## SURVEY AND ISOLASTION OF HISTAMINE PRODUCING BACTERIA FROM FAYOUM CITY, EGYPT Gehad H.S. Hassan ; Kh. M. Atalla ; O. A. Seoudi and Y.F. Abdelaliem

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#### ABSTRACT

Histamine food poisoning is found to be associated with consumption of foods containing unusually high levels of histamine. Fish belonging to scombroid and non-scombroid fish groups may also cause histamine poisoning. In this study, histamine forming bacteria in the commercial food samples of local markets of Fayoum region were investigated. A total of 185 unique isolates were selected on the basis of colony morphology, 28 of which were found to be prominent histamine producers on Niven's medium. The percentage of histamine producing bacteria was 15.13% of the total bacterial load. The dominant microflora was found to be Gram positive bacilli, Gram negative bacilli and cocci. Out of the 185 isolates, 28 were from salted fish, 17 from sardine, 13 from mullet, 12 from herring, 24 from mish, 16 from raw milk, 15 from fermented milk, 21 from rumi cheese, 14 from white cheese, 11 from palm paste and 14 isolates were from pickle. Results indicated that, regarding to fish samples, the highest content of histamine was obtained from mullet followed by salted fish and sardine products by 0.359, 0.316 and 0.259 mg/100g, respectively. Meanwhile, herring fish sample recoded the minimum histamine levels. For dairy samples, the highest content of histamine was obtained from rumi cheese and the lowest histamine concentration recorded in raw milk being 0.379 and 0.133 mg/100g, respectively. Moreover, histamine concentration was 0.119 and 0.110 mg/100g, recorded from pickles and date palm paste, respectively.

## INTRODUCTION

The bacterial spoilage of scombroid and nonscombroid fishes and certain other foods have high levels of free histidine in their tissues is sometimes accompanied by the formation of high levels of histamine in the edible tissues of their types of food (**Frank et al., 1981**). The formation of histamine is particularly noteworthy because the presence of high levels of histamine in spoiled fish and certain other foods have been associated with outbreaks of food poisoning also known as scombroid fish poisoning (**Arnold and Brown 1978**). Histamine is generated from histidine during spoilage by bacteria that possess the requisite enzyme histidine decarboxylase (**Omura et al., 1978 and Yoshinaga and Frank, 1982**).

Histamine, 4-(2-aminoethyl) imidazole, is a primary amine arising from the decarboxylation of the amino acid, L-histidine.

The biogenic amines are well known for their implication in serious human intoxications, associated with the consumption of spoiled food. Even though the isolation and characterization of bacterial strains producing biogenic amines in general and particularly histamine knew a great evolution, the methods used for their detection in foods depend on many parameters, such as the nature of food and the bacterial flora. The early methods used were based on the measure of the carbon dioxide produced with the decarboxylation of amino acids. This process is inaccurate and is now abandoned. Thereafter, several authors proposed the use of selective culture media, in order to obtain a rapid selection of the biogenic amines producing bacteria, such as histamine Niven's agar medium supplemented with L-histidine (**Niven et al., 1981**).

Many different bacterial species are known to possess histidine decarboxylase (Arnold and Brown, 1978 and Yoshinaga and Frank, 1982). However, only *Proteus morganii* (Sakabe, 1973), *Klebsiella pneumoniae* (Taylor *et al.*, 1979), and *Hafnia alvei* (Havelka, 1967) have been isolated from fish incriminated in scombroid fish poisoning incidents. The purpose of this study was to survey, isolate and identify histamine producing bacteria, as well as quantification of histamine content in certain food sources sold from the local markets of Fayoum region, Egypt.

## MATERIALS AND METHODS

#### Samples and raw materials

Samples used included fishes such as Salted Fish, Mullet, Sardine and Herring fish, as well as dairy products like Mesh, Rumi cheese, and Raw Milk, Fermented Milk and White cheese. Otherwise, Pickles and Date Palm Paste were purchased from different retail and wholesale markets in Fayoum Governorate. Ten gram of each sample was mixed with 90 ml of sterile physiological saline (0.85% (w/v) NaCl), homogenized in a stomacher for 2 min and then further diluted in physiological saline at 1:10 dilutions then serial dilutions were made from 10-1 to 10-5. The diluted sample solutions were spread on Niven's medium agar (**Niven** *et al.*, **1981 and Mah** *et al.*, **2001**) to qualitatively detect histamine produce ability of the bacterial isolates.

