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Isolation of the *Pseudomonas* spp. to Cause of Microbial Spoilage of Fishes

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Abstract

In this study, fish samples such as trout (Oncorhynchus mykiss), gilt head bream (Sparus auratus), sea bass (Dicentrarchus labrax), mullet (Mugil cephalus) and whiting (Merlangus euxinus) supplied from fish sales centers arround the country of Izmir and Manisa, Turkey were such as bacterial flora of the investigated samples of carcass and gills.

isolation and identification applied on fish samples. Vitek 2 (Biomerieux) system were used to identification of *Pseudomonas* spp. At the end of identification, counting of *Pseudomonas* spp. carried out. According to the results of counting determined between values of P<105-3.2x107 cfu/g on gills and P<105-2.8x107 cfu/g on carcass of trout; P<105-2.3x107 cfu/g on gills and P<105-2.7x107 cfu/g on carcass of gilt head bream; P<105-2.8x107 cfu/g on gills and P<105-1.6x107 cfu/g on carcass of sea bass; P<105-1.0x107 cfu/g on gills and P<105-1.1x107 cfu/g on carcass of mullet; 3.0x105-1.5x106 cfu/g on gills and 7.2x105-9.0x105 cfu/g on carcass of whiting. *Pseudomonas* spp. identified from fishes were *Pseudomonas* fluorescence and *Pseudomonas* putida.

Keywords: *Pseudomonas*, microbial spoilage, fish, isolation

INTRODUCTION

In the situation of spoilage of fishes; after the process of fishing, the fish is changed with the otholithic-enzymatic, oxydative, microbiological effects. The compounds formed after this change is not composed after a regular fragmentization as in the human digestive system. For these chemical changes are effective on human health as a toxication, these changes are known as being stale in the fish [1, 2].

Fish are usually 3 types of degradation: enzymatic, chemical and microbial degradation. This is the most important distortions of microbial deterioration. Microbial degradation, caused by microorganisms, the loss of food qualities, composition and character is defined as losses caused by exchange of properties.

The biological reactions start to occur after the death of the fish. These reactions determine the changes in the first phase of the storage memories [3, 4]. This quality changes vary from type to type of the microbial content of the water where the fish inhabit rather than fish species [5-9] Microbial flora of fish is determined via fish type, caught the media the degree of pollution, temperature, the shape of the fish caught and performed after fish was caught processing methods [7-10].

In general, newly caught a healthy fish meat is sterile microorganisms are located normally in the outer protective layer, gills and intestinal of fish. The research has revealed that fresh fish usually have some bacteria and yeasts such as *Alcaligenes* spp., *Achromobacter* spp.,*Bacillus* spp., *Corynebacterium* spp., *Clostridium* spp., *Escherichia* spp., *Flavobacterium* spp., *Gaffkya* spp., *Micrococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Photobacterium* spp., *Kurthia* spp., *Serratia* spp [11].

Meat and meat products deterioration is characterized to felt taking various scents. These scents can be defined sometimes as onions and garlic, the reason is that they are due to the formation sulfur compounds, methanethiol and dimethyl disulfide. These sulfur compounds usually occurs after the consumption of meat from the surface of glucose and amino acid metabolism began. *Pseudomonas*, which is responsible for the production of this compounds on meat under aerobic conditions, is the main group of microorganisms. Because it partly prevents the growth of other bacteria its bacteriosins, even it destroys them. Therefore psychrophiles between *P. aeruginosa* and *P. fluorescens* are usually dominant on chilled meats [12, 13].

In this study fish samples, such as trout (Oncorhynchus mykiss), gilt head bream (Sparus auratus), sea bass (Dicentrarchus labrax), mullet (Mugil cephalus), whiting (Merlangus euxinus), offered consumption various places of Izmir and Manisa, Turkey of market and bazaar, they were carried out to determine the presence of *Pseudomonas* species that cause spoilage.

