EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 7
249-256
Year: 2015

# BACTERIAL DIVERSITY IN SOME EDIBLE MARINE CRABS AT KOVALAM BEACH, EAST COAST OF CHENNAI

**Lipshi Deb[1], Bharathi Ravikrishnan[2], Jayanthi, J[1] and Ragunathan M.G*[3]**

[1]Gill Research Institute, Guru Nanak College, Chennai – 42.
[2]Department of Biotechnology, Hindustan College of Arts &Science, Chennai.
[3]Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai – 42.

***Author for Correspondence: Ragunathan M.G**
Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai – 42.

**Article Received on 26/09/2015** **Article Revised on 15/10/2015** **Article Accepted on 05/11/2015**

## ABSTRACT
Different species of both male and female edible marine crabs were collected from Kovalam fish landing centre, East coast of Southern Chennai. The hemolymph was collected from each crab. Then, the crabs were dissected and different tissues like muscle, hepatopancreas and gut were extracted. Isolation of bacteria was performed from these dissected parts, using certain basal and selective media i.e., Nutrient agar and Zobell's marine agar medium. The biochemical results were compared with Bergey's Manual to confirm the genus and species. In the present study, the bacterial diversity of different edible marine crabs were identified as coliforms such as Escherichia coli, Klebsiella sp., Proteus sp., Salmonella sp., Shigella sp. and other Gram negative bacteria like Pseudomonas sp. and Gram positive bacteria like Staphylococcus aureus and Bacillus sp.

**KEYWORDS**: Edible Crab, Bacterial diversity, Biochemical parameters.

## INTRODUCTION
Crustacean cuisines have increased worldwide and spreading of zoonotic diseases through these crustacean meats in humans have also increased in parallel. Thus aquaculture industry of the world is facing serious problem due to microbial load in the edible crabs (Soundarapandian and Sowmiya, 2013). Inorganic and Organic contaminants entering coastal waters may be biomagnified within the tissues of the edible marine organisms to varying degrees from non-portable water, their food or sediments (Fowler, 1982; Nair et al., 1987; Mahalaxmi et al., 2013). The collection site around human habitations like harbour contains diversified bacterial flora of coliform and gram positive bacteria (Jones and summer 1998). Many new pathogens are being reported with significant pathogenicity and adverse effect on the production of mud crabs across the world (Jithendran et al., 2010). Major microbial flora isolated from the cultivable crustacean members includes Vibrio, Pseudomonas and Staphylococcus species.

It was recorded by Lakshmi et al., (2014) that the major species of commercially important brachyuran crabs in the landings were Portunus sanguinolentus, Portunus argentatus, Portunus gladiator, Charybdis lucifera and Charybdis hoplites by trawlers or as a by-catch at Chennai Fisheries Harbour. The most important variety of crabs that are caught at Pulicat Lake is mud crab Scylla serrata and Portunus pelagicus (Ramesh et al., 2008). According to Sivakumar et al., 2014, the Emerita asiatica sample is abundantly found at the coast line of Kovalam beach, South east coast of Chennai.

The present study was done to identify the bacterial diversity in different tissues of edible crabs collected from Kovalam beach East coast of Chennai.

## METHODOLOGY
### Collection of Samples
The most common edible crabs like Charybdis lucifera, Portunus pelagicus and Portunus sanguinolentus were purchased in live conditions from the fish landing centre of Kovalam, East coast of Southern Chennai in juvenile state of their growth. The crab samples were immediately transferred to the laboratory within 24 hours where they were subjected to bacterial flora analysis.

### Preparation of samples
From both male and female crabs, hemolymph was collected with suitable anticoagulant. The hemolymph was centrifuged at 1000 rpm for 20 minutes at room temperature and the supernatant was collected in a separate tube with a small pinch of sodium azide in it and the samples were stored in aliquots at 4°C for further use. The crabs were dissected for different parts like muscle, gut and hepatopancreas were suspended in sterile Phosphate Buffer Saline solution and were incubated at 75 rpm orbital shaker for one hour at room

temperature. 100µl of each samples were subjected to serial dilution. $10^{-5}$ dilution was used as the sample for further bacteriological studies.

