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EFFECT OF BACTERIA ON SURVIVAL AND ISOZYMES IN NILE TILAPIA, *OREOCHROMIS NILOTICUS* FINGERLINGS

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ABSTRACT

Citrobacter freundii, Bacillus pumilus, and *Bacillus firmus* were isolated from apparently healthy Nile tilapia (*Oreochromis niloticus*) and these isolates were harmless to *O. niloticus*. Six hundred apparently healthy fish (average body weight 10 ±1 g) were divided into five equal groups (120 fish each). Fish of the 1st group served as a control and was fed un-supplemented feed during the entire period of the experiment. Fish of the 2nd, 3rd and 4th groups were fed with feed containing 10^7cells/g of *C. freundii, B. pumilus* and *B. firmus*, respectively. The 5th group was fed with a mixed feed containing an equal number (10^7/g) of the three tested bacteria. After 14 days of feeding, the fish were challenged intraperitoneally (IP) by *Aeromonas hydrophila*. The survival rate was recorded and fish samples were examined for the effects of pathogenic bacteria on some isozymes expression in liver and muscle tissues. The starch gel electrophoresis was used to study the changes on relative mobility of isozymes as an expression of gene product. The survival rate was significantly high in the fish group that fed a diet containing *B. pumilus* in contrast to the group fed diet containing *B. firmus*. The control fish showed the lowest survival rate. There were differences between the treated and control fish regarding the relative mobility of electrophoretic bands. An increase in the number of electrophoretic bands of the treated fish was observed compared to that of the control fish. This confirmed the effect of bacteria on genes expression represented by the presence of different forms of bands of liver tissue. Isozyme results agreed with those for the survival ratio, where α-esterase in *Aer. hydrophila* and *B. firmus* gave the lowest ratio of survival which were in the line with isozyme results that expressed α-esterase, at low electrophoretcic relative mobility. Positive and strong correlation coefficient was shown between survival rate and relative mobility for *C. freundii* in muscles Lactic Dehydrogenase (LDH) and Malate Dehydrogenase (MDH) and liver general esterase, whereas it was weak for muscles α-Esterase. *B. pumilus* was demonstrated negative correlation coefficient between survival rate and relative mobility for all tested isozymes from liver and muscles. *A. hydrophila* revealed negative correlation coefficient with muscles LDH compared with, a low and moderate in the rest of tested isozymes.

Keywords: Bacteria, pathogenic, probiotic, fish, *Oreochromis niloticus*, survival, electrophoresis, isozyme, gene expression, correlation.

INTRODUCTION

In fish farms and hatcheries, bad water quality, handling and stress, may lead to infection of fish by bacteria. Most of the bacterial pathogens cause serious disease problems in tilapia production and may lead up to 80% mortality during the rearing cycle (Shoemaker *et al.*, 2000). *Aeromonas* species are causative agents responsible for septicemia and local inflammations and necrosis on skin, muscles and soft tissue (Řehuka, 2002). They are known as important pathogens for humans and lower vertebrates, including amphibians, reptiles and fish (Janda and Abbott, 1998). The characteristic of *Aeromonas* species or its extracellular product is their ability to secrete a wide variety of enzymes associated with pathogenicity (Pemberton *et al.*, 1997; Rey *et al.*, 2009). Other bacteria in the mean time, naturally occurred in aquaculture, are useful for fish and may be used as probiotics to enhance fish growth and immunity having antibacterial effect (Abd El-Rhman et al., 2009). *Bacillus pumilus* release protein and carbohydrates at certain conditions (Sung Deuk *et al.*, 2003). Moreover, *B. firmus* and another *Bacillus* species were capable of producing haloperoxidases (Hunter-Cevera *et al.* 1991) and could produce enzymes needed in the degradation of fish protein and in flavor compound development in pates (Sanchez and Klitsaneephaiboon, 1983).

The electrophoretic molecular variations, which may be happened due to the environmental disturbances, are largely genetically controlled and co-dominant (McAndrew and Majumdar, 1983). For example, parasites, diseases and pollution can affect the level of enzymse activities resulting in some tissues damaged and may release tissue specific-enzymes into blood stream. Consequently, disease and pollution can affect levels of enzyme activity, and electrophoretic results can be affected both quantitatively and qualitatively (Poly, 1997).

