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BACTERIAL-FLORA OF EGYPTIAN SALTED *MUGIL CEPHALUS* FISH (FESSIEKH) PCR - IDENTIFICATION

(With 4 Tables and One Picture)

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بكتريا اسماك البوري المملحة المصرية (الفسيخ) التصنيف بتفاعل البلمرة المتسلسل

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أجريت هذه الدراسة على 17 عينة من اسماك البوري المملحة المصرية (الفسيخ) حيث تم فحص العينات حسيا وكيميائيا وبكتريولوجيا. وقد أظهرت نتائج الفحص الحسي للعينات أن 15 (88.23%) عينة كانت مقبولة حسيا بينما 2 (11.77%) عينة فقط كانت غير مقبولة. أما الفحص الكيميائي فقد أظهرت نتائجه أن قيم تركيز أيون الهيدروجين ومحتوى ملح الطعام للعينات تتراوح بين 6.1 إلى 8.1 و بين 6.13 إلى 10.11 ،على التوالي. وأظهرت نتائج الفحص البكتريولوجي للعينات أن قيم العد الكلي للبكتريا الهوائية ، البكتريا أليفة الملوحة ، والبكتريا اللاهوائية تراوحت بين 2.64 إلى 6.91 ، 4.64 إلى 7.93 ، وبين 3.3 إلى 6.23 لو$_{10}$ خلية/جم ، على التوالي. هذا وقد تم استخلاص عدد 29 عتره من البكتريا الهوائية ، 31 عتره أليفة الملوحة ، 36 عتره لاهوائية بطريقة عشوائيه من أطباق الزرع المختلفة. تم تصنيف هذه العترات باستخدام تقنية تفاعل البلمرة المتسلسل- تتابع الحمض النووي. تم تصنيف عترات (29) البكتريا الهوائية إلى 18 عتره *Staphylococcus equorum* srain JH6 ، 6 عترات *Staph.* sp. L50 ، 2 عتره *Bacillus subtilis subspecies subtilis* strain BCRC 10255 ، 2 عتره *Lactobacillus* sp. CWBI/B-659/(E912) و 1 عترة *Bacillus subtilis* ، وتم تصنيف ال 31 عتره من البكتريا أليفة الملوحة إلى 16 عتره *Staphylococcus equorum* srain JH6 ، 10 عترات *Staph.* sp. L50 ، و 5 عترات *Teratogenococcus halophilus* ، هذا وقد تم تصنيف عترات (36) البكتريا اللاهوائية إلى 23 عتره *Clostridium bifermentans* strain IBUN 188 ، 7 عترات *Clostridium bifermentans* strain IBUN 179 ، 3 عترات *Clostridium* sp. Zx5 ، 1 عتره *Clostridium butyricum* strain W4 ، 1 عتره *Clostridium cochlearium* ، و1 عتره *Clostridium* sp. DF2C1.

SUMMARY

A total of 17 samples of Egyptian salted *Mugil cephalus* fish (fessiekh) were examined sensorial for appearance, juiciness, saltiness, rancidity, flavor and general acceptability; chemically for pH and NaCl content; and bacteriologically for aerobic plate count, halophilic bacterial count and anaerobic bacterial count. The sensory evaluation of the samples revealed that 15 (88.23%) of them were organoleptically accepted, while only 2 (11.77%) were unaccepted. The pH and NaCl content of the examined samples ranged from 6.1 to 8.1 and from 6.13 to 10.11, respectively. The aerobic, halophilic and anaerobic bacterial counts of the examined samples ranged from 2.64 to 6.91, 4.64 to 7.93, and from 3.3 to 6.23 $\log_{10}$ cfu/g , respectively. A total of 29, 31 and 36 colonies were picked randomly from aerobic, halophilic and anaerobic plates, respectively. The colonies were purified and being identified using PCR-sequencing technique. The 29 identified aerobic bacteria were 18 strains *Staphylococcus equorum* srain JH6, 6 strains *Staph.* sp. L50, 2 strain *Bacillus subtilis subspecies subtilis* strain BCRC 10255, 2 strains *Lactobacillus* sp. CWBI/B-659/(E912), and 1 strains *Bacillus subtilis*. The identified 31 halophilic bacteria included 16 strains *Staphylococcus equorum* srain JH6, 10 strains *Staph.* sp. L50, and 5 strains *Teratogenococcus halophilus*. The 36 identified anaerobes were *Clostridium bifer*mentans strain IBUN 188 (23 strains), *Clostridium bifermentans* strain IBUN 179 (7 strains), *Clostridium* sp. Zx5 (3 strains), *Clostridium butyricum* strain W4 (1 strain), *Clostridium cochlearium* (1 strain), and *Clostridium* sp. DF2C1 (1 strain).