#### Enumeration of histamine producing bacteria

For total aerobic histamine producing bacterial count, 1.0 ml portion of each dilution was poured onto petri dishes and then 15 ml of Niven's agar medium containing 0.5 % NaCl at 45 °C was added and gently mixed. The poured plates were allowed to solidify and incubated at 37 °C for 48 hours. Bacterial counts from the different samples were expressed as colony forming units (CFU/g). To confirm histamine production, 1.0 ml of each dilution was spread on histamine Niven's agar medium supplemented with

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L-histidine. After incubation of plates for 48 hours at 37°C, colonies with blue or purple color were picked and further streaked on Niven's agar medium to obtain pure isolates.

#### **Detection of histamine producing bacteria (HPB)**

Histamine producing bacteria (HPB) detection was done by Niven's methods (Niven *et al.*, 1981). Histamine-producing bacteria were inoculated on agar plates containing Niven's medium (0.5% tryptone, 0.5% yeast extract, 2.0% L-histidine-monohydrochloride was purchased from Sigma (Sigma Aldrich), 0.5% NaCl, 0.1% CaCO3, 2.0% agar and 0.006% bromo cresol purple, at pH 5.3) which was sterilized at 121°C for 10 min. The plates were incubated at 37°C for 48 hrs. aerobically (Lopes –Sabater *et al.*, 1996). Purple zone appeared around colony is an indicator of histamine producing bacteria. When the indicator showed an increase of pH on agar plates containing Niven's medium. To determine histamine qualitative assay of isolates, the purple zone diameter (mm) was measured. The colonies of these zones were transferred to new plates, and the morphology of the colonies was observed under a microscope following Gram staining.

#### Histamine food quantification

Quantification of histamine was carried out using colorimetric method reported by Patange et al., (2005). In this method, 1 ml of the muscle extract was taken into a glass-stoppered test tube and diluted to 2 ml with saline and 0.5 g of salt mixture containing 6.25 g of anhydrous sodium sulfate to 1 g trisodium phosphate monohydrate was added. The tubes were stoppered and thoroughly shaken. 2 ml of n-butanol was then added and the tubes shaken vigorously for 1 min and allowed to stand for 2 min and then shaken briefly to break the protein gel. The tubes were further shaken vigorously for few seconds and then centrifuged at 3100 rpm for 10 min. The upper butanol layer (only 1 ml) was transferred into a clean and dry test tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of distilled water. In a clean tube 5 ml of 1.1% sodium carbonate solution was taken and 2 ml of the chilled reagent, pphenyldiazonium sulfonate was added slowly and mixed. It was then added to the tube containing 1 ml solution of the residue collected in the extraction process. The absorbance of the color produced was measured immediately after 5 min at 496 nm. The concentration of histamine in sample was obtained from the standard curve for the corresponding absorbance measured at 496 nm. The histamine concentration in sample was estimated using the following formula.

# Histamine $(mg/100g) = \frac{A \times 2 \times 25 \times 100}{5 \times 1000}$

Where: A is the value of histamine obtained in  $(\mu g/ml)$  from the standard curve.

#### **Determination of histamine production**

For histamine determination, Gram positive and Gram negative bacterial isolates were streaked on triplicates on tryptic soy agar (TSA) plates containing 2% NaCl supplemented with 2% L-histidine and incubated at 37°C for 24 h. Representative colony from each of the plate was inoculated into 9 ml of tryptic soy broth (TSB) with 2% NaCl concentration, 2% histidine and 0.0005% pyridoxal-HCl (pH 5.8) and incubated at 37°C for 24h. 1 ml sample of each TSB+ suspension was transferred into a tube of fresh TSB + media and incubated at 37°C for 48 h. 3 ml subsample of this final culture was transferred into polypropylene centrifuge tubes and centrifuged at 1100 rpm for 20 min (**Joshi and Bhoir , 2011**). Supernatants were diluted 1 to 10 in saline solution and quantification of histamine was carried out using colorimetric method reported by **Patange** *et al.*, (2005).

