Research Article

Tee, J.S., Y. Kaya, and F. Huyop, Isolation of bacteria from Tuz Gölü lake that can grow on high salt concentration International Journal of Life Sciences and Biotechnology, 2019. 2(3): p. 158-164.

Isolation of bacteria from Tuz Gölü lake that can grow on high salt concentration

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ABSTRACT

Today, extremophile is widely studied by the scientist due to its strong survival features that allow them to survive under extreme environment. Halophile is one example that inhabit high salt concentration environment. Isolation of bacteria from the area of Tuz Gölü lake, also known as hypersaline lake in the central plateau of Turkey, led to the isolation of 4 halotolerant bacteria, which were able to grow optimally in media with 0–10% of salt. Surprisingly, the strain A-4 isolate was successfully isolated from the Tuz Gölü lake water on the minimal media that consists of 2,2-dichloropropionic acid (2,2-DCP) as a carbon source. This indicated that the strain A-4 was very useful in the environmental remediation due to its capability to break down 2,2-DCP, a halocarboxylic found in herbicide. Further analysis such as biochemical tests and 16S rRNA sequence analysis were necessary to further identification of the species of the bacteria.

ARTICLE HISTORY
Received
21 June 2019
Accepted
05 August 2019

KEYWORDS
Halophile, halotolerant bacteria, Tuz Gölü

Introduction

In the central plateau of Turkey, there is a hypersaline lake with approximately 30% of NaCl concentration. This lake known as Tuz Gölü which it is the second largest lake of Turkey with the total surface area of 1665km^2. The width of the lake is 35 km and the length of the lake is 90km [1]. Despite the large area of hypersaline lake, this lake is very shallow with the height around 0.5 to 1 metre deep. In dry summer, huge amount of water will be evaporated, leaving a 30cm thick of salt layers in the lake surface. The thick of salt layers have a high market value. Each year, the salt production from Tuz Gölü lake is estimated 300,000 tons which is equal to 60 % of the total salt production in Turkey [2]. The density of this hypersaline lake water is slightly higher than the water density. This lake water density and water density are 1.225g/cm^3 and 0.997g/cm^3 respectively.

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Besides that, the annual rainfall in the Tuz Gölü lake area is very low which is only 250 mm per year [3].

Today's scientists have raised the interest in studying the extremophilic microorganisms such as thermoacidophiles, hyperthermophiles and hyper-halophiles [4]. The main reason of studying these organisms is to understand the biochemical mechanisms of the ability to withstand the extremophilic extreme environment. Many useful enzymes or molecules that synthesis by these organisms can be very useful in the bioremediation process, food industry and bioprocess industry. Among these extremophilic microorganisms, thermophiles have been studied and reported the most. However, halophilic bacteria have received less focus and attention in the scientific study.

In fact, the enzyme synthesis from the halophilic bacteria are very valuable due to the bacteria has high contamination resistance characteristic. Today, the cost of production and the downstream purification of enzyme from microorganisms are high because of the high energy consumption. Moreover, microbial contamination is another problem which cause a big loss to the industry. This problem will greatly affect the economy as well as the process effectiveness of the industry [5]. Hence, it is important to look for the contamination resistance microorganisms in order to reduce the energy consumption that apply for the sterilization purpose.

Recently, contamination resistant microorganisms like extremophiles which including halophilic, acidophilic, thermophilic and alkaliphilic bacteria are popular in bioproduction industry. There is successful example that applying the halophilic bacteria for the production of biopolymer, protein and chemicals. For instance, the bacteria *Halomonas campaniensis* that use in the production of poly(3-hydroxybutyrate) (PHB) was cultured and grown under an open and continuous fermentation process. Surprisingly, there is no contamination of culture for over 65 days [6]. Hence, it is crucial for the researcher to look for the novel halophilic bacteria that able to use in bioproduction industry in order to reduce the cost of operation.

Isolation of the halophilic bacteria is an important field of study because microorganisms that can grow in hypersaline environments are not only resistant to high salt concentration but also resistant to other stresses like pH, nutrients depletion and temperature [7]. For example, psychrophilic microorganisms isolated from the saline lakes in cold climate region like Arctic can grow in both extreme salt concentration and cold temperature [8].