Key words: *Salted fish, fesseikh, assessment, sensory, chemical, bacterial-flora, PCR-Identification.*

INTRODUCTION

The salting process is one of oldest methods in preservation of fish, and this process has still been used in many places in the world, either for economic reasons, owing to its low production costs, or in order to satisfy consumer habits (Yang *et al.*, 1981; Wheaton and Lawson, 1985). The effect of salt are obstructed growth of the microorganism or they destroyed, and in this way the fish meat gets durability (Tarr, 1969; Filsinger, 1987).

Historically, fish fermentation has been associated with the treatment of fish with salt and allowing the product to mature over several months (Kemp, 1973; Filsinger *et al.*, 1982). The final product

has a characteristic combination of appearance, odor, flavor and texture. The salting process is highly manipulative and with high potential for environmental transfer of microorganisms to the product. In addition some industrial abuses can occur, one of them being an excess of impurities in salt used for salting, e.g. $CaCl_2$ and $MgCl_2$ which retard the penetration of NaCl, enhancing fish bacterial spoilage (Van Klaveren and Legendre, 1965).

In Egypt ´Fesiekh´ is the Arabic name for a salted fermented Bouri (*Mugil cephalus*) fish. It is quite a popular food product, especially during certain occasions. Fessiekh is not a product of modern times considering its processing chain. Whole non-eviscerated fish are washed with tap water, and left to decompose for a day before salting. The salting process varies depending on the processor and so does the quality of the finished product; in general it involves stuffing of the gills and covering of the entire fish with approximately 15 – 25% salt by weight (El-Sebaiy and Metwalli, 1989). The process is done by unscientific methods with poor quality salt and unhygienic conditions.

As fessiekh constitute an important part of the diet of great portion of consumers in Egypt; and have been subjected to many risks of contamination from various sources (El-Sebaey *et al.,* 1999), so it is very important to evaluate the hygienic quality of such fish product.

Determination of bacterial-flora of Fessiekh using culture methods has been reported (Morshdy *et al.,* 1982; Abd-Allah 2008). Due to the known limitations of cultivation methods many recent studies have used culture independent 16S rRNA-based PCR techniques (Singh *et al.,* 2009) including PCR-Denaturing Gradient Gel Electrophoresis (DGGE) and PCR-Single Strand Conformation Polymorphism (PCR-SSCP) to determine the microflora of various traditional fermented Foods. (Chamkha *et al.,* 2008; Chen *et al.,* 2008; Kim *et al.,* 2009).

In general, the identification of bacteria based on phenotypic analysis needs special knowledge and judgment is often subjective. It is considered that the evolutionary history of bacteria is memorized in their ribosomal RNAs, so that the systematic classification and identification of bacteria in recent years have been based on the analysis of rRNAs sequences (JPF, 2003).

The objective of this study was to determine the sensory; pH and sodium chloride content; as well as bacterial-flora of the Egyptian salted fish *Mugil cephalus* (fessiekh) and identifying the bacterial-flora isolates

using a molecular approach, based on PCR amplification of divergent regions of the 16S rRNA gene followed by sequencing and comparison of the sequences with those in the database (PCR-sequencing technique) for accurate definition of these flora.

MATERIALS and METHODS

In this study Egyptian salted *Mugil cephalus* fish (fessiekh) were used. The samples were obtained from the different salted-fish markets in Cairo - Egypt and totally 17 samples were collected. The samples were placed in sterile polyethylene bags and stored at 4 ± 1°C until they were analysed.