Rey et al. (2009) described the tissue distribution and morphological changes induced by *Aeromonas hydrophila* or its extracellular products after the experimental infection of two fish species; white cachama (*Piaractus brachypomus*) and tilapia hybrids (*Oreochromis species*). *B. firmus* gave the highest mortality rate

in contrast to *B. pumilus* that gave significantly low mortality rate, moreover, there were variations in allozymes banding phenotype, relative mobility and optical density of tilapia treated with bacteria (Kamel and Abd El-Rhman, 2005). The specific activities of esterase and certain other molecular properties indicated that the electrophoretic variations of these enzymes in bacterial populations resulted in allelic variations at specific gene loci (Goullet and Picard, 1995).

The aim of the current work was to study the effect of harmless *(Citrobacter freundii, Bacillus pumilus,* and *Bacillus firmus*) and pathogenic bacteria (*Aer. hydrophila*) on survival rate. The correlations between these bacteria and the isozymes as expression of gene products were used to detect the pathogensity of bacteria on Nile tilapia, *Oreochromis niloticus* fingerlings.

Materials and Methods

Bacterial isolation

Fifty Nile tilapia, *Oreochromis niloticus*, fingerlings (average body weight 10 ±1 g) were randomly collected from the earthen ponds in Abbassa Fish Farm; Abbassa, Abu-Hammad, Sharkia Governorate, Egypt. Bacteriological examination of the collected fish samples was done through the inspection of the internal organs (liver, kidney, gonad, stomach, and intestine). Gills were cultured on treptic soya broth (TSB) and incubated at 30°C for 24-48 h afterwards, inocula from broth were inoculated on treptic soya agar (TSA). Purification and the identification of the isolates were conducted using biochemical tests according to Bergey *et al.* (1984). API 20 E strip system (Bio Merieux) was used for *Enterobactereaceae* identification.

Experimental infection

The experimental infection was done using the three bacterial isolates to study their pathogenicity among Nile tilapia samples. A random sample of 240 clinically healthy fish (Av. weight 10 ±1 g) was distributed among 24 glass aquaria (40×70×80 cm) at a stocking density of ten fish per aquarium. Fish were acclimatized to lab conditions for two weeks period after which they were divided into 8 equal groups (with three replicates per treatment). Fish from groups 1, 2 & 3 (T1 – T3) were inoculated intramuscularly (IM) with 0.2 ml of saline solution containing 10^7 cells/ml of each of Citrobacter *freundii*, *Bacillus pumilus*, and *Bacillus firmus*, respectively, whereas fish from groups 4, 5 & 6 (T4 – T6) were inoculated

intrapretonially (IP) with 0.2 ml containing 10^7 cells/ml of each of *C. freundii, B. pumilus* and *B. firmus*, respectively. Fish from Group 7 (T7) were inoculated with IM with 0.2 ml of sterile saline solution and those of group 8 (T8) were inoculated IP with 0.2 ml of sterile saline solution as negative control groups. All groups were kept under observation for 14 days and mortality rates were recorded. At the end of the experiment, fish were subjected to laboratory thorough examination and bacterial re-isolation.

Feeding experiments

Six hundred clinically healthy Nile tilapia, *O. niloticus*, fingerlings (Av. weight 10 ±1 g) were acclimatized for two weeks; after which they were randomly distributed into five equal groups (120 fish each) among five tanks supplied with dechlorinated tap water and continuous aeration. Fish of the 1st group served as a control (CTR) and was fed with unsupplemented food during the entire period. Fish of 2nd, 3rd & 4th groups were fed with feed containing 10^7/g of *C. freundii, B. pumilus,* and *B. firmus*, respectively. The 5th group was fed with mixed feed containing an equal number (10^7/g) of the three bacteria under examination. Fish were fed until apparent visual satiation three times daily for 14 days. At the end of feeding trial, fish of each group were divided into two subgroups. The first subgroup of each treatment was divided into three replicates (20 fish each). This subgroup was injected IP with *Aer. hydrophila* (0.2 ml of 10^7 cells/ml) to evaluate the resistance of fish to *Aer. hydrophila*. The second subgroup was injected IP by 0.2 ml of a sterile saline solution as a control. *Aer. hydrophila* was obtained from Fish Disease Department, Central Laboratory for Aquaculture Research, Abbassa, Abu-Hammad, Sharkia. This species was isolated from the liver of diseased Nile tilapia. Both subgroups were kept under observation for 14 days to record the daily mortality rate and re-isolate the bacteria that were existed.