**Isolation of bacteria**

$10^{-5}$ dilution of the bacteria obtained from the crab samples were initially inoculated in basal media such as nutrient agar and Zobell's marine agar. The total bacterial concentration that was analysed from the various organs using MacFarland reagent and O.D. was recorded at 630nm. The bacterial flora was characterised and confirmed by standard parameters as per Bergey's manual (Garrity et al., 2005).

**Microscopic examination**

The bacterial flora were subjected to various staining like Gram staining, Spore Staining (Schaeffer-Fulton Method) and Capsular polysaccharide staining, examined under 100X microscope to determine shape and size. The microbes were also subjected to hanging drop technique and the patterns of motility, if any was examined and recorded.

**Biochemical characterization**

The standard biochemical tests were performed for all the bacterial isolates which are isolated from different parts of all the three edible crabs. The biochemical tests like indole production test, methyl red test, Voges Proskauer test, citrate utilization test, catalase test, urease test, $H_2S$ production test, lactose fermentation, oxidase test, ONPG, TSI, starch hydrolysis and carbohydrate utilization test in which glucose, fructose, lactose, maltose and mannitol and the growth pattern in luminescent agar medium was performed and the results were tabulated (Cappuccino and Sherman, 1999).

**Confirmatory test**

The isolates were inoculated on selective media like Eosin-Methylene blue agar, Pseudomonas agar (APHA, 1998), blood agar, Aeromonas agar with ampicillin, starch hydrolysis agar, mannitol salt agar, Salmonella-Shigella agar, luminescent agar and TCBS agar to confirm the bacteria and the readings were recorded.

**RESULT AND DISCUSSION**

The coastal areas are highly contaminated with fecal coliform and other pathogenic microorganisms due to sewage effluents, anthropogenic sources, domestic wastes, urban runoff and rapid industrialization. Environmental loading of fecal by-products from humans and their associated animals is highly significant and can affect the quality of water and food resources (Fayer, 2004; Kim et al., 2004). There is a high possibility of transmission of bacterial infections from the edible crabs available in the coastal area and can represent a significant public health problem (Krantz et al., 1969). Therefore in the present study the microbial diversity was analysed in the different parts of the edible marine crabs like Charybdis lucifera, Portunus pelagicus and Portunus sanguinolentus.

Different tissues such as hemolymph, muscle, gut and hepatopancreas, total microbial load of both the male and the female specimen of each species of crabs was analysed by the colorimetric method. The results are tabulated in Table 1. Among all the tissues examined the highest bacterial count was observed in hemolymph and the least bacterial count was observed in hepatopancreas (Table 1). It was evident that maximum bacterial load was observed in the hemolymph of female crab, P. pelagicus and the minimum was identified in the hemolymph of male crab, Charybdis lucifera.

Soundarapandian and Sowmiya (2013) have analysed the microbial diversity in crabs, P. sanguinolentus and P. pelagicus and the predominant microbes were V. parahaemolyticus P. fluorescens Pseudomonas, Staphylococcus aureus, S. saprophyticus and Acinetobacter calcoaceticus. The present study focuses on the microbial communities isolated from different tissues (Hemolymph, Muscle, Hepatopancreas and Gut) of crabs collected from the study area showed a highly diverse and varied microbial population. The microbial analysis done by morphological and biochemical characterization is tabulated in Tables 2 and 3.

