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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

USEFULNESS OF API TEST STRIPS FOR IDENTIFICATION OF BACTERIAL FLORA IN BLUE CRAB (*Callinectes sapidus*) CAUGHT FROM AKYATAN LAGOON (ADANA-TURKEY)

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Abstract: Total 109 bacterial strains were isolated and 20 different bacterial species were identified from fresh blue crab. In order to confirm bacterial species, Analytical Profile Index Test Strips (API) was used. Bacterial flora from three different parts (crab surface, body meat and claw meat) of blue crab was identified by using API-20E, API-20NE and API Listeria Test Kits. *Pseudomonads* were largest isolated group and constituted 50%, 40% and 29.8% of total bacteria on surface, in claw and body meat of blue crab, respectively. Although *Listeria* was not found on surface of blue crab, *Listeria grayi* (10%) and *Listeria seeligeri* (3.3%) were isolated from claw meat and body meat of blue crab, respectively. Percentage of unidentified bacteria was 12.5% for surface, 30% for claw meat and 30% for body meat of blue crab.

Keywords: Blue crab, API, bacteria

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Introduction

In some comparative studies, API system was reported to be more accurate, simple and rapid than other methods used for bacterial identification. Previously it was indicated that those system could be replaced with conventional biochemical methods (Oberhofer, 1979; Poutrel and Ryniewiczt, 1984). The API-20NE is reported to be useful for identification of environmental bacteria (Truu et al., 1999), while the API 20E system provide a reliable means to identify members of the family *Enterobacteriaceae* (Edwards and Ewing, 1972; Edinger et al., 1985).

It was known that the micro-organisms associated with most fishery products indicate the microbial flora in their aquatic environment (Ashie et al., 1996; Gram and Huss, 1996). It has been estimated that world ocean contain more than 10^{29} microbes (Whitman et al., 1998). Concentrations of microorganism of the sea depend on substantially water depth (Karl and Dore, 2001). Blue crab lives in soft-bottomed estuaries, bays and deltas, which females of the blue crab migrate offshore to spawn (FFWCC, 2005). One of the commercial important species in Akyatan lagoon on the Mediterranean coast is blue crab (Anonymous, 2008).

Fish and shellfish spoil rapidly primarily because of bacterial action (Colby et al., 1993; Sivertski et al., 2002). The shellfish flesh provides good substrate for the microorganisms due to their meat properties. Fish and shellfish may be carry pathogenic bacteria which is found in naturally aquatic environments, contaminated water or handling environments (Huss et al., 2003; Gillespie et al., 2001; Herrera et al., 2006). Thus, the purpose of this study was to investigate enteric and non-enteric bacterial flora from three different parts (crab surface, body meat and claw meat) of freshly caught blue crab using API-20E, API-20NE and API Listeria Test Kits.

Materials and Methods

Wild blue crabs (*Callinectes sapidus*) were caught by dip net in Akyatan Lagoon in the south of the Mediterranean Sea and transferred to laboratory. The mean carapace length and width of blue crab were 6.19±0.04 and 12.03± 0.17 cm, respectively. Blue crab samples were taken from three part of the crab which surface, body meat and claw meat. Surface of the blue crab was sampled using sterile swap and transferred onto agar plate. Body meat and claw meat of crab were aseptically weighed (10 g) and mixed with 90 mL of Ringer solution and then stomached for 3 min. Further decimal dilutions were made and then 0.1 mL of each dilution was pipetted onto the surface of agar plate in triplicate. Petri dishes used for each groups were consist of Plate Count Agar (PCA), Violet Red Bile Agar (VRBA), *Listeria* Sellective Agar (LSA), *Salmonella-Shigella* Agar (SSA). All groups were incubated for 2 days at 30ºC. Each of chosen individual bacterial colonies was spread out several times on the agar plate using sterile loop in order to produce pure colonies. Isolates were identified according to the manufacturer's instructions of API 20E, API 20NE and API Listeria strip system (BioMereux, France). The inoculated strip was incubated for 16-24h and the colour reactions were noted either positive or negative. The result obtained were analysed using the APILAB PLUS software (Biomerieux, France).

