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Review Article

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Review on Pathogenic Vibrios from Marine Food Resources

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Introduction

Fish and seafood constitute an important food constituent for a large section of the world population. They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein (Wafaa *et al.*, 2011). The marine biota is presently choaking and drowing in its own feces. All aquatic systems have become universal recipients of the human waste that have erupted from modernization. The advent of blue revolution, in marine and estuarine realms as important potential area of commercially important finfish and shellfish are now facing the danger of extensive use of seashore areas as convenient means for the disposal of treated and untreated wastes in a most unscrupulous fashion (Banupriya *et al.*, 2014).

Sea foods are prone to bacterial contamination, especially filter feeders such as mussels and oysters, which concentrate these bacteria in their filtration systems and therefore, are ideally suited to trap all bacteria and viruses, pathogenic or otherwise that live in the water (Popovic *et al.*, 2010). They also occur in both marine and fresh water habitats and in associations with aquatic animals. Some species are pathogens of fish, eels and frogs as well as other vertebrates and invertebrates (Todar, 2005).

The potential of seafood to harbor microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Wright, 2004). Water-related diseases continue to be one of the major health problems globally. It is estimated that 80% of all illnesses are linked to use of food and water of poor microbiological quality. Treatment of farm produce such as seafood with chlorinated water reduces populations of pathogenic and other microorganisms on fresh produce but cannot eliminate them. Reduction of risk for human illness associated with raw produce can be better achieved through controlling points of potential contamination in the field, during harvesting, during processing or distribution, or in retail markets, food service facilities, or the home (FAO/WHO, 2007).

Seafood especially shellfish is a food substrate for some zoonotic Vibrios of which these microorganisms, cause food poisoning and diarrhea in human (Merwad *et al.*, 2011). Sea foods are prone to bacterial contamination and could cause health risk to consumers (Wafaa *et al.*, 2011). Vibrios are associated with live seafood as they form part of the indigenous microflora of the marine environment (Adeleye *et al.*, 2010). The aquatic ecosystem is the natural habitat of microorganisms belonging to the *Vibrio* genus and the most known is *Vibrio cholerae*, the causative agent of cholera. Otherwise, many species are able to transmit intestinal or extra intestinal diseases to human (Rodrigues *et al.,* 2001).

There have been several reports on the health risks associated with the consumption of processed seafood ranging from allergic reactions, stomach and intestinal cancerous growths and a general degeneration of cancerous growths and a general degeneration of peripheral cellular tissues, to gradual breakdown of the digestive and excretive systems in a statistically high percentage of people examined. Few of these reports however, have looked at the likely risks from a microbiological food safety point of view (Okonko *et al.*, 2009).

Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade (Wafaa *et al.*, 2011). *V. parahaemolyticus* and *V. vulnificus* are part of the natural flora of estuarine and coastal marine environments worldwide and have been isolated from sea and brackish water of both tropical and temperate regions, sediments and a variety of seafood especially shellfish and bivalve mollusks (Kirs *et al.*, 2010).

Vibrio infections are generally acquired either through ingestion of foods and water contaminated with human faeces or sewage, raw fish and seafood, or they are associated with the exposure of skin lesions, such as cuts, open wounds and abrasions, to aquatic environments and marine animals (Toti *et al.,* 1996; Lee and Younger, 2002). Food borne hazards are still of great concern for human health and in particular the risks connected with shellfish and seafood consumption continue to be important both in developing and developed countries despite the advances in technology, changes in food processing and packaging (Feldhusen, 2000; Huss *et al.,* 2000; Egli *et al.,* 2002).

Today the world is witnessing the resurgence in the consumption of shrimp. Shrimp farms have developed during the past twenty years (Pazir *et al*., 2011). Substantial evidence suggests that seafood is high on the list of foods associated with outbreaks of food borne diseases (Mousavi *et al*., 2011). It is worth noting that the microbial status of seafood after being caught is closely related to environmental conditions and microbiological quality of the water (Mousavi *et al.*, 2011; Khamesipour *et al.*, 2013).

During the last several decades, researchers have reported cases of food borne infections in humans caused by the consumption of contaminated fresh raw shellfish, shrimp and other sea foods. Occasionally, *Vibrio* species has been identified as the most significant cause of food borne hospitalizations, even it has been named as the cause of death for some people in some epidemics (Colakoglu *et al*., 2006; Raissy *et al*., 2011; Khamesipour *et al*., 2013).