Such surviving characteristics are very useful in the food industries, detergent and environmental bioremediation.

In order to successfully achieve this objective, the ideal place for sample collection that has been chosen is Tuz Gölü lake due to its hypersaline condition. In theory, there is difference between halotolerant bacteria and halophiles. Halotolerant bacteria can grow and survive without the need of NaCl, but they still can grow under saline condition [9]. However, halophiles cannot grow without the NaCl. In facts, there are three groups of halophiles which classified based on the responding of the halophiles to the NaCl. The first group is slight halophiles which the bacteria can grow well at 2 to 5% NaCl (0.34 to 0.85 M). The second group is the moderate halophiles with bacteria that can survive at 5 to 20% NaCl (0.85 to 3.4 M). Lastly, the extreme halophiles, a group of bacteria that can grow at 20 to 30% NaCl. (3.4 to 5.1 M) [10].

In this study, the main aim is to isolate the bacteria that can grow on high salt concentrations. Therefore, the isolated pure culture bacteria can be used to study the biochemical mechanisms or applying in the bioproduction industry in the future.

Material and Methods

Water sampling and reagent used

Hypersaline lake water samples were obtained aseptically from the hypersaline lake Tuz Gölü. In this study, distilled water was used to prepare different types of media. The chemicals used in the experiment and their brand will be listed in Table 1.

Table 1 List of chemicals used in the experiment

Chemicals	**Brand**
Dipotassium hydrogen phosphate trihydrate $K_2HPO_4 \cdot 3H_2O$	QRec
Ammonium sulfate $(NH_4)_2SO_4$	Wako
Sodium dihydrogen phosphate dihydrate $NaH_2PO_4 \cdot 2H_2O$	Wako
Iron(II) sulfate heptahydrate $FeSO_4 \cdot 7H_2O$	Wako
Manganese(II) sulfate tetrahydrate $MnSO_4 \cdot 4H_2O$	Wako
Magnesium sulfate $MgSO_4$	Sigma-Aldrich
Zinc Sulfate monohydrate $ZnSO_4 \cdot H_2O$	Wako
Nitrilotriacetic acid	Sigma-Aldrich

$C_6H_9NO_6$	
Cobalt(II) chloride hexahydrate $CoCl_2 \cdot 6H_2O$	Merck
2,2-dichloropropionic acid sodium salt $C_3H_3Cl_2O_2Na$	Merck

Media preparation

There were two types of media used in this experiment which were nutrient media and minimal media. A standard nutrient media was prepared which each 28 g of nutrient agar powder were suspended in a litre of distilled water.

Besides that, the preparation of minimal media involved the mixture of two different stock solutions. The first stock solution was basal salts, made of $K_2HPO_4 \cdot 3H_2O$ (42.5 g l^{-1}), $(NH_4)_2SO_4$ (25.0 g l^{-1}), and $NaH_2PO_4 \cdot 2H_2O$ (10.0 g l^{-1}). The second stock solution was made up by trace metal salts, $FeSO_4 \cdot 7H_2O$ (120.0 g l^{-1}) , $MnSO_4 \cdot 4H_2O$ (30.0 g l^{-1}), $MgSO_4$ (2.0 g l^{-1}), $ZnSO_4 \cdot H_2O$ (30 g l^{-1}), nitrilotriacetic acid (1.0 g l^{-1}) and $CoCl_2 \cdot 6H_2O$ (10 g l^{-1}) [9]. The liquid minimal media was prepared in the 250ml of conical flask. 10ml of the basal salts and 10ml of trace salt were mixed followed by the addition of 2ml of 1M 2,2-dichloropropionic acid sodium salt solution. Then, the mixture was top up to 100ml by distilled water. The preparation of a solid minimal medium was prepared by mixing liquid minimal media with 1.5% (w/v) Oxoid bacteriological agar No. 1.

All the media were autoclaved at 121°C, 15psi for 15 minutes.

