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Research Paper

Microbial Load of Lake Van under pH and Salt Stress

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Abstract

Alkaline environments are rare habitats on Earth that result from a combination of geologic, geographic, and climatic conditions. Therefore, their way of life is often unique. This study investigated the effects of different growth conditions on the cultivable sediment microorganisms of Lake Van. Aerobic, alkaliphilic microorganisms were cultivated from sediment samples collected in the autumn season, November 2018 from alkaline Lake Van, Turkey, with a pH 9.7. The total number of microorganisms in the sediment samples was found to be 10^5 cfu g-1. Different pH and salinity conditions were designed to examine the effects of stress. As a result, it has been shown that the number of microorganisms decreases in the presence of high salt concentrations and high or neutral pH values.

Keywords: Microbial ecology of lakes, soda lakes, lake Van, ph stress, salt stress

1. Introduction

Soda lakes are special salt lakes in which sodium carbonate/bicarbonate ions are concentrated as salt. Salinity rates vary according to the location of the lakes [1, 2]. Lake Van is the world's largest soda lake [3]. The water of Lake Van is quite alkaline (9.7 pH), and its salinity is as low as 2.17%, unlike other soda lakes. This is mostly due to calcium, sodium, chlorine, and carbonate ions and a small amount of sulfate, potassium, and magnesium ions [4, 5]. As a result, Lake Van has a special place among other soda lakes with its geological location and unique water chemistry.

Alkaliphiles are organisms that show optimum growth at high pH values (usually >9 pH values). This group includes members of the three domains Bacteria, Archaea, and Eukaryota. Soda lakes are rare natural environments that provide suitable conditions for obligate alkaliphiles and alkali-tolerant microorganisms [1, 2]. Microbial communities play an important role in alkaline-saline ecosystems, especially in organic matter cycles [6]. Therefore, these environments are ecosystems rich in microbial diversity [7, 8]. Lake Van not only has the largest known microbial diversity in the world [4, 5] but is also an important ecosystem for the Van fish, an endemic species of pearl mullet.

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Microbial communities form the basis of food web dynamics. Therefore, it is important to determine the population density changes of microbial communities, under different stress conditions. In the present study, environmental stress factors such as pH and salinity were applied to determine the quantitative response of aerobic, cultivable microbial populations in Lake Van.

2. Materials and Methods

2.1. Sampling

Sediment samples were collected from the shore of Lake Van (38°55'30.9"N 43°37'53.4"E) in November 2018. All samples were immediately placed on ice and stored at 4 °C until analysis.

2.2. Cultivation and Enumeration

Determination of total viable count of microorganisms was carried out using nutrient agar (NA) media of various pH (7, 9, 10, 11, and 12) by the spread plate technique. The pH of the medium was adjusted using 1N NaOH solution. The effect of different salt concentrations (0, 5, 10, 15, 18 and 20%) was also studied in the medium by addition of NaCl at constant pH (9). Sediment samples were diluted with phosphate buffer saline (PBS) and 0.1 mL aliquots were spread on agar plates in triplicate. Cultures were incubated at 28 °C for 7 days. Colony forming units (cfu) were counted as cfu g-.

2.3. Statistical Analysis

All statistics were conducted in GraphPad Prism (Version 6). Tukey's multiple comparisons test was performed on sediment sample CFUs at a 95% confidence interval to determine which pH and salt values differed significantly from each other and the control group.

3. Results and Discussion

The aerobic total number of microorganisms from the sediment samples was found to be 105 cfu g-1. Regarding microbial population density in soda lakes, our result was similar to previous studies [1, 6]. This result was obtained using NA media with a pH of 9 and no added salt. This group was considered as the control group for the salt and pH stress experiments to evaluate how the increased NaCl concentration and pH changes affect the cultivable lake sediment community (Figures 1 & 2).