The members of Vibrionaceae are the natural pathogens of the aquatic environment, which is also inhabited by shellfish, shrimp and other aquatic organisms (Colakoglu *et al*., 2006; Mahbub *et al*., 2011; Ebrahimzadeh Mousavi *et al*., 2011; Raissy *et al.,* 2011). Vibrios are among the most important bacterial pathogens of cultured shrimp responsible for a number of diseases, and mortalities up to 100% have been reported due to this disease. *Vibrio* species are a normal part of the bacterial flora in aquatic environments and formerly considered to be mostly opportunistic pathogens (Mahbub *et al.,* 2011; Raissy *et al.*, 2011).

Vibrio is a genus of bacteria indigenous to the aquatic environment also contaminant of raw or under cooked seafood. Some bacterial species of this genus are now considered as emerging pathogens, involved in food-borne infections in humans (Gopal *et al.,* 2005; China *et al.,* 2003). The genus *Vibrio* are Gram negative, curved, rod shaped, halophilic, non-spore forming bacteria, autochthonous inhabitants of the marine and estuarine environment. They occur in saline aquatic environments, both free in the water and bound to animate and inanimate surfaces (Huq *et al.,* 1983; Montanaria *et al.,* 1999).

The members of the family Vibrionaceae are a significant component of the microflora which includes more than 64 species (Thompson *et al.*, 2005). Few of these reports however, have looked at the likely risks from a microbiological food safety point of view (Okonko *et al.*, 2009). In the last 20 years, many halophilic *Vibrio* species such as *V.parahaemolyticus*, *V.alginolyticus*, *V.vulnificus*, *V. hollisae*, *V. fluvialis V.mimicus*, *V. furnissii* and *V. damsella*, have been implicated inhuman enteric infections, wound infections and septicemia due to the consumption of shellfish and exposure to seawater (Thompson *et al.,* 2004; Merward *et al.,* 2011).

The species are distributed worldwide in sea water and are associated with the resident aquatic organisms. Their presence is independent of anthropogenic pollution, but is dependent on salinity, temperature and organic matter (Hervio-Heath *et al.*, 2002). Their concentration in the aquatic environment and in foods of marine origin is a function of the geographic and hydrographic conditions in the area, and varies according to the time of year and location within the lagoon systems (Sousa *et al.*, 2004).

Bacterial diseases, mainly due to *Vibrio*, have been reported in Penaeid shrimp culture systems implicating at least 14 species and they are *V. harveyi, V.splendidus*, *V.parahaemolyticus*, *V.alginolyticus*, *V.mimicus*, *V.anguillarum*, *V. vulnificus*, *V.campbelli*, *V. fischeri*, *V. damsella*, *V.pelagicus*, *V. orientalis*, *V. ordalii*, *V. mediterrani* and *V. logei* (Raissy *et al.*, 2011; Raissy *et al.*, 2012). Of these, *V. harveyi* is the causative agent of luminous disease, which resulted in 80 to 100% mortality in *Penaeus monodon* hatcheries. *V. harveyi* is found in coastal and marine waters, in association with surface and gut of marine and estuarine organisms and also in shrimp pond water and sediment (Reboucas *et al.*, 2011; Raissy *et al.*, 2011). *V. harveyi* was also reported as the causative agent of vibriosis in tiger shrimp (*P. monodon*), kuruma shrimp (*Penaeus japanicus*) and pearl oyster (*Pinctada maxima*) (Raissy *et al.*, 2011; Caipang and Aguana, 2011). *V.anguillarum*, *V.campbelli*, *V. nereis*, *V. cholerae* and *V. splendidus* have also been reported in association with disease outbreaks in crustaceans (Ansari and Raissy 2010; Raissy *et al.*, 2011). *V. parahaemolyticus*, *V.vulnificus* and *V. alginolyticus* are halophilic organisms that resemble phenotypically, and they exist in warm seawater, river mouths and seafood (Jaksic *et al.*, 2002). Vibriosis has been the main cause of production loss due to bacterial disease in shrimp farms in south Iran in recent years (Hosseini *et al.*, 2004).