Isolation of Bacteria

Isolation of bacteria were done by transferring 0.1ml of the Tuz Gölü lake water sample onto the nutrient agar and minimal media that containing 2,2-DCP followed by spread plate technique. The culture plates were incubated for 24-48 hours at 30^{o}C followed by the selection of bacterial colonies according to their distinctive morphologies. Isolated strains were further purified to obtain a pure culture using a standard microbiological technique.

NaCl tolerance test

The pure culture of different isolated strains was tested in the nutrient broth that supplemented with various concentrations of filter-sterilized solutions of NaCl (0, 35, 50, and 100 g·l^{-1}) to check the resistant for each strain in high salinity.

Results and Discussions

There were four bacteria strains were isolated based on distinguishable features like pigment production, size and shape. These bacteria were designated as strain A-1, A-2, A-3 and A-4. Each strain was tested for morphological and NaCl tolerance. Initially, all strains were grown well on nutrient agar media incubated at 30°C over 24 to 48 hours of incubation period. The results of morphological and NaCl tolerance were summarised in Table 2 and 3 respectively.

Table 2 Morphological characterization of bacteria from hypersaline lake water in the nutrient media after 48 hours incubation

Strains	**Colony morphology**
A-1	Pale yellow, irregular, flat, entire
A-2	Yellow, circular, convex, entire
A-3	red, circular, raised, entire
A-4	Creamy white, circular, raised, entire

Table 3 NaCl tolerance test for strains A-1, A-2, A-3 and A-4 in the nutrient broth that supplemented with various concentrations of filter-sterilized solutions of NaCl after 24 hours incubation

Strains	0% (w/v) NaCl	3.5% (w/v) NaCl	5% (w/v) NaCl	10% (w/v) NaCl
A-1(Pale yellow)	+	+	+	+
A-2(yellow)	+	-	-	-
A-3(red)	+	+	-	-
A-4(creamy white)	+	+	+	+

'+'=positive grow; '-'= negative grow

Based on the morphological characterization analysis, A-3 strains had the similar morphological characteristics with the *Pseudomonas halophila HX* with red pigmentation, circular form, raised elevation and entire margin [12]. However, further test on NaCl tolerance, A-3 strain could not withstand high salinity as reported with *P. halophila HX* due to that of A-3 strain was unable to grow on 25% of NaCl (w/v) (250g

l-1, 4.27M). Hence, A-3 strain was not belong to *P. halophila* group because the *P. halophila HX* can tolerate 25%(w/v) of NaCl and grow well on the media as reported earlier [12].

In this study all 4 isolates designated as strain A-1, A-2, A-3 and A4 were classified as halotolerant bacteria. They were not halophile because they were still able to grow well without NaCl in nutrient media. The presence of halotolerant bacteria in Tuz Gölü lake may likely due to the heterogenicity of the lake. The NaCl level of Tuz Gölü lake was fluctuating regularly, favouring the growth of Euryhaline microorganisms [13].

Interestingly, one isolate could grow well in the minimal media with 2,2-DCP as a carbon source. This isolate was designated as strain A-4. Since it was capable to grow on the 2,2-DCP, suggesting A-4 strain had the ability to produce dehalogenase enzyme for degradation of the organic chlorinated compound. This finding also suggested that strain A-4 could be one of the useful strains in environmental remediation. Current study elaborated the phenotypic characterization of all strains A-1 - A-4. In future further studies might involve the basic biochemical tests and the 16S rRNA sequencing.

Conclusion

Among all isolates, strain A-4 was important for further biological characterization as these bacteria were great potential source for discovery of enzymes for industrial applications or environmental remediation. Future work will be focused on identification and isolation of the potential enzymes with commercial importance.

Abbreviations

2,2-DCP: 2,2-dichloropropionic acid; NaCl: Sodium chloride; 16S rRNA: 16S ribosomal Ribonucleic acid

Acknowledgements

This work was financially supported by the Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia for undergraduate research project. Authors also thank TUBITAK for financial awards for sampling.

Availability of data and material

Please contact the corresponding author for any data request.

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