2.1. Sensory assessment

Three consumer-based sensory panels were conducted to evaluate the appearance, juiciness, saltiness, rancidity, flavor and general acceptability of the examined salted fish samples (fessiekh) using a 5-point Hedonic Scale according to Ikeme (1986).

2.2 chemical analysis

The pH values of samples were determined by using a pH meter (IQ 120) at 25 ± 1°C (Lyhs *et al.,* 1998). The salt content of the samples was determined according to the method described by AOAC (1980).

2.3. Microbiological Analysis

Decimal dilutions (up to 10^6) of fish samples were prepared using sterile 0.1% peptone water solution. The appropriate dilutions were surface plated on appropriate media for enumeration of bacteria according to APHA (1984). The microbiological media and incubation conditions used for enumeration of microorganisms were Plate Count agar (PCA) for mesophilic aerobic microorganisms, (at 35 ± 1°C, 48 hours) (APHA, 1984). The count of halophilic bacteria was determined on plate count agar with added 6% (w/v) NaCl (35 ± 1°C, 48 hours) **(**APHA, 1984). Gifu anaerobic medium (GAM agar, Nissui Co**.,** Tokyo, Japan) was used to determine the anaerobic bacterial count (35 ± 1°C, 72 hours under anaerobic condition). The composition of the GAM medium was as follows (gm/L): peptone, 10; soy bean peptone, 3; proteose peptone, 10; serum powder, 13.5; yeast extract, 5; beef extract powder, 2.2; liver extract powder, 1.2; glucose, 3, KH_2PO_4, 2.5; NaCl, 3; soluble starch, 5; L-cystine hydrochloride, 0.3; sodium thioglycolate, 0.3; agar, 15.

2.4 Purification and characterization of the bacterial isolates

2.4.1. Isolates purification

Random number of colonies was selected from growing plates according to their phenotypic differences (color, size and shape). For purification each colony was subculture onto the surface of Luria-Bertani (LB) agar plates (Sambrook and Russell, 2001) and incubated at appropriate conditions (35 ± 1°C, 24 hours). The media composition is as follow (gm/L): bacteriological tryptone, 10; bacteriological yeast extract, 5; NaCl, 10; agar, 15. A separate and well isolated colony were picked up, inoculated into 5 ml LB broth (bacteriological tryptone, 10; bacteriological yeast extract, 5; NaCl, 10; DW, 1L) and incubated in shaking incubator (100 rpm) at 35 ± 1°C, for 24 hours. Either purified colonies on LB agar or in broth culture can be used to prepare template-DNA. For anaerobes, separate colonies was picked directly from GAM agar and used fro preparation of template-DNA.

2.4.2. Template-DNA preparation

Either colonies or its broth culture were used to prepare the template-DNA using heating in DNA-releasing solution at 100°C for 10 min according to JPF (2003).

2.4.3. PCR running

The used PCR primers were: 10F (sense primer) 5`-GTTTGATCCTGGCTCA-3` and 800R (anti-sense primer) 5`-TACCAGGGTATCTAATCC-3` (JPF, 2003**)**. The PCR mixture per sample was as follow: 10X buffer, 5.0 µl; dNTPs (2.5 mM each), 5.0 µl; 10 F primer (100 pmol/µl), 0.75 µl; 800R primer (100 pmol/µl), 0.75 µl; Taq polymerase (5U/ µl), 0.25 µl; sterile bi-distilled water, 34.5 µl. The running program were: segment 1 (initial denaturation), 94°C, 1 min; segment 2 (amplification cycles), 94°C/30sec, 55°C/1min, 72°C/1min for 30 cycles; segment 3 (final extension) 72°C, 2min followed by 4°C, 99.9 min. The PCR-product size ranged from 700-800 base pair "bp".

2.4.4. PCR product confirmation, purification and quantification.

The PCR product confirmation was done by the gel documentation technique (Sambrook and Russell, 2001). Purification of the product was done using polyethylene glycol (PEG) precipitation technique
(http://www.auburn.edu/~santosr/protocols/PEGTAEProtocol.pdf).