They account for a significant proportion of human infections such as gastroenteritis usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Adeleye *et al.*, 2010). Most of these vibrios secrete enterotoxins in foods, water or in the gastrointestinal tract (Nishibuchi and DePaola, 2005). Antibiotics and other chemotherapeutic agents are commonly used in fish farms either as feed additives or immersion baths to achieve either prophylaxis or therapy. It was observed that individual and multiple antibiotic resistances were associated with antimicrobial use.

Acquired antibiotic resistance in bacteria is generally mediated by extra-chromosomal plasmids and are transmitted to the next generation (vertical gene transfer) and also exchanged among different bacterial population (horizontal gene transfer). Extensive use of these antibiotics has resulted in an increase of drug-resistant bacteria as well as R-plasmids (Son *et al.*, 1997).

Marine *Vibrio* species are known to produce various extracellular products, some of which are known pathogenicity factors (Hasegawa *et al.*, 2008). These toxic proteins include cytolysins, proteases, lipases, siderophores, exopolysaccharides, and effectors delivered via Type III secretion systems (Hasegawa *et al.*, 2008). Secades and Guijarro (1999) reported that stress environmental conditions could play an important role in the induction or repression of the enzyme by specific compounds. Production of extracellular proteases has been shown to be sensitive to repression by different carbohydrate and nitrogen sources (Haulon *et al.*, 1982). Faced with starvation conditions in seawater, *V. alginolyticus* cells have been shown to undergo strong structural and metabolic modifications under laboratory conditions (Ben Kahla-Nakbi *et al.*, 2006).

The occurrence of Vibrio species in raw shellfish is common, especially shellfish from regions with temperate climates around the world from both natural and farm environments and all seafood types (Baffone *et al.*, 2000). The four species most frequently isolated in clinical microbiology laboratories are *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V.alginolyticus* (Fournier and Quilici, 2002).

V. parahaemolyticus has been frequently involved in outbreaks of food borne diseases worldwide (Crump *et al.*, 2003; Dalsgaard *et al.*, 1999). *V.parahaemolyticus* is often isolated from seawater, sediment and a variety of seafood including shrimp, crab, oyster and clam due to its halophilic characteristics (Merwad, *et al.*, 2011). This bacterium is one of the leading causes of food borne gastroenteritis associated with ingestion of undercooked shellfish throughout the world including the United States, China, Japan and Korea (Merwad *et al.*, 2011). Also, this microbial infection is characterized by diarrhea, vomiting, nausea, abdominal cramps and low grade fever (Pinto *et al.*, 2008).

V. vulnifus is another organism of great concern in seafood safety due to the severity of the disease and the high mortality rate it can cause (Chun *et al.*, 2009). In addition *V. vulnificus* is a potentially lethal food borne pathogen and capable of causing primary septicemia and necrotizing wound infections in susceptible individuals (Harwood *et al.*, 2004; Merwad *et al.*, 2011). Other species that have been increasingly recognized as food pathogens in recent years are *V. mimicus* and *V. alginolyticus. V. mimicus* has genetic and many biochemical similarities to *V. cholerae* and its pathogenicity involves several toxins including that of *V. cholerae*. Many food-borne outbreak cases involving *V. mimicus* have been reported (Hlady and Klontz, 1996).

V. alginolyticus is a halophilic *Vibrio* first recognized as being pathogenic in humans in 1973 (Zen-Yoji *et al.*, 1973). Wound infections account for 71% of *V. alginolyticus* infections. Ear infections are also seen with this organism. Gastroenteritis was thought to be a rare presentation of *V.alginolyticus* infection, but it accounted for 12% of infections in one series (Hlady and Klontz, 1996). Other clinical syndromes reported in association with *V. alginolyticus* infection include chronic diarrhea in a patient with AIDS (Caccemese and Rastegar, 1999), conjunctivitis (Lessner *et al.*, 1985) and post-traumatic intracranial Infection (Opal and Saxon, 1986). Resistance to tetracycline and chloramphenicol has been reported in a few isolates of *V.alginolyticus*, but all strains appear to be sensitive to ciprofloxacin (French, 1990).