Isozymes electrophoretic study

Samples preparation

Ten random fish samples were collected from each treatment at the end of the feeding experiment and after the fish injected by *Aer. hydrophila*. Fish were apparently healthy and stored at −30 ^{0}C for analysis. Additional fish samples were used as a control from the original stock without any treatment. Muscles and liver

tissues were used for isozymes electrophoretic analyses. Equal volume of distilled water was added to each sample prior to mechanical homogenization using homogenizer (Virtishear, Visrt,Campany. Homogenizer, INC. serial no. 206325, USA). Each tube with homogenate was centrifuged at 3000 g for 30 min at 5 ^{0}C. The supernatant was transferred into another tube and kept refrigerated until being used.

Gels preparation and isozymes electrophoresis

All chemicals reagents used in the present study were obtained from Sigma Chemicals Co. (St Louis, MO). Starch potato was used to prepare the gels. Different systems of buffer were used for isozymes electrophoretic analysis. Gels were cooked one day before use and kept in a refrigerator at 4 °C. After loading the samples, gels were run at 5°C according to Kamel (1999) and enzymes were visualized, using staining methods of Shaw and Prasad (1970). The protein loci that encoded enzymes were tested to show the isozymes variations by resolving four enzymes, namely, Lactic Dehydrogenase (MDH)) (LHD*(EC1.1.1.27)), Malate Dehydrogenase (MDH* (EC1.1.1.37)), α-Esterase, α EST* and general EST* (EC3.1.1.-). Locus and alleles nomenclature were conducted following Shaklee *et al.* (1990) technique.

Data analysis

Gels were analyzed using Gel Analyzer Ver. 3 program software (2007, http://www.geocities.com/egygene) and software (UVIgeltec Ver.12.3) to calculate the relative electrophoretic mobility for each individual.

Statistical analysis was performed using one way and two ways analysis of variance (ANOVA). Duncan's Multiple Range Test was applied as a post-hoc test to determine differences among treatment means at a significance level of $P<0.05$. Standard errors were also estimated. All statistics were run using the SAS program (SAS, 2000).

RESULTS

The phenotypic and biochemical characteristics of the bacterial isolates were detected as *Citrobacter freundii, Bacillus pumilus*, and *Bacillus firmus*. *C. freundii* was enterobacteriaceae, Gram-negative bacilli, motile, fermentative, and oxidase negative. *B. pumilus* was Gram-positive bacilli, variable with oxidase reaction but citrate, Voges-Proskauer, and ornithine decarboxylase positive. *B. firmus* was Gram-

positive bacilli and did not give any reaction with oxidase, citrate, Voges-Proskauer, or ornithine decarboxylase. *B. firmus* and *C. freundii* were isolated from fish's stomachs, while *B. pumilus* was isolated from fish's gonads.

Mortality rate and clinical signs

From the experimental infection of the three bacterial isolates, *C. freundii* was harmless via IM route, while IP injection mortality was 6.7% without clinical signs (Table 1). *B. pumilus* was harmless to fish where no clinical signs or mortalities were seen following the injection of IP or IM. Moreover, although *B. firmus* had no mortalities, the injected fish with IP or IM had clinical signs (black coloration all over the fish body). Bacterial analyses at the end of the experiment revealed the isolation of those bacteria from the corresponding injected fish group and no other pathogens were isolated.

Table 1. The experimental infection to evaluate the pathogenicity of bacterial isolates in Nile tilapia and the resulted mortality.

Group	Bacteria/saline	Route of injection	Mortality (%)
T_1	*Citrobacter freundii*	IM	0.0
T_2	*Bacillus pumilus*	IM	0.0
T_3	*Bacillus firmus*	IM	0.0
T_4	*Citrobacter freundii*	IP	6.7
T_5	*Bacillus pumilus*	IP	0.0
T_6	*Bacillus firmus*	IP	0.0
T_7	Sterile saline (Control)	IM	3.3
T_8	Sterile saline (Control)	IP	10.0

IP = intra-peritoneal, IM = intra-muscular, * 0.2 ml of 10^7 bacterial cells/ml.
Each group contained three replicates of ten fish per each.