The concentration of the purified DNA was measured using the spectrophotometer (Pharmacia Gene Quant RNA/DNA calculator) at

260 nm wave length, and the concentration was then adjusted to 50 ng/ µl using sterile bi-distilled water.

2.4.5. The DNA sequencing and data analysis

The purified DNA was loaded into the sequencing plates and dispatched to sequencing company (Takara Biotechnology "Dalian" Co., Ltd.). The obtained data were analyzed using the PhredPhrap genetic software (Fukushi H., Laboratory of Veterinary Microbiology, Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan). The analyzed sequences were then compared with data in the BLAST database (http://www.ncbi.nlm.nih.gov/blast/). When sequencing data show ≥ 99% identity with a sequence in the database, higher ranked species was judged as identified species.

RESULTS

Sensory assessment of the examined fessiekh samples revealed that most (88.23%) of them were organoleptically accepted, where 5 (29.41%) and 10 (58.82%) out of the 17 samples were categorized as of good and medium quality, respectively, while 2 (11.77%) were of bad quality. The 2 bad samples were evaluated highly to extremely rancid (results not shown). The mean values of the sensory assessment for appearance, juiciness, rancidity, flavor and general acceptability of the examined salted-fish samples were shown in Table 1.

The chemical analysis (Table 2) revealed that the mean values for pH and NaCl content of the examined samples were 6.63 ± 0.16 and 8.09 ± 0.33, respectively. The minimum and maximum values were 6.1 and 8.1; 6.13 and 10.11 for pH and Nacl, respectively.

As for bacteriological analysis, mean values (log_{10} cfu/g) of 4.66 ± 0.53, 6.05 ±0.25, and 4.63 ± 0.2 were found for total mesophilic aerobes, halophiles, and total anaerobes, respectively. The bacterial counts (log_{10} cfu/g) ranged from 2.64 to 6.91 for total mesophilic aerobes; from 4.46 to 7.93 for halophiles and from 3.3 to 6.23 for total anaerobes (Table 3). Out of the 17 examined sample, only 10 (58.82%) show detectable count of mesophilic aerobes (> 2 log_{10} cfu/g), while the other 7 (41.18%) samples show non detectable count (< 2 log_{10} cfu/g) (results not showen).

As clarified in Table 4 a total of 96 bacterial strains isolated from the examined salted fish samples was characterized using PCR-sequencing technique. Out of the 96 isolated strains, 29 (30.21%) were from mesophilic aerobes, 31 (32.29%)) were from halophiles, and 36 (37.5%) were from anaerobes. The 29 strains of mesophilic aerobes were 18 (62.07%) strains *Staphylococcus equorum* srain JH6, 6 (20.69%) strains *Staph.* sp. L50, 2 (6.9%) strains each of *Bacillus subtilis subspecies subtilis* strain BCRC 10255 and *Lactobacillus* sp. CWBI/B-659/(E912), and 1 (3.45) strain *Bacillus subtilis*.

As for halophiles, the 31 PCR identified strains included 16 (51.61%) strains *Staphylococcus equorum* srain JH6, 10 (32.26%) strains *Staph.* sp. L50, and 5 (16.13%) strains *Teratogenococcus halophilus*. Out of the 36 characterized anaerobic strains, 23 (63.89%) were *Clostridium bifermentans* strain IBUN 188, 7 (19.44%) were *Clostridium bifermentans* strain IBUN 179, 3 (8.33%) were *Clostridium* sp. Zx5, and 1 (2.78%) was each of *Clostridium butyricum* strain W4, *Clostridium cochlearium*, and *Clostridium* sp. DF2C1 (Table 4) .