## **RESULTS AND DISCUSSION**

#### Histamine contents of tested food samples

Biogenic amines content expressed as histamine (mg/100g) in different Egyptian food samples, which were obtained from retail markets of Fayoum city shown in **Table (1)**. Obtained results indicated that, there were differences in the contents of biogenic amines in different food samples. Histamine concentration of tested food types was among 0.110 to 0.379 mg/100 g. In related to fish samples, the highest content of histamine was obtained from mullet followed by salted fish and sardine products being 0.359, 0.316 and 0.259 mg/100g, respectively. Meanwhile, herring fish sample recoded the minimum histamine levels. For dairy samples, the highest content of histamine was obtained from rumi cheese and the lowest histamine concentration recorded in raw milk was 0.379 and 0.133 mg/100g, respectively. As shown in **Table (1)** the pickle histamine concentration was 0.119 mg/100g, while date palm paste histamine concentration recorded 0.11 mg/100g.

The differences in the biogenic amines concentrations of food samples could be due to the hygienic quality of raw material, manufacturing practices, the specific bacteria, ripening period and the type of culture. The biogenic amines concentration may be used as a quality index for these kinds of products. Handling of raw materials and production technology for fermented foods and fish products are relatively primitive in Egypt. These results indicate that the natural fermentation process used for dairy products and other food samples may result in accumulation of high levels of biogenic amines. The brand specific variation suggests that different environmental conditions have some effect on histamine content. Although the natural fermentation process and storing conditions used in the preparation of these products (*i.e.* fish and dairy products) probably did not involve in growth of any major biogenic amines-producing bacteria. The

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lack of quality control in their production and the use of natural fermentation make selection of desirable organism's difficult (**Bodmer** *et al.*, 1999).

Determination of the exact toxicity threshold of biogenic amines in individuals is extremely difficult, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual. Although the toxicity of biogenic amines to man is a controversial subject, ingestion of from 70 to 1000 mg histamine will usually cause clinical symptoms intoxication (**Henry**, **1960**).

Food Drug Administration (FDA) has established a hazard action concentration for histamine in tuna fish of 50 mg of histamine/100 g (**Stratton** *et al.*, **1991**). Moreover, it must be noted that smaller amounts of biogenic amines may cause poisoning particularly if the person is vulnerable, because of the inhibition of the biogenic amines detoxification mechanism in the body due to reasons such as personal predisposition, gastrointestinal diseases, the use of certain medicines and alcohol intake and the existence of other amines (Bodmer *et al.*, **1999 and Joosten and Van-Boekel 1988**).

Fermentation of milk, a considerable increase of histamine content often occurs, leading to contents of up to 7  $\mu$ g/ml histamine in sour cream and even slightly higher levels in yoghurt. Finally, in cheese production a rather drastic increase of histamine content often occurs, leading to maximum levels of histamine of up to 2500 ppm in aged cheese (**Bodmer** *et al.*, **1999**).

	Sources	Histamine concentrations (mg/100g)
	Salted fish	0.316
Fish	Sardine	0.259
Products	Mullet	0.359
	Herring fish	0.128
Dairy Products	Mish	0.193
	Raw milk	0.133
	Fermented milk	0.207
Products	Rumi cheese	0.379
	White cheese	0.191
Pickles	Pickle	0.119
Fruits	Date palm paste	0.110

Table (1): Histamine concentration in different types of food samples (mg/100 g wet weight).

#### Microbiological profile Total counts of bacteria

Histamine is a natural constituent of fermented foods generated by microbial activity. Microbiological profile of different food sources *i.e.* fish, dairy, pickles and fruits were studied. The average number of aerobic plate count (APC) of histamine forming bacteria CFU/g in salted fish sample was

 $18.0 \times 10^5$ , salted sardine sample was  $10.7 \times 10^5$ , mullet sample was  $3.3 \times 10^3$ , herring fish sample was  $10.2 \times 10^5$ , mish sample was  $14.0 \times 10^3$ , raw milk sample was  $10.6 \times 10^4$ , fermented milk sample was  $15.8 \times 10^3$ , rumi cheese sample was  $11.4 \times 10^4$ , white cheese sample was  $14.3 \times 10^3$ , date palm paste sample was  $10.1 \times 10^3$  and pickle sample was  $14.0 \times 10^4$ . In the present study, a total of 185 isolates were obtained from 11 types of fish and dairy products.

