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Halotolerance of Heterotrophic Bacteria Isolated from Tropical Coastal Waters

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Abstract

To initiate an understanding of the halotolerance of indigenous coastal water bacteria, the salt tolerance of a total of one hundred and seventy (170) surface water heterotrophic bacteria isolated from three (Badagry Creek, Ologe Lagoon and Lagos Lagoon) coastal waters in Lagos, Nigeria was evaluated. All isolates proliferated at NaCl concentrations of 0 to 4%, however, as the concentration of salt increased, the percentage of organisms able to grow decreased. At 15% salt concentration, only 10.7, 16.7 and 6.1% of the isolates from Badagry Creek, Ologe Lagoon and Lagos Lagoon respectively grew. Majority (51%) of the isolates grew at salt concentrations of 0 to 8% and were classified as moderately halotolerant, 35% were highly halotolerant growing beyond 8% (10 to 15%) salt concentrations, while 14% which grew between 0 and 4% were grouped as slightly halotolerant. The highest number of highly halotolerant bacteria was isolated from surface water with salinity 0.01 to 0.1‰, meanwhile, none of the organisms isolated at the highest salinity range (30 to 33‰) grew at 15% salt concentration. The widespread of halotolerant bacteria in these water bodies is of great significance for future research and biotechnological development.

Keywords: salt tolerance; salinity; Lagos Lagoon; Badagry Creek; Ologe Lagoon

1. Introduction

All forms of life require salt for growth and metabolism, and life is known to exist in the diverse salt concentrations encountered in all natural habitats, from fresh water environment to hyper-saline lakes [1].

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Microorganisms including bacteria are among the forms of life able to grow at these different salt concentrations. The tolerance of NaCl exhibited by microbes surpasses that of any other organism, prokaryotes and eukaryotes. They may be classified according to their salt requirements: non-halophiles grow optimally at NaCl concentration of between 0 - 2%, slight halophiles grow at 2-3% NaCl; moderate halophiles grow at 5 - 10% NaCl; and extremely halophilic organisms grow at salt concentrations greater than 10%. On the other hand, halotolerant organisms are capable of growing in the absence as well as in the presence of salt [2, 3, 4, 1, 5,6].

The ability of halotolerant microorganisms to grow in a very wide salt concentration has recently increased attention to their prospective potentials. These organisms are of enormous significant in nutrient cycling of environments with fluctuating salinity. They also play an essential role in the production of fermented food and food supplements; the degradation or transformation of organic pollutant and the production of alternative energy [7, 2, 8]. A good number of halotolerant bacteria have been observed these belong to several genera such as *Pseudomonas, Flavobacterium, Virgibacillus, Staphylococcus, Oceanobacillus, Acinetobacter, Vibrio, Micrococcus, Alteromonas, Bacillus and Escherichia coli* [9, 10, 6, 12, 8].

The salinity of coastal and estuarine systems varies considerably as a result of run-off, rain and in flow of fresh water from rivers. And salinity strongly affects aquatic organisms; some bacteria exhibit certain morphological changes when salinity rises above 5%, and further increases hinder reproduction [13]. In addition, microbial diversity is known to decrease with increased salinity [14]. Therefore, microorganisms inhabiting these environments develop osmoadaptive strategies to cope with such fluctuations in salinity [15].

The extent of diversity of halophilic and halotolerant microorganisms in nature is yet to be known [1]. Nevertheless, our understanding of the adaptability of the surface water heterotrophic bacteria to salinity is limited. In addition, the variability of the salinity observed in Nigerian coastal waters suggests the existence of interesting salt tolerant organisms which can be of great biotechnological importance. Hence, this study was carried out to elucidate the salt tolerance of the culturable aerobic heterotrophic bacteria of three coastal waters in Lagos State, Nigeria as an initial step towards future research.

2. Materials and Methods

2.1 Sample collection

Surface water samples were obtained at different sampling events between May 2012 to July 2013 at Badagry Creek, Ologe Lagoon and Lagos Lagoon (Figure 1). About 1000 ml of surface water were collected in clean sterile bottles. The containers were kept in ice-chest box, transported to the laboratory and processed within 24 hrs. The salinity of surface water was determined immediately in the Laboratory using the Horiba U-10 multiple water checker.