Table 1: Sensory assessment of the examined salted-fish samples

	Appearance	Juiciness	Saltiness	Rancidity	Flavor	General acceptability
Mean ± SE	3.24 ± 0.14	3.24 ± 0.14	2.41 ± 0.12	3.88 ± 0.27	3.00 ± 0.21	3.18 ± 0.15

Table 2: Chemical analysis values of the examined salted-fish samples

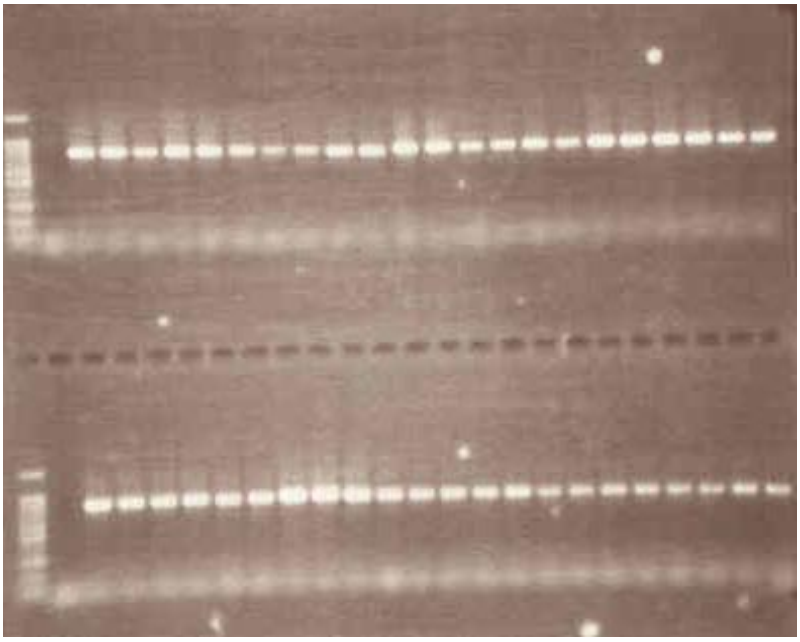
Chemical analysis	Mean ± SE	Minimum	Maximum
pH	6.63 ± 0.16	6.1	8.1
Salt (%)	8.09 ± 0.33	6.13	10.11

Table 3: Bacteriological analysis results (log_{10} cfu/g) of the examined salted-fish samples

Microorganisms	Mean ± SE	Minimum	Maximum
Total Mesophilic Aerobes (n = 10)*	4.66 ± 0.53	2.64	6.91
Halophiles	6.05 ± 0.25	4.46	7.93
Total Anaerobes	4.63 ± 0.2	3.3	6.23

Only 10 samples showed detectable count for mesophilic aerobes, the other 7 samples show count <2 log_{10} cfu/g

M-ve 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 1920 21 22



The PCR product for the amplified partial sequence of the 16S rRNA gene of the unknown bacteria.

M = Marker (100bp) -ve = Negative control
1, 2, 3…= sample numbers

Table 4: Identified strains isolated from the examined salted-fish samples by PCR-sequencing technique

Identified strains	Mesophilic Aerobes		Halophiles		Anaerobes	
	No.	%	No.	%	No.	%
Bacillus subtilis	1	3.45	—	—	—	—
Bacillus subtilis subsp. subtilis strain BCRC10255	2	6.9	—	—	—	—
Clostridium bifermentans strain IBUN 179	—	—	—	—	7	19.44
Clostridium bifermentans strain IBUN 188	—	—	—	—	23	63.89
Clostridium butyricum strain W4	—	—	—	—	1	2.78
Clostridium cochlearium	—	—	—	—	1	2.78
Clostridium sp.DF2C1	—	—	—	—	1	2.78
Clostridium sp. Zx5	—	—	—	—	3	8.33
Lactobacillus sp. CWBI/B-659/(E912)	2	6.9	—	—	—	—
Staphylococcus equorum strain JH6	18	62.07	16	51.61	—	—
Staphylococcus sp. L50	6	20.69	10	32.26	—	—
Teratogenococcus halophilus	—	—	5	16.13	—	—
Total	29	100	31	100	36	100

DISCUSSION

In this study sensory evaluation results of the examined fessiekh samples seemed to be nearly similar to that obtained by El-Morshdy *et al.* (1981) who found that 10% of the examined fessiekh samples were unfit for human consumption. On the other hand, it was lower than that obtained by Nayel (2007), who found that 20% of the examined fessiekh samples showed putrefied odor and rancid taste.