Results and Discussion

Enteric and non-enteric bacterial flora from three different parts (crab surface, body meat and claw meat) of blue crabs were given in Table 1, 2 and 3. Total 109 bacterial strains were isolated and 20 different bacterial species were identified from fresh blue crabs. Dominant bacteria species for surface, body and claw meat of blue crab were *Pseudomonas putida* (25%, 6.6%, 10%), *Pseudomonas luteola* (12.5%, 13.3%, 10%), *Pseudomonas oryzihabitans* (6.25%, 6.6%, 10%), respectively. Although microflora of molluscan shellfish is more variable, the dominant bacteria were reported as pseudomonades and *Moraxella*/*Acinetobacter* (Ashie et al., 1996; Jay, 2000, Linton et al. 2003).

Table 1. Enteric and non-enteric bacterial flora on surface of blue crab

Microorganisms	**Number of isolates**
Aeromonas salmonicida salmonicida	1 (6.25%)
Bergeyella zoohelcum	1 (6.25%)
Proteus penneri	1 (6.25%)
Pseudomonas fluorescens/ putida	1 (6.25%)
Pseudomonas luteola	2 (12.5%)
Pseudomonas oryzihabitans	1 (6.25%)
Pseudomonas putida	4 (25%)
Ochrobactrum anthropi	1 (6.25%)
Stenotrophomonas maltophilia	1 (6.25%)
*Wau*tersia *paucula*	1 (6.25%)
Undetected	2 (12.5%)
Total number of isolates	16

Table 2. Enteric and non enteric bacterial flora in claw meat of blue crab

Microorganisms	**Number of isolates**
Bordatella /Alcaligenes /Moraxella spp	1 (10%)
Enterobacter sakazakii	1 (10%)
Listeria grayi	1 (10%)
Pseudomonas fluorescens/ putida	1 (10%)
Pseudomonas luteola	1 (10%)
Pseudomonas oryzihabitans	1 (10%)
Pseudomonas putida	1 (10%)
Undetected	3 (30%)
Total number of isolates	10

Table 3. Enteric and non enteric bacterial flora in body meat of blue crab

Microorganisms	Number of isolates
Acinetobacter baumannii/calcoaceticus	1 (3.3%)
Aeromonas hydrophila/caviae	1 (3.3%)
Listeria seeligeri	1 (3.3%)
Pasteurella pneumotropica/haemolytica	1 (3.3%)
Providencia rettgeri	1 (3.3%)
Pseudomonas fluorescens/putida	1 (3.3%)
Pseudomonas luteola	4 (13.3%)
Pseudomonas oryzihabitans	2 (6.6%)
Pseudomonas putida	2 (6.6%)
Ochrobactrum anthropi	4 (13.3%)
Stenotrophomonas maltophilia	1 (3.3%)
Vibrio fluvialis	1 (3.3%)
Vibrio metschnikovii	1 (3.3%)
Undetected	*9 (*30%)
Total number of isolates	30

Bacteria of the pseudomonas genus isolated from surface of blue crab were *Pseudomonas fluorescens*/ *putida*, *Pseudomonas luteola* , *Pseudomonas oryzihabitans* and *Pseudomonas putida*. The remaining 37.5 % of total isolated bacteria belonged to the genera *Aeromonas*, *Bergeyella, Proteus, Ochrobactrum, Stenotrophomonas, Wautersia*. *Wautersia paucula* is a gram-negative environmental bacterium and causes human infection sporadically (Kwon et al., 2007), while *Bergeyella zoohelcum* is frequently isolated as pathogen bacteria from the upper respiratory tract of dogs, cats and other mammals (Shukla et al., 2004; Lin et al., 2007). Percentage of each of this pathogen was 6.25%. From claw meat of blue crab, *Pseudomonas* spp and other gram negative bacteria species such as *Bordatella* /*Alcaligenes* /*Moraxella* spp, *Enterobacter sakazakii* and *Listeria grayi* were isolated. *Enterobacter sakazakii* known as an opportunistic pathogen (FAO/WHO, 2004) recognised representing a risk to newborns, especially low birth weight and immunocompromised infants (Cordier, 2006). *Listeria grayi* was the only one gram positive bacteria species among the isolated strains. Jallewar et al. (2007) found 39 isolates of *Listeria* spp. in walking catfish *(Clarias batrachus*) which consisted of *Listeria monocytogenes (*67%), *Listeria seeligeri* (21%), *Listeria grayi* (8%) and *Listeria welshimeri* (5%). *Listeria* species isolated from fish were *L. monocytogenes* (1), L. *innocua* (6) and *L. welshimeri* (1) (Molla et al. 2004).