Survival of fish fed the experimental diets after challenging with *Aer. hydrophila*

There was no evidence of disease in any of the fish groups received the three bacteria during the 14 days of the experimental challenge. The survival rate was significantly increased among groups fed on diets containing these bacteria compared to the control group (Fig 1). After *Aer. hydrophila* challenge, fish survival rate was relatively high in fish fed diets containing *B. pumilus*, mixture of the three

bacteria, and *C. freundii* (78.3±4.4, 73.3±1.7, and 70±2.9%, respectively). Fish group fed diet supplemented with *B. firmus* had a relatively lower survival rate (43.3± 6.0%), but this was still higher than that of the control group (21.7±6.0%).

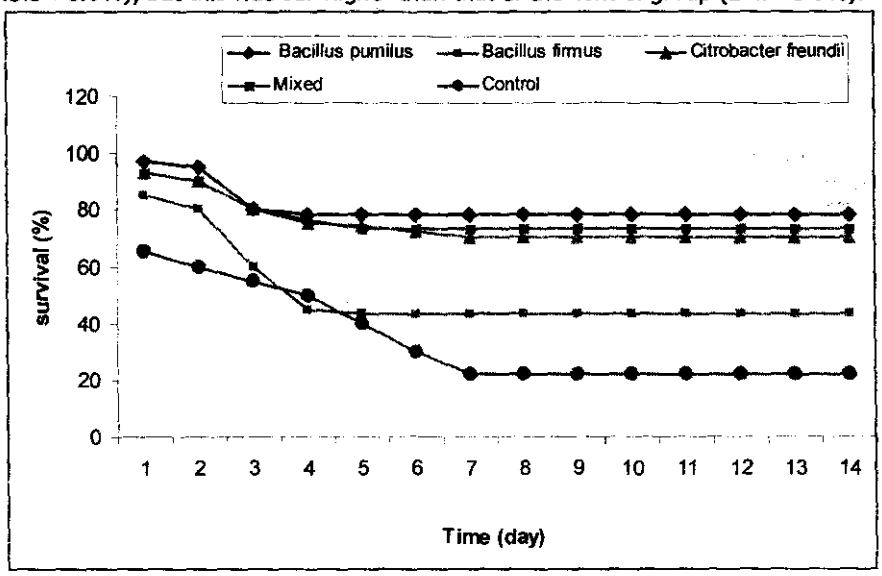

Fig 1. Survival (%) of Nile tilapia fingerlings fed on diet supplemented by each of harmless bacteria (*Citrobacter freundii, Bacillus pumilus* and *Bacillus firmus*) for 14 days and challenged by pathogenic *Aeromonas hydrophila* for further 14 day.

Isozymes electrophoresis

Results of samples analyses for expression of gene products and relative mobility; and the differences among the treated groups (pathogenic, harmless bacteria, and their mixtures) compared to that of the control are shown in Tables 2 and 3 and graphically represented in Figure 2.

Liver esterase

***α*- esterase**

The results of injection of the pathogenic, harmless bacteria, or their mixtures groups on liver *α*-esterase isozyme showed an increase in the number of bands compared to that of the control group. The decrease in the relative mobility of liver α-esterase in treated fish was recorded for *EST*-2* (locus 2) isozyme band.

The lowest relative mobility for *EST*-1* (locus 1) isozyme band was recorded for *Aer. hydrophila and B. firmus*. There was negative correlation between the survival rate and relative mobility of bands for *B. pumilus* (Table 3). The relationship between the survival rate and relative mobility was positive with no significant correlation for B. *firmus* (r=0.292).

Table 2. Effect of bacteria (*Citrobacter freundii, Bacillus pumilus Bacillus firmus* and their mixture and *Aeromonas hydrophila*) on relative mobility for liver (α-esterase, general esterase), and muscles (α-esterase, lactic dehydrogenase (LDH) and malate dehydrogenase (MDH))