V. alginolyticus is considered one of the most frequent species living freely in water and sediment and can survive in sea water even in famine conditions while maintaining their virulence (Kahla-Nakbia *et al.*, 2007). The first reports identifying *V. alginolyticus* as possessing the *trh* gene occurred in Alaska and in Tunisia. In addition, it has been shown that strains of *V.alginolyticus* carry the *trh* gene and the pathogenic *V.alginolyticus* strains is recognised as a potential reservoir of many known virulence genes of other *Vibrio* species in the aquatic environment which have been demonstrated to contributes to the onset of wound infections, enteric pathologies, septicaemia and peritonitis in humans by exposure to seawater (Masini *et al.*, 2007).

V.alginolyticus is a Gram negative, asporogenous rod that is either straight or has a single rigid curve. Cells are motile, mostly having singular polar flagellum when grown in liquid medium. Most members of the genus *Vibrio* produce oxidase and catalase enzymes and ferment glucose without producing gas, besides being pathogenic (Kaysner and DePaola, 2001). *V.alginolyticus* is an opportunistic pathogen to human and marine animals (Zhao *et al.*, 2010; Reilly *et al.*, 2011). The outbreaks of *V. alginolyticus* infections increase rapidly during summer seasons (Sganga *et al.*, 2009). Most of these infections stemmed from contact of cuts to contaminated seawater (Reilly *et al.*, 2011). Infection by *V. alginolyticus* causes otitis, endophthalmitis and wound infections (Austin, 2010; Reilly *et al.*, 2011).

V. alginolyticus is considered to be a part of normal marine flora (Austin *et al.*, 1995; Vandenberghe *et al.*, 1998). However, some studies have shown its virulence to aquatic animals (Lighter, 1993). Despite the boom of fish farming in Guangdong, China, the whole industry was badly hampered by the fish mortality because of vibrosis, where *V. alginolyticus* is the dominant causative species. 16S genes and part of the toxR sequence of *V.alginolyticus* and *V. parahaemolyticus* are highly homogeneous (Osorio and Klose, 2000). Previous studies have shown that *V. parahaemolyticus* is closely related to either tdh or trh or both genes (Iida *et al.,* 1998), but no results have been reported yet clarifying which virulence genes of *V.alginolyticus* are critical for its pathogenicity. Only a few studies were conducted to search *V. cholerae* virulence genes in *V. alginolyticus* in Sardinia, Italy (Sechi *et al.,* 2000), but none in Guangdong. No information is available on the presence of homologous *V. parahaemolyticus* virulence genes in *V. alginolyticus*. Therefore, it is necessary to investigate whether *V.parahaemolyticus* and *V.cholerae* virulence genes present in *V.alginolyticus* in Guangdong. Additionally, we have to assess whether *V.alginolyticus* pathogenic strains have arisen, and if there is a correlation between the virulence and possession of *V. parahaemolyticus* and/ or *V.cholera* virulence genes in *V. alginolyticus*.

V. alginolyticus, a ubiquitous organism in seawater, has been isolated from different marine organisms as part of the saprophytic microbiota (Carli *et al.*, 1993). However, it has also been suggested that this species is a pathogen of several marine animals and humans (Rikelme *et al.*, 1996). There is controversy about the precise role of *V. alginolyticus* as a fish pathogen. This species has been reported to be the causal agent of outbreaks of vibriosis in grouper and sea bream and also has been associated with other *Vibrio* species in high-mortality outbreaks related to abdominal swelling in larvae of several fish species (Colorni *et al.*, 1981; Sedano *et al.*, 1996).

As a representative of the halophilic vibrios, *V. alginolyticus* is isolated from coastal waters and sediments all over the world (Uchiyama, 2000; Hsieh *et al.*, 2007) and is considered to be part of the normal marine microflora. However, *V. alginolyticus* is an important bacterial pathogen of humans, causing wound infections, otitis media, otitis externa, endophthalmitis and gastrointestinal infection (Reina *et al.*, 1995; Li *et al.*, 2010). This bacterium also belongs to the most important opportunistic pathogens of aquatic animals, including fish, shellfish, crustaceans, coral and echinoids, causing serious disease and damage in cultured fish and important economic losses (Liu *et al.*, 2004). Several virulence factors, including the iron uptake system (Litwin and Calderwood, 1993), extracellular haemolysin (Lee *et al.*, 1996) and proteases are suggested as the major contributors to pathogenicity in this species.