MATERIALS AND METHODS

In this study, between the dates July 2010 - March 2011 various places on market and bazaars of Izmir and Manisa, as the material obtained from sample of 100 fish were examined. As a material was examined samples of fish in 41 samples of trout (Oncorhynchus mykiss), 24 samples of gilt head bream (Sparus auratus), 23 samples of sea bass (Dicentrarchus labrax), 10 samples of mullet (Mugil cephalus) and 2 samples of whiting (Merlangus euxinus).

Aliquots of different dilutions of each carcass and gills of a fish sample under aseptic conditions were spread on Cetrimide agar, than all the samples were incubated at 35°C for 48 hours. As a result of incubation, breeding colonies examined for 366 nm wavelength under ultraviolet light, yellowish-green color pigment-producing colonies detected were counted in figure 1 (Analysis, ISO 13720: Meat and meat products was performed according to the method of enumeration of *Pseudomonas* spp.).

After counting, each selected colony, streak purified on nutrient agar plates with line sowing method, were incubated at 25°C for 24 hours. Bacterial strains, gram stains and oxidase tests were subjected to biochemical validation. As

a result of identification at the level of genus, the colonies with cells which are pink-red color on Gram stain, react with a positive oxidase and are seen in the form of *Bacillus* (rods) and *Coccobacillus* are evaluated as *Pseudomonas* spp. colonies. On the stage of the level identification of species, these colonies are described at a level of species via using colorimetric GN ID card of VITEK 2 Compact a fully automated biochemical identification device.

RESULTS

77% of the total of 100 fish, which were provided from market and bazaars of Izmir and Manisa, Turkey in the variety of different times, were isolated from *Pseudomonas* spp. Fish isolated from *Pseudomonas* species; *Pseudomonas fluorescence* and *Pseudomonas* putida.Isolated *Pseudomonas* species rates in the carcass and gills of each fish samples have also been identified. Isolated *Pseudomonas* species from the distribution of fish organs is shown in the Table 1. The percentage distribution of isolated *Pseudomonas* species from fish is 69% for gill and 67% for carcass.

In figure 2, isolated *Pseudomonas* species from fish were examined in the seasonal distribution. Isolated *Pseudomonas* species are investigated for every season and it is seen that isolated ones exist 50% (N=34) in summer, 79% (N=33) in autumn and 98% (N=33) in winter.

Separately from each carcass and gills of fish samples counted isolated *Pseudomonas* species. In figure 3 was shown that gill and carcass of fish samples on count range of isolated *Pseudomonas* species. Accordingly, the rate of count results were found between P<105-2.7x107 cfu/g on carcass and P<105-3.2x107 cfu/g on gills of fish.

DISCUSSION

In this study, market and bazaars of Izmir and Manisa, Turkey in the variety of different times provided 100 pieces of the fish of gill and carcass examples were analyzed for *Pseudomonas* spp. In study determined between values of P<105-3.2x107 cfu/g on gills and P<105-2.8x107 cfu/g on carcass of trout; P<105-2.3x107 cfu/g on gills and P<105-2.7x107 cfu/g on carcass of gilt head bream; P<105-2.8x107 cfu/g on gills and P<105-1.6x107 cfu/g on carcass of sea bass; P<105-1.0x107 cfu/g on gills and P<105-1.1x107 cfu/g on carcass of mullet; 3.0x105-1.5x106 cfu/g on gills and 7.2x105-9.0x105 cfu/g on carcass of whiting.

Dainty et al. [14] reported that meat and meat products was found at the level of *Pseudomonas* 106-108 cfu/g have identified cause of deterioration. Terzi [15] detected that some rainbow trout farms of Ankara, Turkey of skin samples of fish was determined the number of *Pseudomonas* spp. 1.0x104 cfu/cm^2, 2.1x105 cfu/cm^2, 3.7x102 cfu/cm^2. Balboa et al. [16] reported that Atlantic cod fish (Gadus morhua) have isolated *Pseudomonas anguilliseptica* in Northern Europe. These strains of bacteria were counted and results were found between that 7.6x104-5x107cfu/g [16,17]. stated that some rainbow trout (Oncorhynchus mykiss) farms of Erzurum, Turkey of skin samples of fish on numbers of *Pseudomonas* determined between values of P<102-103 cfu/cm^2 on A business, 102-104 cfu/cm^2 on B and C business, 102-103 cfu/cm^2 on D and E business, P<102 cfu/cm^2 on F business in April and June, 9,0x102 cfu/cm^2 on F business in May [17, 18]. reported that fresh fish sold in supermarkets in the state of Khartoum were isolated from 62% of *Pseudomonas* spp. and 55.3% Enterobacteriacaea. Isolated *Pseudomonas* was found between values of 5x107-2x109 cfu/g on gills, 7x105-3x107 cfu/g on intestinal and 4x105-3x107 cfu/cm^2 on skin [18]. The data obtained in this study, the microbial degradation of fish and fish products, valued at the border (P>106–107 cfu/g)19. In the light of these results, the reason for the variable number of microorganisms isolated from fish, can be connected to fish hunting in environmental conditions, captured fish and fish products bring point of sales and conserve point of sales.