#### Isolation of histamine producing isolates

Different types of food sources were screened for the detection of histamine-producing bacteria (HPB) as shown in **Table (2)**. A total of 185 unique isolates were selected on the basis of colony morphology, 28 of which were positive producers on Niven's media. The dominant microflora was found to be gram positive bacilli, gram negative bacilli and cocci. Out of the 185 isolates, 28 were from salted fish, 17 from sardine, 13 from mullet, 12 from herring fish, 24 from mish, 16 from raw milk, 15 from fermented milk, 21 from rumi cheese, 14 from white cheese, 11 from date palm paste and 14 isolates were from pickle. Presumptive colonies were isolated from the Niven's medium plates and screened for histamine production.

	Sources	Number of isolates	Number of histamine producing isolates	Percentage of histamine producing isolates
	Salted fish	28	12	42.85
Fish	Sardine	17	5	29.41
Products	Mullet	13	0	0.00
	Herring fish	12	0	0.00
	Mish	24	4	16.66
Datar	Raw milk	16	3	18.75
Dairy Products	Fermented milk	15	2	13.33
Froducts	Rumi cheese	21	1	4.76
	White cheese	14	0	0.00
Pickles	Pickle	14	0	0.00
Fruits	Date palm paste	11	1	9.09
	Total	185	28	15.13%

 Table (2): Microbiological profile of food sources *i.e.* fish, dairy, pickles and fruits products.

The percentage of histamine producing bacteria was 15.13% of the total bacterial load. Similar results obtained by **Koohdar** *et al.*, (2011) who found that counts of mesophilic and psychrophilic producers of 45 samples of skipjack were  $7.2 \times 10^6$  and  $2.9 \times 10^6$  CFU/g, respectively. The mean count

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of histamine forming bacteria was about  $2.8 \times 10^2$  CFU/g and it was 0.004% and 0.009% of the total and psychrophilic bacterial loads, respectively. Purple halo around Niven's medium is an indicator of positive histamine producing bacteria HPB (Chong *et al.*, 2011).

Based on positive/negative Niven's media test, among the isolates obtained previously, 12 isolates were associated with salted fish, 5 were associated with sardine, 4 were associated with mish, 3 were associated with raw milk, 2 were associated with fermented milk, and only one was associated with rumi cheese or date palm paste were positive for histamine formation. On the other hand, the isolates obtained from mullet, herring, white cheese and pickles were negative for histamine production.

#### Histamine qualitative assay

One of the aims of this study was to isolate and characterize the histamine forming bacteria in traditional Egyptian foods included fish, dairy and date palm past. In all cases of fish products, the histamine-producing isolates were obtained from muscles. As mentioned previously in **Table (1)** a total of 185 isolates were tested to verify their ability to produce histamine based on Niven's positive test. Only 28 tentative histamine-producing isolates were initially obtained from the Niven agar (NA) plates and confirmed as histamine producer as indicated by halo-purple zone assay, as shown in **Table (3)**. The decarboxylating Niven agar medium is due to the corresponding precursor amino acids (histidine monohydrochloride). The pH of the medium was adjusted to 5.3, and it was sterilized at 121°C for 10 min. The plates were incubated at 37°C for 7 days under anaerobic or aerobic conditions. During this time, halos formed around colonies on histamine medium on a yellowish background were measured.

#### Quantitative determination of histamine

Out of 28 Niven's positive isolates were tested to verify their ability to produce histamine. Only 11 isolates were chosen as the strongest histamine producers in addition to reference strain *Enterobacter aerogenes* when determined by colorimetric assay of histamine. As presented in **Table (4)** among salted fish isolates His 18 was the most efficient histamine producer by (97.00  $\mu$ g/ml) and equal to *Enterobacter aerogenes*. As well, histamine isolate obtained from fermented milk with code His 8 showed the highest histamine producer by (94.00  $\mu$ g/ml) in comparison with the other histamine isolates of dairy products. Meanwhile, the lowest histamine producing isolates by (90.00  $\mu$ g/ml) were obtained from raw milk and date palm past.