2.2 Isolation and salt tolerance of culturable heterotrophic bacteria

The different culturable bacteria were isolated using the traditional spread plate method on standard plate count agar after initial serial dilution of the water samples. After incubation for 24 hrs at 28 ± 2^{o}C, single colonies were selected randomly selected and further purified on nutrient agar and stored on nutrient slants for subsequent analysis.

Fig. 1. Map of sampling sites

Pure cultures of a total of one hundred and seventy (170) isolates were grown in tryptose soya broth and incubated at 28 ± 2^{o}C for 24 hrs. The cells were harvested by centrifugation at 4000 rpm for 7 minutes, washed twice with phosphate buffer and re-suspended in the same phosphate buffer solution. The optical density of the bacterial suspensions was determined by measuring the absorbance of the suspension samples at 600nm and relating the value to a calibration curve (10^{10}cfu ml^{-1} = 1 OD unit). About 0.1 ml of the bacterial suspension was used to inoculate tryptose soya broth containing the various concentrations (0, 2, 4, 6, 8, 10, 12 and 15). All experiments were carried out in duplicate, with un-inoculated tubes serving as control experiments. After incubation at28 ±2^{o}C for 24 hrs, growth of isolates was monitored by visual turbidity.

2.3 Identification of bacterial isolates

Pure cultures of randomly selected bacterial isolates were identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the method of Cowan and Steel [16].

3.0 Results

3.1 Halotolerance of heterotrophic bacteria

As depicted in Table 1, all the bacteria isolated in this study from the three different water bodies grew on media with 0 to 4% salt concentration. Generally, the number of isolates capable of growing at the various NaCl concentrations decreased as the concentration of the salt increased. At Badagry Creek, 89.3 and 78.6% of the isolates grew at 6 and 8% salt concentration respectively. A drastic reduction to 35.7% was noted at 10% salt concentration, while 12 and 15% salt concentration had a minimal 14.7 and 10.7% of the isolates growing. Likewise, at Ologe Lagoon, 93.3% of the isolates were able to grow at 6% salt concentration. The number steadily decreased from 66.7 to 16.7% as the salt concentration was increased from 8 to 12%. The same pattern of response to increase in salt concentration was encountered at Lagos Lagoon. 79.6, 55.1, 32.7 and 22.5% of the isolates grew at the salt concentration was increased from 6, 8, 10 and 12% respectively. The least percentage (6.1%) of isolates was noted with further increase of the salt concentration to 15%.

Table 1 Percentage of isolates with the ability to grow at the different salt concentrations

NaCl concentration (%)	Badagry Creek	Ologe Lagoon	Lagos Lagoon
0 - 4	100	100	100
6	89.3	93.3	79.6
8	78.6	66.7	55.1
10	35.7	36.7	32.7
12	14.3	30	22.5
15	10.7	16.7	6.1

3.2 Halotolerance classification

The data in Figure 2 represents the classification of all the halotolerant isolates from this study based on the concentration of salt they can grow on. A small number (14%) of these surface water isolates were designated slightly halotolerant and could only tolerate salt concentrations from 0 to 4%. Majority of the isolates (51%) were moderately halotolerant growing in salt concentrations of up to 10%. Moreover, a good number (35%) of them were able to proliferate beyond 10% salt concentration and so were classified as highly halotolerant.