V. alginolyticus is so named because of taxonomic rules. A clear difference between the two biochemical species is found in the ability of sucrose fermentation which is negative for *V. parahaemolyticus* while it is positive for *V. alginolyticus* (Shinoda, 2011). *V. alginolyticus* has a large geographic distribution in marine and estuarine waters especially in bathing areas (Barbieri *et al.*, 1999; Baffone *et al.*, 2000). It is normal host mussels and was also isolated fish and seafood variety. Indeed, the vibrios support high concentrations of salt and water to survive in different environments (sea and brackish water). Resistance to sea salt which is explained by their character is more pronounced halophilic *V. alginolyticus* than *V. choleae* with which it shares many characters (Denis *et al.*, 2007). Several studies have shown a wide distribution of genes in *V. cholerae* and *V. parahaemolyticus* between strains of *V. alginolyticus* into the environment (Snoussi *et al.*, 2008). Studies have shown that *V. alginolyticus* is considered the most frequent species living freely in water and sediments (Harriague *et al.*, 2008) and can survive in sea water even under conditions of nutrient stress while maintaining their virulence (Kahla-Nakbi *et al.*, 2007).

V. alginolyticus associated with several diseases of marine animals including fish, crustaceans and molluscs (Balebona *et al.*, 1998; Gay, 2004; Gómez-León *et al.*, 2005; Kahla-Nakbi *et al.*, 2006). This reflects both the increasing pressure of human-induced coastal areas and the genetic and phenotypic plasticity of *Vibrio* quickly adapt to changing environmental conditions (Saux *et al.*, 2002). The mode of infection and transmission of this species remains to be studied, a transmission paths is probably seawater (Kahla-Nakbi *et al.*, 2007). Studies have considered strains of *V. alginolyticus* as a potential reservoir of many virulence genes known in other *Vibrio* species in the aquatic environment (Masini *et al.*, 2007; Lafisca *et al.*, 2008). The first reports showed that identification of *V. alginolyticus* has the trh gene occurred in Alaska (Escalona *et al.*, 2006) and Tunisia (Kahla-Nakbi *et al.*, 2006). Other studies have highlighted the virulence of *V. alginolyticus* in China (Xie *et al.*, 2005), confirming reports from other countries in Europe and America (Barbieri *et al.*, 1999; Matte *et al.*, 1994; Robert-Pillot *et al.*, 2002; Hervio-Heath *et al.*, 2002). In addition, it has been shown that in Morocco, strains of *V. alginolyticus* carry the trh gene is a virulence gene associated with a positive test Kanagawa phenomenon (Sabir Mustapha *et al.,* 2012).

Indeed, in humans, *V. alginolyticus* can be isolated from skin infections, often as a result of contact with sea water (Scheftel *et al.,* 2006). Previous work has shown the potential risks to health associated with the use of non-sterile products such as alginate gels derived from seaweed prepared at home for cleaning wounds that could incriminate *V. alginolyticus* infections as a cause serious wounds especially among vulnerable groups such as older people and those with conditions underlying risk (Reilly *et al.*, 2011). Similarly, *V. alginolyticus* was isolated from the pus of ear and spitting, while being responsible for conjunctivitis infection and tissue necrosis and opacification of the sphenoid sinus (Lopes *et al.*, 1993).

In addition, *V.alginolyticus* has been shown responsible for gastroenteritis (Darbas *et al.*, 1992; Reina *et al.*, 1995) and peritonitis (Rod *et al.*, 1981) in humans. Studies have report infection with *V. alginolyticus* have caused mortality in immunecompromised patients (Campanelli *et al.*, 2008). The first case of septic shock due to *V. alginolyticus* in a cirrhotic patient has been reported in Korea after eating seafood (Dong-Young *et al.*, 2008).