Concerning the Egyptian standards (EOSQS, 1996) for pH value of salted fish (pH 6.0 – 6.5), 29.41% of the examined samples had pH values higher than the standards (results not shown). This was lower than that previously reported by El-Sheshnagui (2006), who found that 45% of the examined fessiekh samples had a pH exceeded the Egyptian standards. The pH range and mean values of the examined samples were slightly differed from those previously obtained by El-Sheshnagui (2006). However it was generally higher than those reported by Silla-Santos (1996); Hernandez-Herrero (1999b); Majumdar *et al.* (2006); Patir *et al.* (2006); Yung-Hsiang *et al.* (2006); Koffi-Nevry *et al.* (2011) for a variety of salted fish products. The obtained values were generally lower than those recorded by Steinkraus (1983) and Anihouvi *et al.* (2006).

Salt is introduced into fish in processing at two levels either for flavoring at about 2 percent as in kippers etc.; or for long term preservation at about 18 – 20 percent, as in salt cod (Ranken, 1986). As for the examined fessiekh all the samples had NaCl content more than 6% which comply with the established Egyptian standards (EOSQC, 1996) for salted Fish (NaCl content not less than 6%). This was differ from that reported by El-Sheshnagui (2006), who recorded that the salt content of 60% of the examined fessiekh samples were below the Egyptian standards limit. The obtained results of Nacl mean and range values were higher than those recorded by Sakai *et al.* (1983); Anihouvi *et al.* (2006); El-Sheshnagui (2006); Koffi-Nevry *et al.* (2011). However, they were lower than those recorded by El-Morshdy *et al.* (1981); Abd El-Rahman (1988); Sanni *et al.* (2002); Patir *et al.* (2006); Yung-Hsiang *et al.* (2006) for a variety of salted fish products.

It is generally accepted that in order to get a picture of the bacterial activity on the fish the easiest and most practical method is to determine the total bacterial count (FAO, 1981). As there is no Egyptian standards for aerobic plate count in salted fish and in comparing the obtained results with Spanish specifications (MSC, 1991) which allow counts up to 5 $\log_{10}$ cfu/g for mesophilic microorganisms in salted fish products, it is noticed that 23.53% of the examined fessiekh samples not comply with this specification (results not shown). Nayel (2007) found that 44% of the examined fessiekh samples had aerobic bacterial count of more than 5 $\log_{10}$ cfu/g, which seemed higher than the current obtained result.

As for recorded mesophilic aerobes count, the mean and range values were nearly similar to that recorded by Vilhemsson *et al.* (1996)

for bachalao (salted cod). However, higher values were reported by El-Morshdy *et al.* (1981); Paludan-Muller *et al.* (2002); Sanni *et al.* (2002); Anihouvi *et al.* (2006) and lower ones were reported by Achinewhu and Oboh (2002); Patir *et al.* (2006); Yung-Hsiang *et al.* (2006) for a variety of salted fish products.

Although salt prevents growth of spoilage bacteria, other microorganisms may not be affected by its presence. Microorganisms have been conveniently divided into four groups based on their sensitivity to salt: halotolerant, slight halophiles, moderate halophiles and extreme halophiles. Most halotolerant microorganisms are isolated when foods are tested for slight or moderate halophiles (Baross and Lenovich, 1992). Some halotolerant microorganisms are involved in spoilage of salted foods, whereas others, such as *Staph. aureus*, are human pathogens (APHA, 1984).

The obtained results of the halophilic bacterial count of the examined fessiekh samples revealed that 88.24% of them showed count more than 5 $\log_{10}$ cfu/g. Meanwhile, 41.18% of the samples showed halophiles count more than 6 $\log_{10}$ cfu/g (results not shown).

The recorded mean and range values for halophiles were higher than those registered by Knockel and Huss (1984); Surono and Hosono (1994); Hernandez-Herrero *et al.* (1999a); Anihouvi *et al.* (2006); Patir *et al.* (2006) for a various types of salted fish products. However the mean value was lower than that reported by Ahmed and El-Kazzaz (2005) for the examined fesseikh samples.