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Code of isolates	Sources of isolates	Histamine producer (zone diameter mm)
1	Date palm Paste	30
2	Rumi Cheese	25
3	Salted Fish	25
4	Raw Milk	20
5	Fermented Milk	15
6	Salted Fish	20
7	Salted Fish	25
8	Mish	30
9	Raw Milk	15
10	Fermented Milk	25
11	Salted Fish	20
12	Raw Milk	15
13	Sardine	20
14	Salted Fish	20
15	Sardine	20
16	Sardine	15
17	Sardine	15
18	Salted Fish	15
19	Salted Fish	10
20	Sardine	10
21	Mish	15
22	Mish	15
23	Mish	10
24	Salted Fish	15
25	Sardine	10
26	Salted Fish	15
27	Salted Fish	10
28	Salted Fish	10

Table (3): Histamine qualitative assay by histamine producing<br/>isolates after 48 hours.

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Isolates	Source	Histamine Concentration (µg/ml)
Enterobacter aerogenes*	Reference strain	97.00
His 1	Date palm Paste	90.00
His 8	Mish	93.00
His 9	Raw Milk	91.00
His 10	Fermented Milk	94.00
His 11	Salted Fish	93.00
His 12	Raw Milk	90.00
His 13	Sardine	91.00
His 15	Sardine	92.50
His 16	Sardine	91.00
His 18	Salted Fish	97.00
His 19	Salted Fish	93.00

\* reference strain

**Koohdar** *et al.*, (2011) found that fourteen bacterial strains with histidine decarboxylase activity were isolated and then tested for their ability to produce histamine, of which 8 strains (57.14%) of these tentative isolates showed positive results. Sixteen of the total 45 samples of frozen skipjacks contained less than 20  $\mu$ g/ml histamine, but these amounts were 20-50  $\mu$ g/ml and more than 50  $\mu$ g/ml in 10 and 19 samples, respectively.

There were clear differences in histamine contents in samples with different counts of histamine forming bacteria, so that the samples with high counts of histamine forming bacteria had relatively higher levels of histamine than other samples.

## Identification of the most two histamine bacterial isolates using 16SrDNA gene

The highly talented of the two histamine bacterial isolates with code of His 10 and His 18 were selected to identification based on their pronounced results. The 16SrDNA gene sequence of isolates were determined and compared with sequences in Gene-Bank database. A phylogenetic tree based on 16SrDNA sequences (Fig 1 and 2) revealed affiliation of His 10 to *Escherichia coli* and His 18 to *Pseudomonas otitidis*. **Nucleotide of 16SrDNA of His 10** 

GCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTC TGGGAAACTGCCTGATGGAGGGGGGATAACTACTGGAAACGGT AGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGGACCT TCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAG TAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGT CTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCA GACTCCTACGGGAGGCAGCAGTGGGGGAATATTGCACAATGGG CGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTT CGGGTTGTAAAGTACTTTCAGCGGGGGGGGAGGAAGGGAGTAAAGT TAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGG CTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTT GTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGC ATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGGGTAGAAT TCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATAC CGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGG TGCGAAAGCGTGGGGGGGCAAACAGGATTAGATACCCTGGTAG TCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAG GCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGA GTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGGCC CGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCG AAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGA TGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGG CTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCA AACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGG GATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACA 

#### Nucleotide of 16SrDNA of His 18

GCAGTCGAGCGCAGGAATCGACGGAACCCTTCGGGGGGGAAGT CGACGGAATGAGCGGCGGACGGGTGAGTAACACGTAAAGAAC CTGCCCTCAGGTCTGGGATAACCACGAGAAATCGGGGGCTAAT ACCGGATGGGTCATCGGACCGCATGGTCCGAGGATGAAAGGC GCTTCGGCGTCGCCTGGGGGATGGCTTTGCGGTGCATTAGCTAG TTGGTGGGGTAATGGCCCACCAAGGCGACGATGCATAGCCGA CCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGG ACGAAAGTCTGATGGAGCAACGCCGCGTGAACGATGAAGGCC TTCGGGTCGTAAAGTTCTGTTGTAAGGGAAGAACAAGTGCCGC AGGCAATGGCGGCACCTTGACGGTACCTTGCGAGAAAGCCAC GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA AGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGG CCTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGG GCCATTGGAAACTGGGAGGCTTGAGTATAGGAGAGAAGAGTG GAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA ACACCAGTGGCGAAGGCGACTCTTTGGCCTATAACTGACGCTG AGGCGCGAAAGCGTGGGGGGGCAAACAGGATTAGATACCCTGG TAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTT CCGCCCTTCAGTGCTGAAGCTAACGCATTAAGCACTCCGCCTG GGGAGTACGGTCGCAAGGCTGAAACTCAAAGGAATTGACGGG GACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAA CGCGAAGAACCTTACCAACTCTTGACATCCCCCTGACCGGTAC AGAGATGTACCTTCCCCTTCGGGGGGCAGGGGGGGACAGGTGGT GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT CCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCACCATTCAG TTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAG GTGGGGATGACGTCAAATCATCATGCCCCTTATGAGTTGGGCT ACACACGTGCTACAATGGACGGTACAAAGGGCAGCGAAGCCG CGAGGTGGAGCCAATCCCAGAAAGCCGTTCTCAGTTCGGATTG CAGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAATC GCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGTCTTGTAC ACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCG GTGAGGTAACCTTA