Total number of isolates from body meat of blue crab was higher than that of other parts of the blue crab. These include *Acinetobacter*, *Aeromonas*, *Listeria*, *Pasteurella*, *Providencia*, *Pseudomonas*, *Ochrobactrum*, *Stenotrophomonas* and *Vibrio* strains. Percentage of *Pseudomonas luteola* and *Ochrobactrum anthropi* was 13.3%, which is higher than other isolated strains. *Ochrobactrum anthropi*, formerly classified as *Achromobacter* is a ubiquitous pathogen organism, which is widely distributed in the environment and water sources (Kettaneh et al., 2003) and may be part of the normal flora of the large intestine of human (Galanakis et al., 2002; Wi et al., 2007).

Aeromonads have been isolated from various aquatic environments, including fresh and marine (saline) water systems (Krovacek and Faris,

2003). Among members of this group, *Aeromonas salmonicida salmonicida* and *Aeromonas hydrophila/caviae* constituted 6.25 % and 3.3% of total bacteria on surface and in body meat of blue crab. Although human beings are minor contributors of *Aeromonas salmonicida salmonicida* in the environment, aeromonads multiply to significant numbers in sewage systems (Araujo et al., 1991).

Pseudomonas, Vibrio, Aeromonas, Pasteurella, Ochrobacterium, Achromobacter, Flavobacterium, and *Micrococcus* were reported as dominant bacteria species in the other shellfish species such as oyster and mussel meat (Tanikawa, 1937; Colwell and Liston, 1960; Croci et al., 2001). Moreover, *Serratia* spp, *Proteus* spp. *Clostridium* spp. and *Bacillus* spp. were shown to present in crustacean meat (Jay, 2000). Bacterial flora in fresh Indian white shrimp meat was reported to be *Aeromonas, Pseudomonas, Vibrio, Flavobacterium* and *Serratia*. In that study, *Aeromonas* constituted about 38% of the flora (Jeyasekaran et al., 2006).

Pathogenic bacteria have been found in shellfish, including *Vibrio parahaemolyticus, Salmonella, Clostridium, Escherichia coli, Campylobacter, Aeromonas* and *Listeria monocytogenes* (Ward, 1991; Gillespie et al., 2001; Huss et al., 2000). However, in the present study, different pathogen bacteria species were isolated. The occurrence of pathogenic microorganisms in seawater or in shellfish could exist anytime sewage from human or animal origin would be discharged to the coast (Metcalf, 1982). The presence of pathogens in the environment mainly depends on the density of the coastal urban and animal populations (Pommepuy et al., 2005). The Akyatan lagoon is rich in fish and bird species. There are extensive farming activities in lagoon enviroment and receives domestic and industrial wastes. These factors could be responsible for contamination of blue crab with these different pathogen species.

Conclusions

The hygienic handing of blue crab from the moment of capture to the point of consumption is crucial to ensure good quality and long shelf life. Bacterial contamination of blue crab flesh is a main cause of spoilage and is of particular importance if the blue crab is contaminated with pathogenic bacteria, which causes food poisoning or even death amongst consumers. Therefore, blue crab should be kept clean and chill temperature which can help to minimise or delay the growth of bacteria. Good hygiene practice and proper handling are necessary to prevent food poisoning associated with consumption of blue crab.

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