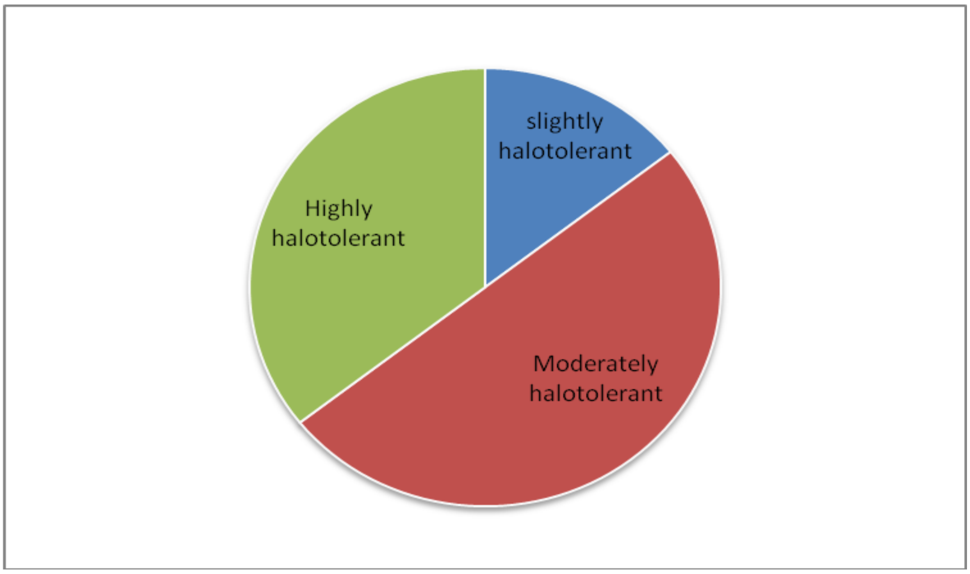

Fig. 2. Salt tolerance classification

3.3 Influence of surface water salinity on salt tolerance

Analyzing the salt tolerance of the heterotrophic bacteria based on the salinity of the surface as of the time of isolation, it was observed that all organisms isolated at 0‰ salinity, grew at 0 to 4% salt concentration (Table 2). A decrease in the percentage of tolerant isolates was noted as the salt concentration increased, however 26.67 and 13.3% of these organisms still tolerated 12 and 15% salt concentrations respectively. Remarkably, all the organisms (100%) isolated within salinity of 9 to 13.8‰ tolerated up to 8% salt concentration. Besides, 91.7 and 83.3% was recorded for 10 and 12 % salt concentration respectively. This particular group also had a reasonable (25%) percentage of tolerant isolates for 15% salt concentration. The isolates under salinity values of 0.01 to 0.1‰ and 6 to 7.9 ‰ also showed a widespread tolerance with each group showing 30 and 18.2% tolerant isolates at the highest salt concentration (15%) tested. On the contrary, none of the isolates within 0.2 to 0.36‰ salinity grew at elevated salt concentrations of 12 and 15%; nevertheless, a few grew at 10% salt concentration. Furthermore, at 30 -33‰ salinity, all the isolates were unable to grow at 15% salt concentration, but 4.2, 8.3 and 45.8% of the isolates tolerated 12, 10 and 8% salt concentrations respectively.

Table 2 Halotolerance of bacteria isolated at different surface water salinity values

	Salt Concentrations (%)					
Salinity(‰)	**4**	**6**	**8**	**10**	**12**	**15**
0	100	86.67	66.67	33.3	26.67	13.3
0.01 - 0.1	100	100	90	60	50	30
0.2 -0.26	100	100	70	10	0	0
0.34 - 0.36	100	100	75	10	0	0
6 - 7.9	100	72.7	72.7	54.55	27.27	18.2
9 - 13.8	100	100	100	91.7	83.3	25
18 - 25	100	64.3	42.9	35.7	14.3	7.14
30 -33	100	83.3	45.8	8.3	4.2	0

3.4 Bacterial identification

The presumptive identification of the heterotrophic bacteria based on their cultural/morphological characteristics, Gram's reaction and various biochemical tests, showed that they belonged to the following genera *Zymonomas, Micrococcus, Pseudomonas, Aeromonas, corynebacterium, Acinetobacter, Alcaligenes, Chryseomonas, Bacillus, Serratia, Vibrio* including *Escherichia coli.*