The obtained counts of anaerobic bacteria from the examined fessiekh samples declared that 44.18% of the samples had anaerobic count exceeded 5 $\log_{10}$ cfu/g, while only 5.88% of them had count exceeded 6 $\log_{10}$ cfu/g. The result of range and mean values for anaerobes was nearly similar to those recorded by Ahmed (1976) for a variety of salted fish products. However, Anihouvi *et al.* (2006) recorded a lower range value in lanhouin "a traditionally processed fermented fish in Benin"

In recent years many studies have used culture independent 16S rRNA-based PCR techniques to determine the microflora of various traditional salt-fermented fishery products (Ha *et al.,* 2002; Paludan-Muller *et al.,* 2002; Abd-Allah, 2008; Tanasupawat *et al.,* 2010; An *et al.,* 2011)

Using PCR-sequencing technique different species of *Bacillus, Clostridium, and Staphylococcus* could be characterized from the examined fessiekh samples.

The isolation of diverse microorganisms from fessiekh agreed with the finding of Morshdy *et al.* (1982); Abd-Allah (2008); who reported the isolation of *Bacillus, Citrobacter, Lactobacillus, Micrococcus, Proteus,* and *Staphylococcus* amongst others from fessiekh samples. *Bacillus, Clostridium, Enterobacter, Lactobacillus, Micrococcus, Pediococcus, Pseudomonas, Staphylococcus,* and *Streptococcus* had been reported as a microflora amongst others in various salt-fermented fishery products (Ahmed, 1976; Nassar and Ahmed, 1997; Surono and Hosono, 1994; Paludan-Muller *et al.,* 2002; Sanni *et al.,* 2002; Rodrigues *et al.,* 2003; Tanasupawat *et al.,* 2010; An *et al.,* 2011; Koffi-Nevry *et al.,* 2011)

In the current study three strains of *Bacillus* had been isolated from the examined samples, *Bacillus* species are spore-forming bacteria known to thrive high concentrations of NaCl. It was suggested that these spore-forming bacilli may play an active role early in the fermentation process (Crisan and Sands, 1975; Sanni *et al.,* 2002). Some strains of Bacillus spp. isolated from Spanish semi-preserved anchovies had been proved to have histidine decarboxylase activity (Rodriguez-Jerez *et al.,* 1994a).

A total of 36 strains of *Clostridium* had been isolated from the examined samples. *Clostridium* is strict anaerobic spore-forming bacteria that may be pathogenic or spoilage microorganism. None of the known pathogenic strains of *Clostridium* (*C. botulinum* and *C. perferingens*) in salted fish products had been isolated from the current samples. Ahmed (1976); Surono and Hosono (1994); Rodriguez-Jerez *et al.* (1994b); El-Sebaey *et al.* (1999) isolated different species of *Clostridium* from a variety of salted fish products.

Lactic acid bacteria are found as the dominant microorganisms in many fermented fish products (Saisithi *et al.,* 1986; Olympia *et al.,* 1992; Ostergaard *et al.,* 1998; Paludan-Muller *et al.,* 2002; Kopermsub and Yunchalard, 2010). The primary role of *Lactobacillus* is to ferment the available carbohydrates and thereby cause a decrease in pH. Paludan-Muller (2002) reported that the growth of lactic acid bacteria was inhibited in fermented fish with salt concentration more than 10% NaCl. In the current study, three strains of Lactobacillus (2 strains *Lactobacillus* sp. CWBI/B-659/(E912) and 1 strain *Teratogenococcus halophilus*) were isolated from the examined fessiekh samples. *Teratogenococcus halophilus,* is a halophilic lactic acid bacterium active in the fermentation process of salted fish products (Villar *et al.,* 1985; Nishimura *et al.,* 2009). It was previously isolated from fermented fish

products with salt concentration higher than 10 – 15% such as plaa-ra, nam-budu (fish sauce) and nam-plaa (fish paste) (Tanasupawat and Daengsubha, 1983; Ito *et al.,* 1985).