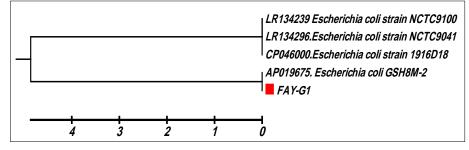


Figure (1): Tree showing the estimated phylogenetic relationships of His 10:

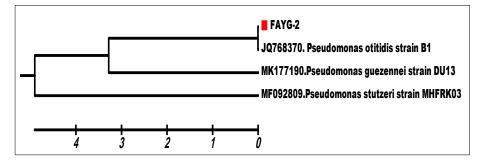


Figure (2): Tree showing the estimated phylogenetic relationships of His 18:

## **CONCLUSION:**

It could be concluded that, histamine concentration of tested food samples was among 0.110 to 0.379 mg/100 g. The highest histamine content 0.379, 0.359 and 0.316 mg/100g were obtained from rumi cheese, mullet and salted fish, respectively, whereas pickles and date palm paste associated with the lowest histamine food content, as compared to the other tested food samples. The use of Niven agar aided considerably in the isolation and identification of the histamine-producing bacteria, the histamine-producing bacteria isolated from fish products was 12 out of 28 from salted fish followed by 5 out of 17 form sardine and 4 out of 24 from mish followed by 3 out of 16 from raw milk as dairy products were tentative histamine producers. It is worth to note that, salted fish as a popular food in Fayoum city pronounced to be the higher food source containing histamine producing isolates with 12 isolates.

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حصر وعزل البكتيريا المنتجة للهستامين من مدينة الفيوم ، مصر جهاد حمدي سيد حسن ، خالد محمد عطالله ، اسامة عبدالتواب سعودي ، ياسر فتحى عبدالعليم

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أجريت هذه الدراسة بهدف حصر مصادر الأغذية ذات المحتوى العالي من الأمينات الحيوية (الهستامين) والشائعة بمحافظة الفيوم ، وكذلك تم عزل البكتيريا التى لها المقدرة على انتاج الهستامين من تلك المصادر ، مثل منتجات الأسماك (السمك المملح ، السردين ، سمك الفسيخ ، السمك المدخن) ومنتجات الألبان (المش ، اللبن الخام ، اللبن المتخمر ، الجبن الرومي ، الجبن الأبيض) ومنتج من المخللات وآخر من عجوه نخيل البلح.

## وكانت النتائج المتحصل عليها كالأتي:

الجبن الرومي وسمك الفسيخ والسمك المملح سجلت 0.379 ، 0.359 ، 0.310 مليجرام هستامين لكل 100 جرام على التوالي. وقد تم عزل ٢٨ سلالة البكتيرية منتجة للهستامين من هذه المصادرالمختبرة من أصل ١٨٥ عزلة بكتيرية. وكانت اكثر العزلات البكتيرية المنتجة للهستامين التي تم الحصول عليها من السمك المملح والسردين بواقع ١٢ و ٥ عزلات البكتيرية على الترتيب. وقد تم اختيار وتعريف أقوى عزلتين منتجة للهستامين تحت كود His I ، 10 ، 18 بالمقارنة بالسلالة البكتيرية المرجعية Enterobacter aerogens. وكانت تعريف العزلة البكتيرية ذات الكود His 10 هي Escherichia coli ، والعزلة البكتيرية ذات الكود His 18 هي His 18 مى His 10 .