4. Discussions

The three coastal waters in this study experience varying salinity which ranges from 0.1 to 0.2‰ at Ologe Lagoon, 0 to 9‰ at Badagry Creek and 6 to 33 at Lagos Lagoon. According to Mc Arthur [12], bacteria have successfully survived in varying environmental conditions; these include a wide range of salinities. Also, microorganisms inhabiting environments with fluctuating salinity have a wider range of tolerance, since they have the selective advantage for growth and metabolism within that salinity range. This therefore could be attributed to the remarkable tolerance to a wide range (0 to 15%) of salt concentrations exhibited by the culturable heterotrophic bacteria from the surface water of these coastal waters (Table 1). Ologe Lagoon had earlier been described as a fresh water body by Ndimele and Kumolu-Johnson [17] and in the present study, it had the least salinity values, yet, about 16.7% of the organisms from this water body tolerated 15% salt concentration. Conversely, a meager 6.1% of the bacteria isolates from Lagos Lagoon which had salinity values ranging from 6 to 33‰ could grow at 15% salt concentration. This suggests that the salinity of an environment might not necessarily determine the salt tolerance of the indigenous bacteria. Although aquatic organisms are affected by the salinity of their environment, tolerance to salinity is to some extent due to the physiological mechanisms and morphological adaptations. In addition, other nutritional and environmental factors which affect the duration of exposure and rate of increase in salinity concentrations may also play a part to salt tolerance. Indeed, salinity tolerance in bacteria is highly influenced by temperature, usually; bacteria exhibit their highest range of salt tolerance at optimum growth temperature. Thus salt tolerance can vary between species, populations and even between individuals of the same population over time [4, 18 and 13].

Contrary to report of Oven [1] in which he noted that halophiles exist among aerobic heterotrophs, none of the surface water heterotrophs from this work was halophilic, since all isolates grew on media without salt but tolerated varying concentrations of salt. A good proportion (51%) of these isolates was moderately halotolerant proliferating at salt concentration of up to 8%. Meanwhile, about 35% tolerated 10 to 15% salt concentration. In addition to ability of halotolerant microbes to grow at high salt concentrations they are easy to grow with simple nutritional requirements; a greater part of them can use a variety of compounds as sole carbon and energy source. Their genetic manipulation seems simple since many of the genetic tools developed for non-halophilic bacteria can be applied to them. Therefore, they proffer potential applications in various fields of biotechnology. These include production of fermented food and food supplements, degradation or transformation of organic pollutants and production of alternative energy [7, 3 and 19]. Furthermore, Solomon and Viswalingam [8] had stated that halotolerant and moderately halophilic bacteria have contributed significantly to the knowledge of phase behavior of lipids, physiological changes during adaptation to different NaCl concentrations and functions of compatible solutes in protecting cells from salts. In other words, the ubiquity of these halotolerant bacteria in our coastal waters might be of great importance for future research and industrial purposes.

In the present study, halotolerance exhibited by the heterotrophic bacteria from a variety of salinity indicates their ability to adapt to fluctuating salinity values. A few highly halotolerant bacteria were among the organisms isolated at zero salinity (Table 2). Vreeland and Hochstein [20] stated that microorganisms inhabiting non-hypersaline environments can tolerate relatively high salt concentration, although with a reduction of their growth rates. High salinity can be deleterious to microbial cells since water is lost to the external medium until osmotic equilibrium is achieved. To prevent loss of cellular water, microbial cells maintain an intracellular osmotic pressure somewhat greater than that of the growth medium in order to generate cell turgor pressure. Therefore, the ability to adapt to fluctuations in salinity is essential for growth and survival, and microorganisms have developed a number of osmo-adaptive strategies to cope with such fluctuations. Generally, halotolerant organisms accumulate high solute concentrations within the cytoplasm [15, 4, and 11]. The bacteria identified in this study were similar to the halotolerant genera earlier reported [21, and 22].

5. Conclusion

Although Badagry Creek, Ologe Lagoon and Lagos Lagoon experience fluctuations in their salinity, their indigenous heterotrophic bacteria demonstrated outstanding tolerance to salt concentrations. This therefore confirms the ubiquity of halotolerant bacteria in non-saline and moderately-saline aquatic environment. These organisms will most probably possess unique salt tolerant adaptive features which will be great potential tools for biotechnological applications of halotolerant bacteria. Future studies will explore further understanding of these potentials for research and industrial use. Also an establishment of the influence of other physico-chemical parameters on salt tolerance will be studied.

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