	locus	Liver		Muscles		
		α-Esterase	Esterase general	α-Esterase	LDH	MDH
Citrobacter freundii	Locus 1	0.24	0.48	0.37	0.65	0.59
	Locus 2	0.41	0.76	0.56	0.73	0.80
	Locus 3	0.63		0.76		
	Locus 4			0.99		
Bacillus firmus	Locus 1	0.17	0.32	0.30	0.55	0.47
	Locus 2	0.55	0.71	0.52	0.71	0.77
	Locus 3	0.75		0.74		
	Locus 4			1.00		
Aeromonas hydrophila	Locus 1	0.02	0.29	0.28	0.55	0.42
	Locus 2	0.36	0.45	0.50	0.73	0.76
	Locus 3	0.57	0.74	0.74		0.96
Mixture	Locus 1	0.27	0.29	0.19	0.48	0.38
	Locus 2	0.41	0.47	0.43	0.70	0.71
	Locus 3	0.57	0.71	0.69		
Bacillus pumilus	Locus 1	0.30	0.35	0.18	0.42	0.30
	Locus 2	0.46	0.55	0.42	0.55	0.63
	Locus 3	0.66	0.79	0.69	0.69	
Control	Locus 1	0.48	0.20	0.08	0.38	0.19
	Locus 2	0.71	0.60	0.34	0.51	0.54
	Locus 3			0.63	0.67	

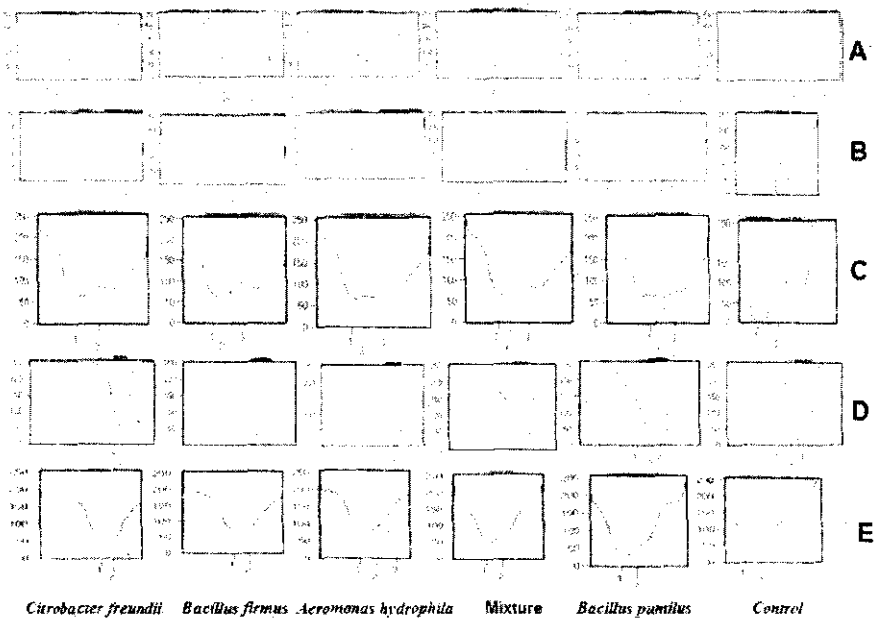

Fig 2. Effect of *Citrobacter freundii, Bacillus pumilus, Bacillus firmus* and their mixture and *Aeromonas hydrophila* on A- liver α esterase, B- liver general esterase, and C- muscles α esterase, D- muscles lactic dehydrogenase and E- muscles malate dehydrogenase.

General esterase *EST** (EC3.1.1.)

B. pumilus, Aer. hydrophila, and bacterial mixture treated fish groups were represented by three loci, whereas, *C. freundii, B. firmus,* and the control groups were represented by only 2 loci. *B. pumilus* showed higher relative mobility for *EST*-3* (locus 3) isozyme. In the same time, the control group represented the lowest relative mobility. Moderate correlation coefficient was found between survival rate and the relative mobility of bands (r= 0.582) for *Aer. hydrophila* group, while very strong correlation coefficient existed between survival rate and relative mobility of bands for *C. freundii* group. In parallel, a negative correlation was obtained for both *B. pumilus* and *B. firmus* groups (Table 3).

Muscles

***α*-esterase**

Three loci were recorded among *Aer. hydrophila group,* mixture bacteria-treated groups and the control. While, *B. firmus* and *C. freundii* showed four loci. *B. firmus*

produced the highest relative mobility among the treatments. Negative correlation was found between survival rate and relative mobility of bands for *B. pumilus* and *B. firmus* groups, while positive and low correlation was recorded for *C. freundii* (Table 3).

Lactic dehydrogenase

B. pumilus and control groups produced three loci, while the other treated groups produced *only two loci. C. freundii* and *A. hydrophila* showed the same relative mobility, which was higher than other groups. Positive and strong correlation coefficient was obtained between the survival rate and relative mobility for *C. freundii* group, whereas negative correlation was found for other treated groups (Table 3).