The bacterial isolates obtained from different tissues of each edible marine crab specimen were found to have variations in their morphological characters like shape (rod and cocci), size, motility and presence of granules. After biochemical characterization and confirmatory tests the specific bacterial isolates were identified with respect to their genus and species. The results of the confirmed bacterial isolates from different tissues of each marine edible crab are tabulated in Table 4. Mahalaxmi et al. (2013) had clearly indicated that edible crabs, P. sanguinolentus and P. pelagicus are contaminated with coliforms and other pathogenic microorganisms such as Pseudomonas aeruginosa, Aeromonas salmonicida, Micrococus luteus, Klebsiella pneumonia, Moraxella lacunata, Bacillus cereus, E.coli, Alcaligenes faecalis and Alcaligenes paradoxus. Lalitha and Nirmala (2012) confirmed that farmed crab carry significant numbers of pathogenic microbes such as Vibrio, Aeromonas, Enterobacteriaceae, Pseudomonas/ Shewanella, Acinetobacter, Bacillus, Micrococcus and Flavobacterium/Cytophaga. Therefore the present study confirms the microbial diversity of Bacillus sp., Escherichia coli, Klebsiella sp., Proteus sp., Pseudomonas sp., Salmonella typhi, Shigella sp. and Staphylococcus aureus isolated from different parts of tissues of marine edible crabs, Charybdis lucifera, Portunus pelagicus and Portunus sanguinolentus.

**Table 1: BACTERIA LOAD IN DIFFERENT TISSUES OF CRABS**

| Sl. No. | Crabs | Tissues | Female | Male |
|---|---|---|---|---|
| 1 | Charybdis lucifera | Hemolymph | 1.5X10-8 CFU/ml | 1.25X10-8 CFU/ml |
| | | Muscle | 0.9X 10-8 CFU/ml | 0.91X10-8 CFU/ml |
| | | Hepatopancreas | 0.7X10-8 CFU/m | 0.63X10-8 CFU/ml |
| | | Gut | 1.01X1-^8 CFU/ml | 0.67X10-8 CFU/ml |
| 2 | Portunus pelagicus | Hemolymph | 1.45X10-8 CFU/ml | 1.06X10-8 CFU/ml |
| | | Muscle | 0.92X10-8 CFU/ml | 0.87X10-8 CFU/ml |
| | | Hepatopancreas | 0.67X10-8 CFU/ml | 0.56X10-8 CFU/ml |
| | | Gut | 0.65X10-8 CFU/ml | 0.54X10-8 CFU/ml |
| 3 | Portunus sanguinolentus | Hemolymph | 0.86X10-8 CFU/ml | 0.80X10-8 CFU/ml |
| | | Muscle | 0.94X10-8 CFU/ml | 0.87X10-8 CFU/ml |
| | | Hepatopancreas | 0.87X10-8 CFU/ml | 0.74X10-8 CFU/ml |
| | | Gut | 0.89X10-8 CFU/ml | 0.78X10-8 CFU/ml |