To determine the effect of salt, the sediment bacteria were inoculated into NA containing 0, 5, 10, 15, 18, and 20% NaCl. The data presented in Figure 1 depict the numbers of bacteria presented as cfu g- after 7 days of incubation at 28 °C. There were significant decreases in all salt concentrations compared to the control group. These reductions were small ($p<0.05$) only at of 5% NaCl, but at the other concentrations (10, 15, 18, and 20% NaCl) it was quite large ($p<0.0001$) compared to the control group. At the same time, the numbers in the presence of 5% NaCl were significantly higher than in all other NaCl groups. These differences were $p<0.05$ between the 5% NaCl and the 10% NaCl groups while they were $p<0.01$ between the 5% NaCl and the other groups (15, 18, and 20% NaCl). The other groups (10, 15, 18, and 20% NaCl) were statistically similar to each other. Halophiles have members in all domains: Bacteria, Archaea, and Eukaryota [9]. They have two strategies to live in saline environments: the uptake of chloride ions and the pumping of neutrally charged ions from the cell [10, 11]. As a result of this study, Lake Van harbors different ranked halophiles such as slight (<10% NaCl) and moderate (<30% NaCl) groups [9, 12]. In addition, since values higher than 20% NaCl were not studied in this study, there is a potential for extreme groups (>30% NaCl) to be found [9, 12]. However, as shown in Figure 1 (control group and 5% NaCl group), slight halophiles or facultative ones are more common in Lake Van than other halophilic groups.

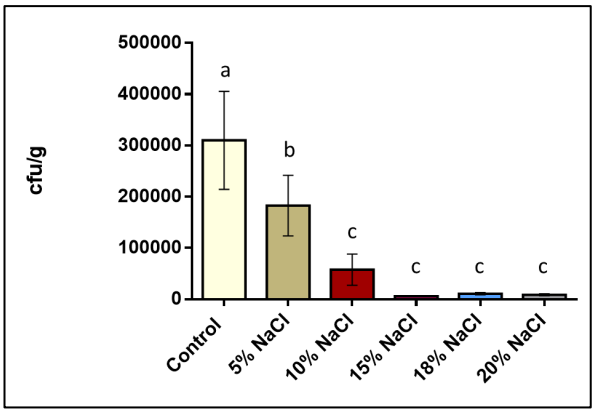

Figure 1. Salt tolerance of cultivable sediment microorganisms of Lake Van (different letters in superscripts indicate a statistical difference ($p<0.05$)).

Inoculations were made in NA at different pHs (7, 9, 10, 11, and 12) to determine the pH tolerance of the sediment microorganisms of Lake Van. The results are shown in Figure 2 after 7 days of incubation at 28 °C. There was a significant ($p<0.001$) decrease with respect to the control group (pH 9), especially the very alkaline pHs (11 and 12). However, while there was no significant difference between pH 9 and pH 7, pH 9 and pH 10, the differences between pH 7 and pH 11 ($p<0.05$) and pH 7 and pH 12 ($p<0.01$) were statistically significant. Similar situations were also shown for pH 10. The decreases between pH 10 and pH 11 and 12 ($p<0.01$) were closer between pH 9 and them. Additionally, there was no statistically significant difference between pH 11 and pH 12. In most studies, it has been noted that isolates from extremely alkaline environments (higher than 11) were only able to grow at more moderate pH values [7, 13, 14], in this study growth occurred even at pH 11 and 12, although the pH of Lake Van is not very high (9.7).

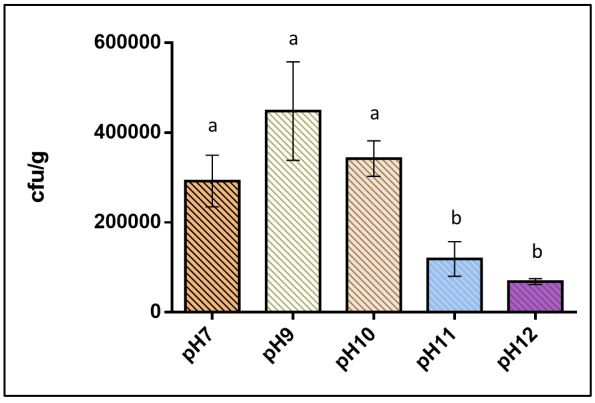

Figure 2. pH tolerance of cultivable sediment microorganisms of Lake Van (different letters in superscripts indicate a statistical difference ($p<0.05$)).