Berthe et al. [19] reported that, in France, sea bass (Dicentrarchus labrax), sea bream (Sparus auratus), turbot (Scophthalmus maximus) as many fish species have isolated *Pseudomonas anguilliseptica* [19-21] detected that trout operation of Denizli, Turkey have isolated a yellow-green pigment producing *Pseudomonas* spp. [20, 21] was stated that in Mediterranean fish stored in gilt head bream (Sparus aurata) isolated *Pseudomonas lundensis*, *Pseudomonas fluorescens*, *Pseudomonas fragi* and *Pseudomonas putida* [21, 22] indicated that the products of salted and dried salted cod fish have identified at a level of species microflora. For level of species identification, Gram-positive and Gram-negative identification cards are used, Vitek 2 used a fully automated identification system. Isolated Gram-positive bacteria were *S. epidermidis, S. hominis, S. warneri, S. haemolyticus, S. simulans and S. saprophyticus*; isolated Gram-negative bacteria were Stenotrophomonas maltophilia, *Vibrio alginolyticus, Pasteurella haemolytica, Enterobacter agglomerans, E. asburiae, E. cloacae, Pseudomonas putida, Pseudomonas fluorescens, Shewanella putrefaciens, Morganella morganii, Actinobacillus urea, Acinetobacter lwoffi/junii and non-fermentative Bacillus* [22]. According to data obtained in this study, fish isolated from *Pseudomonas* species; *Pseudomonas fluorescens* and *Pseudomonas putida*.

Psycrophile, mesophyll and psychrotroph in *Pseudomonas* species, which is also has proteolytic and lipolytic activity. Psycrophile species, led to deterioration in stored foods, such as proteolytic and lipolytic enzymatic activities through the high protein and fat-containing foods leads to the deterioration.

In this study is a result of us, and actually fish and fish products are very perishable foods, basic food items such as fish could threaten human health and in the future factor of potential risk can indicated terms of public health.

As a result, fish is a rich source of protein, vitamins and minerals nutrients; on the other hand, hunting, preservation, processing, sale of fish among, in case of hygienic and technological measures are not taken necessary and sufficient, create a favorable environment for growth and development of microorganisms, leading to deterioration as a result, in terms of human health form a risk.

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Table 1. Isolated *Pseudomonas* species are the distribution of fish organs.

	Gill		Carcass	
	N	%	N	%
Total (100)	69	69.00	67	67.00
Trout (41)	31	75.61	30	73.17
Gilt Head Bream (24)	17	70.83	16	66.67
Sea Bass (23)	14	60.87	14	60.87
Mullet (10)	5	50.00	5	50.00
Whiting (2)	2	100.00	2	100.00

***N**= Number of samples; **%**= Percent

FIGURES:

A **B**

Figure 1. Appearance of colonies of *Pseudomonas* spp. in cetrimide agar medium (A) a normal appearance in the light (B) an ultra-violet appearance in the light.

