial Use: Report of the Second, WHO Expert Meeting, Copenhagen, Denmark.