The isolation of lactic acid bacteria agreed with the findings of Bashir and Agab (1987); Abd El-Rahman *et al.* (1988); Surono and Hosono (1994); Sanni *et al.* (2002); Kopermsub and Yunchalard (2010) who reported the isolation of *Lactobacillus* among others from various salted fish products. Karnop (1988) reported that some *Lactobacillus* strains isolated from semi-preserved anchovies had histidine decarboxylase activity, as well Bover-Cid and Holzapfel (1999) declared that some *Lactobacillus* strains had importance as tryramine producers.

Staphylococcal contamination is very common for a wide variety of foods. The sources of contamination may be from skin, mouth or nose of workers handling the food (Tatcher and Clark, 1978). *Staphylococci* grow well in salted food and in low water activity. They isolated from Thai-fermented fish with salt concentrations above 5% NaCl (Tanasupawat *et al.,* 1991, 1992) and from Korean fermented (hydrolysed) fish with salt concentrations ranging from 8% to 26% NaCl (Um and Lee, 1996). Some strains of *staphylococci* had been previously identified as histamine producers in salted anchovies, beside many other bacterial species (Karnop, 1988; Hernandez-Herrero *et al.,* 1999a). *Staphylococci* were reported as the dominant isolate from various salted fish products including fessiekh (Sanderson *et al.,* 1988; Abd-Allah, 2008). In the current study, out of the 96 identified bacterial strains, 50 (52.08%) were *Staphylococci* (34 strains were *Staphylococcus equorum* strain JH6 and 16 strains were *Staohylococcus* sp. L50).

Steinkraus (1983); Bashir and Agab (1987); Nassar and Ahmed (1997); Hernandez-Herrero *et al.* (1999a); Paludan-Muller *et al.* (2002); Rodrigues *et al.* (2003); Ahmed and El-Kazzaz (2005); An *et al.* (2011) recorded different species of *Staphylococci* (e.g. *S. carmosus, S. chonii, S. epidermidis, S. equorum, S. saprophyticus, S. simulans*) amongst others as a microflora from various salt-fermented fish products. *Staph. equorum* is coagulase-negative cocci currently isolated from fermented sausage (Blaiotta *et al.,* 2004; Rantsiou *et al.,* 2005), curing brines and raw ham. *Staphylococcus equorum* subsp. *equorum* was originally isolated from healthy horses (Schleifer *et al.,* 1984), and later isolates were obtained from the milk of a cow with mastitis and from healthy goats (Meugnier *et al.,* 1996). Another subspecies, *Staph. equorum* subsp. *linens*, was isolated from the surface of ripening cheese (Place *et al.,* 2003). Few *Staph equorum* subsp. *equorum* strains were found in

relevant human clinical materials (Marsou *et al.*, 2001; Alcaraz *et al.*, 2003; Novakova *et al.*, 2006). It is belived that *Staph equorum* may contribute to the development of the meat and cheese products flavor, and it may inhibit Listeri's growth (Place *et al.*, 2003). So, it may be beneficial or might be potential pathogen. To the best of my knowledge this is the first record of *Staph. equorum* in the Egyptian salted *Mugil cephalus* fish (fessiekh).

In summary, this study showed that most of the examined fessiekh samples were of medium quality, as well considerable percentage of them had high pH values (> 6.5), which may reflect some forms of spoilage. Salt content of all samples was more than 6%. However many of the samples showed high aerobic, halophilic and anaerobic bacterial count, which indicates unhygienic conditions during processing and marketing. Halophiles was the dominant microflora recorded the highest count and the highest number of isolates. Most of the identified isolates are spoilage microorganisms (*Bacillus, Clostridium, and Staphylococcus*) that might have impact on product shelf-life. Some of them are proved as histidine decarboxylase producers (*Bacillus* and *Staphlococcus*), which might affect product safety. Others are considered beneficial (*Lactobacillus* and some species of *Bacillus* and *Staphylococcus*) which can be used to improve product quality and sensory characteristics. It is expected that controlled material handling and fermentation will lead to better microbial status in the product. However, new regulations are required for salted fish products to establish microbiological performance standards for aerobes, halophiles and anaerobes contaminations. Characterization of bacteria presented in fessiesk using molecular methods identified new species and opened several possibilities for further research in the field of spoilage, safety and also the influence of the microorganisms onto the sensory characteristics of this product.

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