**TABLE 2: MORPHOLOGICAL CHARACTERS**

| CRABS | PARTS | SEX | ISOLATES | GRAM | SHP | CAP | MOT |
|---|---|---|---|---|---|---|---|
| Charybdis lucifera | Hemolymph | F | 1 | G+ | Rod | + | - |
| | | | 2 | G- | Rod | - | + |
| | | M | 1 | G+ | Rod | + | - |
| | Muscle | F | 1 | G+ | Rod | + | - |
| | | | 2 | G- | Rod | + | + |
| | | M | 1 | G+ | Rod | + | - |
| | | | 2 | G- | Rod | - | + |
| | Hepatopancreas | F | 1 | G- | Rod | - | + |
| | | | 2 | G- | Rod | - | + |
| | | M | 1 | G- | Rod | - | + |
| | | | 2 | G- | Rod | - | - |
| | Gut | F | 1 | G- | Rod | - | + |
| | | | 2 | G- | Rod | - | + |
| | | M | 1 | G- | Rod | - | + |
| | | | 2 | G- | Rod | - | - |
| Portunus pelagicus | Hemolymph | F | 1 | G+ | Cocci | - | - |
| | | M | 1 | G- | Rod | - | - |
| | | | 2 | G- | Rod | - | + |
| | Muscle | F | 1 | G- | Rod | - | + |
| | | | 2 | G- | Rod | - | - |
| | | M | 1 | G- | Rod | - | + |
| | Hepatopancreas | F | 1 | G- | Rod | - | - |
| | | | 2 | G+ | Cocci | - | - |
| | | M | 1 | G- | Rod | - | - |
| | Gut | F | 1 | G- | Rod | - | - |
| | | | 2 | G+ | Cocci | - | - |
| | | M | 1 | G- | Rod | - | - |
| Portunus sanguinolentus | Hemolymph | F | 1 | G+ | Rod | + | - |
| | | | 2 | G+ | Cocci | - | - |
| | | M | 1 | G+ | Rod | + | - |
| | | | 2 | G- | Rod | - | - |
| | Muscle | F | 1 | G+ | Rod | + | - |
| | | M | 1 | G- | Rod | - | - |
| | | | 2 | G+ | Cocci | - | - |
| | Hepatopancreas | F | 1 | G+ | Rod | + | - |
| | | | 2 | G- | Rod | - | + |
| | | M | 1 | G- | Rod | - | - |
| | | | 2 | G- | Rod | - | + |
| | Gut | F | 1 | G+ | Rod | + | - |
| | | | 2 | G- | Rod | - | + |
| | | M | 1 | G- | Rod | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 2 | G- | Rod | - | + |

Note: Gram – Gram staining; Shp – Shape; Cap – Capsule staining; Mot – Motility test; G- - Negative staining; - Negative; +positive

**TABLE.3: BIOCHEMICAL PARAMTERS**

| CRABS | PARTS | SEX | ISOLATES | Cat | Oxi | I | MR | VP | Ci | U | TSI | $H_2S$ Pro | Nitr | ONPG | Glu | Suc | Lac | Mal | Man |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Charybdis lucifera | Hemolymph | F | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | | | 2 | + | - | - | + | - | - | - | k/a | + | - | - | A | - | - | A | A |
| | | M | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | Muscle | F | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | | | 2 | + | - | - | + | - | + | + | k/a | + | + | - | A | - | - | - | - |
| | | M | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | | | 2 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | Hepatopancrease | F | 1 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | - | + | + | k/a | + | + | - | A | - | - | - | - |
| | | M | 1 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | - | - | - | k/a | + | + | - | A+G | - | - | - | A+G |
| | Gut | F | 1 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | - | + | + | k/a | + | + | - | A | - | - | - | - |
| | | M | 1 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | - | - | - | k/a | + | + | - | A+G | - | - | - | A+G |
| Portunus pelagicus | Hemolymph | F | 1 | + | - | - | + | + | - | + | a/a | - | + | - | A | - | - | A | A |
| | | M | 1 | + | - | - | - | + | + | + | a/a | + | - | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | - | - | - | k/a | + | - | - | A | - | - | A | A |
| | Muscle | F | 1 | + | - | - | + | - | - | - | k/a | + | - | - | A | - | - | A | A |
| | | | 2 | + | - | - | + | - | - | - | k/a | + | + | - | A+G | - | - | - | A+G |
| | | M | 1 | + | - | - | - | - | + | - | k/a | + | + | - | A+G | - | - | - | - |
| | Hepatopancrease | F | 1 | + | - | - | - | + | + | + | a/a | + | - | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | + | - | + | a/a | - | + | - | A | - | - | A | A |
| | | M | 1 | + | - | - | + | - | - | - | k/a | + | + | - | A+G | - | - | - | A+G |
| | Gut | F | 1 | + | - | - | - | + | + | + | a/a | + | - | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | + | - | + | a/a | - | + | - | A | - | - | A | A |
| | | M | 1 | + | - | - | + | - | - | - | k/a | + | + | - | A+G | - | - | - | A+G |
| Portunus sanguniolentus | Hemolymph | F | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | | | 2 | + | - | - | + | + | - | + | a/a | - | + | - | A | - | - | A | A |
| | | M | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | | | 2 | + | - | - | - | + | + | + | a/a | + | - | + | A+G | - | A+G | A+G | A+G |
| | Muscle | F | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | | M | 1 | + | - | - | - | + | + | + | a/a | + | - | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | - | - | + | + | - | + | a/a | - | + | - | A | - | - | A | A |
| | Hepatopancrease | F | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | | | 2 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | | M | 1 | + | - | - | - | + | + | + | a/a | + | - | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | + | - | - | - | + | - | k/a | + | + | - | A+G | - | - | - | - |
| | Gut | F | 1 | + | - | - | - | + | - | - | a/a | - | + | - | A | - | A | - | A+G |
| | | | 2 | + | - | + | + | - | - | - | a/a | - | + | + | A+G | - | A+G | A+G | A+G |
| | | M | 1 | + | - | - | - | + | + | + | a/a | + | - | + | A+G | - | A+G | A+G | A+G |
| | | | 2 | + | + | - | - | - | + | - | k/a | + | + | - | A+G | - | - | - | - |