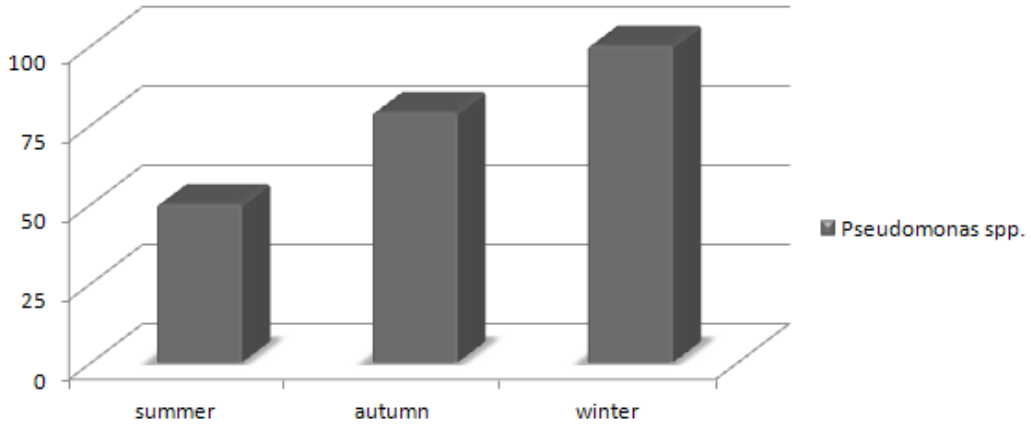

Figure 2. Isolated *Pseudomonas* species in the seasonal distrubition of fish

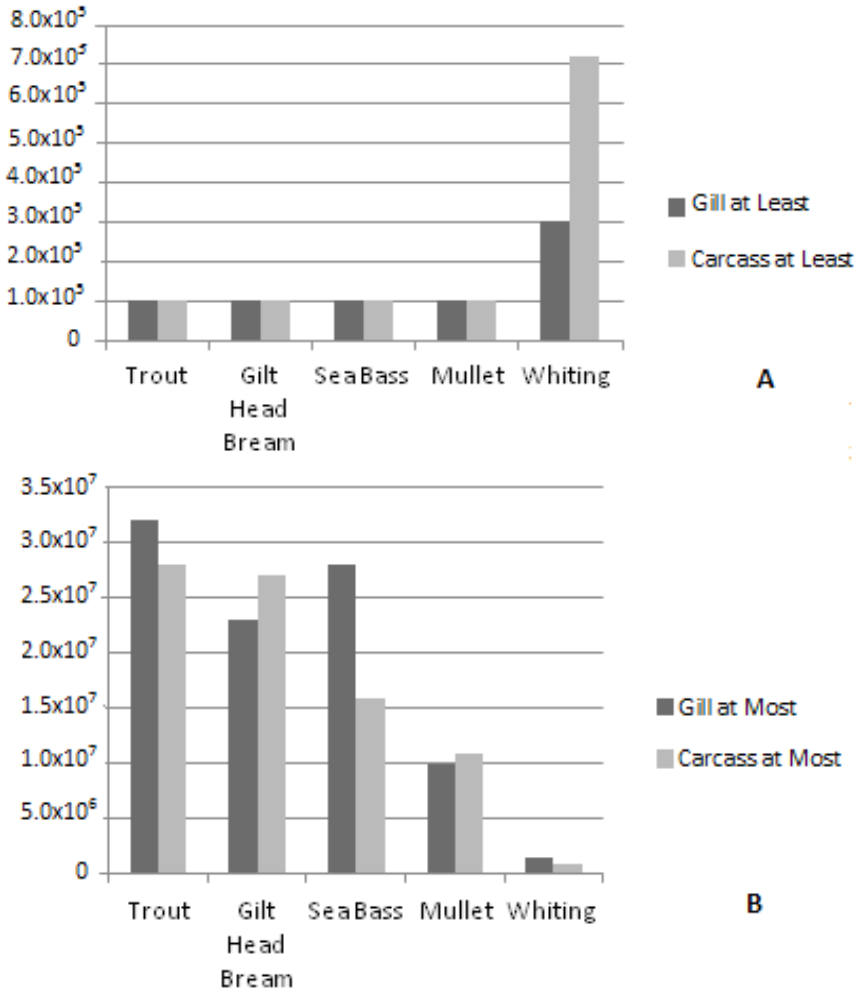

Figure 3. A, B. The gills and carcasses of fish samples on count ranges of isolated *Pseudomonas* species.