4. Conclusion

This study showed that growth conditions affect the cultivable microbial load of Lake Van differently. The greatest microbial CFUs were obtained at pH 9 and no salt addition media. The CFUs of pH 10 and 7 also gave statistically similar results to pH 9. However, growth occurred at high salt concentrations and pH values. Therefore, we can say that Lake Van harbors true halophiles and alkaliphiles. Alkaliphilic enzymes are commonly used in the detergent industry, so tolerance to high alkaline pH and salt is important for biotechnological applications. Therefore, further studies are needed for more accurate determinations of the pH and salt tolerance of microorganisms from Lake Van.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] W. D. Grant, B. E. Jones, W. E. Mwatha, "Alkaliphiles: ecology, diversity and applications", *FEMS Microbiol. Rev*., vol. 75, pp. 255–270, 1990.

[2] D. Y. Sorokin, J. G. Kuenen, G. Muyzer, "The Microbial Sulfur Cycle At Extremely Haloalkaline Conditions Of Soda Lakes", *Frontiers in Microbiology*, vol. 2, pp.1-16, 2011.

[3] G. Landmann, A. Reimer, G. Lemcke, S. Kempe, "Dating Late Glacial Abrubt Climate Changes İn The 14,570 Yr Long Continuous Varve Record Of Lake Van, Turkey", *Palaeo,* vol. 122, pp.107-118, 1996.

[4] S. Kempe, J. Kazmierczak, G. Landmann, T. Konuk, A. Reimer, A. Lipp, "Largest Known Microbialities Discovered İn Lake Van, Turkey", *Letters to Nature***,** vol. 349, pp. 605-608, 1991.

[5] P. Lopez-Garcia, J. Kazmierczak, K. Benzerara, S. Kempe, F. Guyot, D. Moreira, "Bacterial Diversity And Carbonate Precipitation In The Giant Microbialites From The Highly Alkaline Lake Van, Turkey". *Extremophiles,* vol. 9, pp. 263-274, 2005.

[6] A. A. Joshi, P. P. Kanekar, A. S. Kelkar, Y. S. Shouche, A. A. Vani, *et al*., "Cultivable Bacterial Diversity Of Alkaline Lonar Lake, India", *Microb Ecol,* vol. 55, pp.163–172, 2008.

[7] E. B. Jones, W. D. Grant, A. W. Duckworth, G. G. Owenson**,** "Microbial diversity of soda lakes", *Extremophiles*, vol. 2**,** pp**.**191–200, 1998.

[8] S. B. Humayoun, N. Bano, J. T. Hollibaugh, "Depth Distribution Of Microbial Diversity İn Mono Lake, A Meromictic Soda Lake İn California", *Applied and Environmental Microbiology*, vol. 69, pp. 1030-1042, 2003.

[9] M. F. Edbeib, R. A. Wahab, F. Huyop, "Halophiles: biology, adaptation, and their role in decontamination of hypersaline environments", *J. Microbiol. Biotechnol.*, vol. 32, pp. 135. 2016. https://doi.org/10.1007/s11274-016-2081-9.

[10] A. Siggliocolo, A. Paidardini, M. Piscitelli, S. Pascarella, "Structural adaptation of extreme halophilic proteins through decrease of conserved hydrophobic contact surface", *BMC Struct. Biol.*, vol. 11, pp. 50, 2011.https://doi.org/10.1186/1472-6807-11-50.

[11] A. Oren, "Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes", *Front. Microbiol.*, vol. 4, pp. 1–6, 2013.https://doi.org/10.3389/fmicb.2013.00315.

[12] H. Larsen, "Halophilic and halotolerant microorganisms—an overview and historical perspective", *FEMS Microbiol. Rev.* vol. 39, pp. 3–7, 1986.https://doi.org/10.1111/j.1574-6968.1986.tb01835.x.

[13] I. Tiago, A. P. Chung, A. Veríssimo, "Bacterial diversity in a nonsaline alkaline environment: heterotrophic aerobic populations", *Applied and environmental microbiology*, vol. 70, pp. 7378-7387, 2004.

[14] A. W. Duckworth, , S. Grant, W. D. Grant, B. E. Jones, D. Meijer, *"Dietzia natronolimnaios* sp. nov., a new member of the genus *Dietzia* isolated from an East African soda lake", *Extremophiles*, vol. 2, pp. 359–366, 1998.