These bacteria have been found in water samples from marine environment, mussels, fish and their products. Since the end of the 80s, the presence of these microorganisms was correlated to human infections. A large number of *Vibrio* species are pathogenic to humans, marine vertebrates and invertebrates, by producing and secreting various virulence factors such as enterotoxin, haemolysin, cytotoxin, protease, lipase, phospholipase, siderophore, adhesive onto surfaces and haemagglutinins (Austin and Austin, 1999; Shinoda, 1999). The most common type of virulence factors among *Vibrio* species is a haemolysin, an enterotoxin (Lida and Honda, 1997; Shinoda, 1999) which strikes the erythrocyte membranes causing them to lyse and leading to emancipation of iron-binding proteins (haemoglobin) as well as lysing of the blood cells (Lida and Honda, 1997; Shinoda, 1999). The infection sites (the medium ear, conjunctive membrane, open wounds) were hosting a complex microbial flora. Infections caused by *V. alginolyticus* can present themselves as septicemic related to the ingestion of contaminated seawater or seafood products especially in patients as elders, children, carriers of diabetes mellitus or chronic hepatic pathologies (Carli *et al.*, 1993).

Bacterial resistance to antibiotics has become an emerging medical issue threatening the public health because of wide availability of antibiotics and sometimes misuse of drugs without proper prescription (Davies and Amabiles-Cuevas, 2003). More and more pathogenic bacteria have shown resistance to one or many of the antibiotics. It has been observed that antibiotic susceptibility of *Vibrio* species is dynamic and varies with the environment (Ottaviani *et al.*, 2001; Jun *et al.*, 2003). According to Nambabi *et al.* (2010), the ability of *V. alginolyticus* to survive and grow in treated and disinfected seawater, preceded to successive contamination of hake fish, henceforth, be symbolic of related *V. parahaemolyticus*, should this pathogen be present. It is therefore, significant that this center of contamination be controlled to diminish the potential health hazard to consumers.

Vibrios come on the top list of pathogens with direct jeopardy to mariculture development due to high mortalities associated with their invasion to fishes. It is crucial to know that, Vibrios are ubiquitous to marine environment, while clinical disease outbreaks only occur when a sharply stressed fish get exposed to the flaring up infectious agent (Austin and Austin, 2012). Septicemia induced by Vibriosis is characterized by haemorrhages on the base of pectoral fins, exophthalmia, loss of appetite and edematous lesions on the body surface (Toranzo *et al.*, 2005).

V.alginolyticus and *V.parahemolyticus* are responsible for mass mortalities among fish stocks in many marine fish farms throughout the Mediterranean area and severe economic losses in aquaculture worldwide (Snoussi *et al.*, 2008). *V.alginolyticus* causes many epizootic outbreaks among the Gilthead seabream and European sea bass populations, which possess high economic value at the Mediterranean communities (Zorrilla *et al.*, 2003).

In recent year, vibriosis has become one of the most important bacterial diseases in maricultured organisms, affecting a large number of fish and shellfish (Woo and Kelly, 1995; Wu and Pan, 1997). Marine fish's contamination with *Vibrios* results in serious consequences relating to national productivity and development.

Antibiotics and other chemotherapeutic agents are commonly used in fish farms either as feed additives or immersion baths to achieve either prophylaxis or therapy. It was observed that individual and multiple antibiotic resistances were associated with antimicrobial use. Acquired antibiotic resistance in bacteria is generally mediated by extra chromosomal plasmids and are transmitted to the next generation (vertical gene transfer) and also exchanged among different bacterial population (horizontal gene transfer). Extensive use of these antibiotics has resulted in an increase of drug-resistant bacteria as well as R-plasmids (Son *et al.*, 1997).

Public Health Laboratories experience challenges during identification of *Vibrio* species. Conventional phenotypic methods employed during the identification of *Vibrio* species, are found to be time consuming and not so accurate. It is recommended that additional methods, for the identification of *Vibrio* species, including molecular techniques are necessary (Kwok *et al.*, 2002).

Molecular approaches that interrogate the whole genome appears to be a way forward to highlight what may be only minimal differences between strains. PCR method has been widely used in the development of molecular diagnostic techniques for bacteria, because it allows a comparative analysis of genomes between different isolates of the same species by employing distinct molecular markers (Sudeesh *et al.*, 2002).

Based on a mini review by Janda and Abbott (2007), the 16S ribosomal RNA (rRNA) is a highly conserved gene in all prokaryotic organisms. It has been used in phylogenetic and taxonomic studies for several reasons; it exist in every bacterial genome, its function does not change over time that made it a good indicator for evolution and it is long enough (1500 bp) for informatics or genetic determinations.

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