Malate dehydrogenase

All bacteria-treated groups and the control produced two loci, while *Aer. hydrophila* group produced three loci. The highest relative mobility was recorded for *C. freundii* group compared to other treatments. Positive and strong correlation coefficient was found between survival rate and the relative mobility for *C. freundii* group (Table 3), while it was weak for *Aer. hydrophila* group. Negative correlation coefficient was recorded for *B. pumilus*, *B. firmus* and mixture groups.

Table 3. Correlation coefficient between the effect of bacteria *Citrobacter freundii, Bacillus pumilus Bacillus firmus* and their mixture on relative mobility for liver (α-esterase, general esterase), and muscles (α-esterase, lactic dehydrogenase (LDH) and malate dehydrogenase (MDH)) and fish survival rate,

Test Bacteria	**Liver**		Muscles		
	α-Esterase	**General Esterase**	**α-Esterase**	**LDH**	**MDH**
Citrobacter freundii	0.412	1.000	0.284	1.000	1.000
Bacillus firmus	0.293	-1.000	-0.204	-1.000	-1.000
Aeromonas hydrophila	0.319	0.582	0.459	-1.000	0.300
Mixture	0.373	0.414	0.371	-1.000	-1.000
Bacillus pumilus	-0.997	-0.999	-0.999	-1.000	-1.000

Discussion:

The bacterial isolates were identified as *Citrobacter freundii, Bacillus pumilus,* and *Bacillus firmus*. The present work's results were similar to those of Bergey *et al.* (1984), Toranzo *et al.* (1994), Ghosh *et al.* (2002), and Aly *et al.* (2008). *C. freundii,* in the present study, was isolated from the Nile tilapia stomach. however, this species was isolated from other organs in different animals, such as intestine of *Cyprinus carpio, Ctenopharyngodon idella* and *O. niloticus* (Sugita *et al.,* 1985), gastrointestinal tract of soft-shelled turtle (Sugita and Deguchi, 1983), various organs of sunfish (Sato *et al.,* 1982; Pastore, 2008) and from buccal cavity and cloacae of *Caretta caretta* (Foti *et al.* 2009). In the present study, *B. pumilus* was isolated from Nile tilapia gonads. However, this species was isolated from mosquito larvae (Lavrova and Mikhnovskaja, 1978), rohita fingerlings (Ghosh *et al.*, 2002), the shell surface of *Balanus amphitrite* (Khandeparker *et al.*, 2003), *O. niloticus* (Aly *et al.*, 2008), and from crab, oyster and starfish (Parvathi *et al.*, 2009).

Moreover, *B. firmus* was isolated from Nile tilapia stomach in the present work. In prior studies, this species was isolated from mosquito larvae from natural water bodies (Lavrova and Mikhnovskaja, 1978). *Bacillus* genus was also isolated from bivalves (Sugita *et al.*, 1981), marine fish (Sugita *et al.*, 1998), and crustacean's intestine (Rengpipat *et al.,* 2000).

The administration of *C. freundii,* and *B. pumilus* via IM or IP routes did not cause disease signs in Nile tilapia. Fish group fed diet containing *B. firmus* showed no mortality, however, the infected fish had shown disease signs. There was no significant difference for mortality rate among the bacteria-treated groups and control group. However, IP inoculation of *C. freundii* group had induced a mortality rate of 6.7% compared to that of the control groups that produced a mortality rate of 3.3% and 10%, upon IM and IP sterile saline injection, respectively. The mortality rate in the control group could have resulted from the stress of injection or handling and/or other unidentified environmental stressors that was absent in other groups that received *B. pumilus* and *B. firmus*. These results suggest that *B. pumilus* and *B. firmus* might play a role in improving body defense against environmental factors. Moriarty

(1998) claimed that Bacillus species were not associated with pathologies in aquatic organisms. *B. firmus* I-1582 was not toxic, infectious or pathogenic to laboratory animals(EPA, 2008). Moreover, Chowdhury and Wakabayashi (1989) confirmed that *C. freundii* was non pathogenic to fish.