Note: Cat – catalase test; Oxi – Oxidase test; MR – Methyl Red test; VP – Voges Proskauer test; Ci - Citrate Utilization test; Ure - Urease test; TSI – Triple Sugar Ion Test; $H_2S$ - $H_2S$ production test; Nit – Nitrate Test; ONPG – ortho-Nitrophenyl-β-galactoside; Glu – Glucose; Suc – Sucrose; Lac – Lactose: Mal – Maltose and Man – Mannitol.

**TABLE 4: MICROORGANISMS IDENTIFIED FROM TISSUES OF CRABS COLLECTED**

| CRABS | HEMOLYMPH | MUSCLE | HEPATOPANCREAS | GUT |
|---|---|---|---|---|
| Charybdis lucifera | Bacillus cereus | Bacillus cereus | Escherichia coli | Escherichia coli |
| | Salmonella typhi | Proteus vulgaris | Proteus vulgaris | Proteus vulgaris |
| | | Escherichia coli | Shigella dysenteriae | Shigella dysenteriae |
| Portunus pelagicus | Staphylococcus aureus | Salmonella typhi | Klebsiella pneumoniae | Klebsiella pneumoniae |
| | Klebsiella pneumoniae | Shigella dysenteriae | Staphylococcus aureus | Staphylococcus aureus |
| | Salmonella typhi | Pseudomonas aeruginosa | Shigella dysenteriae | Shigella dysenteriae |
| Portunus sanguinolentus | Bacillus cereus | Bacillus cereus | Bacillus cereus | Bacillus cereus |
| | Staphylococcus aureus | Klebsiella pneumoniae | Escherichia coli | Escherichia coli |
| | Klebsiella pneumoniae | Staphylococcus aureus | Klebsiella pneumoniae | Klebsiella pneumoniae |
| | | | Pseudomonas aeruginosa | Pseudomonas aeruginosa |

Klebsiella pneumoniae Proteus vulgaris Escherichia coli Shigella dysenteriae Salmonella typhi Bacillus cereus Staphylococcus aureus Pseudomonas aeruginosa.

## CONCLUSION

The crabs are transported from the landing to market place by keeping them in ice to avoid spoilage by bacterial contamination. If contaminated water is used for the preparation of ice, it is used as a source of microbes leading to spoilage of crabs. Another source of microbial contaminant is due to the disposal of sewage into the sea. They get accumulated in the shore line where these crustacean burrows (Soundarapandian et al., 2013). The occurrence of high counts of pathogens in crustacean flesh may cause zoonotic diseases; especially in individuals who consume sea food raw, lightly or insufficiently cooked. Thus, it is advisable to avoid consuming uncooked crustacean food. The current investigation recommends continuous monitoring of the total microbial load before the crustacean is commercially sold.