The survival rate of fish after challenged with pathogenic bacteria *Aer. hydrophila* indicated that bacterial isolates protected Nile tilapia against *Aer. hydrophila* and resulted in a higher survival rate as compared to that of the control fish. These results are in agreement with those of Abd El-Rhman *et al.* (2009) who recorded that *Micrococcus luteus* protected Nile tilapia against *Aer. hydrophila.* Robertson *et al.* (2000) found that feeding fish with probiotic for 14 days had resulted in an improved survival rate following challenge with pathogenic bacteria (*Aer. salmonicida, Vibrio ordalii* and *Yersinia ruckeri).* Moreover, Duc *et al.* (2004) and Parvathi *et al.* (2009) indicated that *B. pumilus* could produce protease and lipase enzymes and gave high anti-spore immunoglobulin G titers, and bacteriocin-like activity against other Bacillus. This phenomenon may explain the highly significant increase in the survival rate of fish fed *B. pumilus* in the present study. Chowdhury and Wakabayashi (1989) found that *C. freundii* is effective in reducing the number and infectivity of *Flexibacter columnaris.* Similar findings were reported in the present study where *C. freundii* protected fish against pathogenic *Aer. hydrophila* with 70% survival rate. Also, Atta *et al.* (2008) recorded that *B. firmus* contained a precursor of Cyanocobalamin (B12) which is needed for building proteins in the body and red blood cells.

The expression of gene product represented the activity of isozymes, which was varied among the control and bacteria-treated fish groups. The variation in loci and band number in treated fish was higher in liver α-esterase where all bacteria-treated groups gave three loci, while the control gave only two loci. These variations in isozyme data may be due to the damage and change in DNA sequence leading to the change in gene expression and its product. The increase in the activity may be also due to damaged tissues. The variation in gene expression and products was reflected in the variations of relative electrophoretic mobility of bands. These results are in accordance with Kamel and Abd El-Rahman (2005). *Aer. hydrophila* produced hemolysine and five extra cellular enzymes (amylase, lipase, protease, gelatinase and

chitenase) in contrast to *C. freundii* which failed to produce any of the extracellular enzymes or hemolysine (Hung-Hung and Tang-Yao, 1997; Rey *et al.*, 2009).

In the present study, both isozymes and survival rate results supported each other, since the variation in gene expression is attributable to the effect of pathogenic bacteria on DNA sequence (change of α-Esterase in *Aer. hydrophila* and *B. firmus*) gave the lowest survival rate. The lower survival rate agreed with the isozyme results being reflected in low electrophoretcic relative mobility of bands for α esterase. This can give a good example of the effect of bacteria on the locus that encoded this enzyme. Also, the variations in gene products and their relative electrophoretic mobilities among *C. freundii, B. pumilus,* and their mixtures treatments produced different survival rates among treatments due to their different inhibition effect on pathogenic bacteria. These findings are in the same line with these of John and Bauer (1982) who reported low esterase value during the pathogenic status. Grant (1990) reported that a misinterpreted enzyme system may lead to erroneous conclusions about the genetic structures of the taxa under study. Pemberton *et al.* (1997) indicated that *Aeromonas* produces enzymes (β-lactamases, lipases, hemolytic enterotoxins, proteases, chitinases, nucleases, and amylases) and multiple copies of genes encoding each type of enzyme that provide additional biological diversity, except for the chitinases. These multiple copies show little evolutionary relatedness at DNA level and limited similarity at the protein level.

Silverman *et al.* (1995) performed electrophoretic analysis of mussel and bacterial protein demonstrating that intact bacteria were not simply trapped in mussel tissues. *B. pumilus* and their mixtures gave the highest ratio of survival and highest relative electrophoretic mobility. This was due to the ability of Bacillus to persuade production of specific protein, which was supported by Ivanova *et al.* (1993, 1994); Prokof-eva *et al.* (1999) Kalinovskaya *et al.* (2002) and Duc *et al.* (2004). Romanenko *et al.* (2001) have shown that Bacillus had the capability to hydrolyze chitin and induce extracellular RNAses production. Horikoshi (1999) reported that *B. firmus* and *B. pumilus* have antibiotics and enzyme inhibitor. Moreover, several species of *B. pumilus* exhibited cytotoxic and Gram-positive antibiotic activity (Gerard, 1998). The multidrug resistant strain of bacterial

envelopes revealed an antibody response against some protein bands and band sequencing was performed to identify the protein stimulating the immune response. The sequence identity of 80% was seen in 10 amino acid overlaps of 36 kDa bands with a specific gene of *B. firmus*, (Nitzan *et al.*, 2004).

The inspection of *MDH** isozymes pattern among treatments revealed that *Aer. hydrophila* expressed different isozymes pattern of *MDH-3** and these results agreed with those survival rates. There was an increase in relative mobility of MDH in muscle that was correlated with low survival rate. These results agreed with those John and Bauer (1982) who recorded that MDH had increased under the pathogenic conditions due to tissues damage. The recorded higher relative mobility of LDH isozyme when challanged with *Aer. hydrophila* produced lower survival rate in contrast to that of the control. These results agreed with similar results obtained by Elnemaki (2003) who pointed out a significant increase in LDH enzymes in Tilapia species infected with metacercariae of *Pyigidiopsis summa*.

CONCLUSION

This method can be used for detection of pathogeneic bacteria and its virulence. *B. pumilus* and *C. frundii* might play a role in improving the body defense against bacterial infection. These data gave a good example of the effect of bacteria on locus that encoded this enzyme. We can use α esterase as a marker for pathogenity of bacteria on fish and survival.

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تأثير البكتيريا على الحيوية ومشابهات الإنزيمات فى إصبعيات أسماك البلطى النيلى

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الملخص العربى

تم في هذا البحث عزل بكتريا غير ضارة لأسماك البلطى وهى باسلس بوملس، باسلس فيرمس و ستروباكتر فروندای من أسماك ظاهريا سليمة . قسمت ستمائة اصباعية بلطى نيلى بمتوسط وزن ١٠جرام للسمكة إلى خمس مجوعات الأولى استخدمت كمجموعة ضابطة تم تغذيتها بعليقة خالية من البكتيريا ومن الجموعة الثانية حتى الرابعة تم تغذيتها بعليقة تحتوى على $١٠^{٧}$ خلية /جرام من بكتيريا غير مرضية و هى باسلس بوملس، باسلس فيرمس و ستروباكتر فروندای. بينما الجموعة الخامسة تم تغذيتها بعليقة خليط من هذة السلالات البكتيرية الثلاثة. تم تغذية جميع الاصبعيات من الجموعة الاولى حتى الخامسة ثم تم حقنها بالأيريموناس هيدروفيلا.

وقد سجلت النتائج أعلى نسبة حيوية بين أصبعيات البلطى النيلى التى تغذت ببكتريا الباسلس بوملس و اقل نسبة حيوية فى الباسلس فيرمس بينما كانت المجموعة الضابطة هى الاقل فى نسبة الحيوية.

ولتقيم تأثير هذة البكتيريا على بعض مشابهات الإنزيمات كتعبيرللجينات، تم استخدام طرق التفريد الكهربائى لبعض المشابهات الأنزيمية و هى الإستريز المتخصص و غير المتخصص، اللاكتات ديهدروجينيز، المالات ديهدروجينيز لأنسجة العضلات و الكبد لإصبعيات أسماك البلطى النيلى. كان هناك بعض الإختلافات فى التعبير و النشاط الجينى و الذى ظهر واضحا فى الإختلافات بين الهجرة النسبية للتفريد الكهربائى لكل المعاملات كما كان هناك زيادة فى عدد الحزم مقارنة بالمجوعة الضابطة التعبير وقد كان هناك ارتباط واضح بين نسبة الحيوية ونتائج الهجرة النسبية للتفريد الكهربائى وخاصة الإستريز فى باسلس فيرمس و بالأيريموناس هيدروفيلا.

كان هناك ارتباط قوى وموجب بين نسبة الحيوية ونتائج التفريد الكهربائى فى و ستروباكتر فروندای لمشابهات إنزيم اللاكتات ديهدروجينيز، المالات ديهدروجينيز لأنسجة العضلات و الإستريز غير المتخصص فى الكبد بينما اظهرت الإصبعيات التى تغذت ببكتريا الباسلس بوملس ارتباطا سالبا لجميع مشابهات الإنزيمات التى تم دراستها فى العضلات والكبد.

من ناحية إخرى كان هناك ارتباط سالب بين مشابهات انزيم اللاكتات ديهدروجينيز بالأيريموناس هيدروفيلا بالعضلات بينما كان الارتباط متوسط وضعيف بين باقى مشابهات الانزيمات.