## REFERENCES


1. American Public Health Association. Standard methods for the examination of water and wastewater. 20th edn. New York, 1998; 1190.
2. Cappuccino, J. G. and Sherman, N. (1999). Microbiology, a laboratory manual. The Benjamin/ Cummings Publishing Company Inc., California.
3. Fayer, R., Dubey, J.P. and Lindsay, D.S. Zoonotic protozoa: from land to sea. Trends Parasiotol. 2004; 20: 531–536.
4. Fowler, S.W. Biological transfer and transport processes. In: Pollutant transfer and transport in the sea. Kullenberg, G., 1982; 2(1): 1–6. CRC Press, Inc., Boca Raton.
5. Garrity, G.M., Bell, J.A. and Lilburn, T. The revised road map to the manual. In: Brenner, D., Krieg, N.R., Staley, J.T. and Garrity G.M. (eds.). Bergey's Manual of Systematic Bacteriology, 2005; 2(2); 159–220. Springer, New York.
6. Jithendran, K.P., Poornima, M., Balsubramanian, C.P. and Kulasekarapandian, S. Diseases of mud crabs in India. Bull, 2010; 20: 28. Central Institute of Brackishwater and Aquaculture.
7. Jones, S. H. and Summer-Barason, B. Incidence and detection of pathogenic Vibrio sp. in a Northern New England estuary, USA. J.Shell Fish. Res, 1998; 17(5); 1665–1669.
8. Kim. J.H., Grant, S.B., McGee, C.D., Sanders, B.F. and Largier, J.L. Locating sources of surf one pollution: a mass budget analysis of fecal indicator bacteria at Huntington Beach, California. Environ. Sci. technol, 2004; 38: 2626–2636.
9. Krantz, G.E., Colwell R.R. and Lovelace, E. Vibrio parahaemolyticus from the blue crab Callineces sapidus in Chesapeake Bay. Science. 1969; 164: 1286–1287.
10. Lakshmi Pillai, S., Kizhakudan, S.J., Radhakrishnan E.V. and Thirumilu, P. Crustacean bycatch from trawl fishery along north Tamil Nadu coast. Indian J. Fish, 2014; 61(2): 7–13.
11. Lalitha, K.V. and Thampuran, N. Bacterial flora of farmed mud crab, Scylla serrate (Forskal, 1775) and farm environments in Kerala, India. Indian J. Fish, 2012; 59(2): 153–160.
12. Mahalaxmi, B., Revathy, K., Raghunathan, C., Anjalai, K. and Subashini, A. Distribution of microbial population associated with crabs from Ennore seacoast Bay of Bengal north east coast of India. Int. J. Curr. Microbiol. App. Sci. 2013; 2(5): 290–305.
13. Nair, S. and Simidu, U. Distribution and significance of heterotrophic marine bacteria with antibacterial activity. Appl. Environ. Microbiol, 1987; 53: 2957–2962.
14. Ramesh, R., Nammalwar, P. and Gowri, V. R. (2008). Database on coastal information of Tamil Nadu. Environmental Information System (ENVIS) Centre. Department of Environment, Government of Tamil Nadu.
15. Sivakumar, J., Nappinai, B., Jayanthi, J. and Ragunathan, M. G. Effect of temperature on the population of Emerita asiatica (H. Milne Edwards, in the Kovalam beach, East coast, Tamilnadu, India. Indian J. Pharm. Biol. Res, 1837; 2(2): 22–26.
16. Soundarapandian, P. and Sowmiya, S. Microbial contamination of commercially important crabs, Portunus pelagicus (Linnaeus) and P. sanguinolentus (Herbst), 2013; 2. doi:10.4172/scientificreports.593.
17. Wayne, L.G. and Kubica, G. P. Genus Mycobacterium Lehmann and Neumann (1896), 363AL. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt J.G. (eds.). Bergey's Manual of Systematic

Bacteriology, 1986; 2: 1436–1457. The Williams & Wilkins Co., Baltimore.