



## STUDENT WORKSHEET

# THE ROLE OF NITRIFYING BACTERIA

Compiled by: Group 1



For 10th Grade Students

# STUDENT WORKSHEET

**Theme** : **Bacteria**  
**Sub-theme** : **The Role of Bacteria**  
**Grade** : **X**  
**Semester** : .....  
**Group** : .....  
**Member of the Group** : **1.**.....  
**2.**.....  
**3.**.....  
**4.**.....  
**5.**.....

## Learning Objectives:

1. Students are able to classify living things, especially nitrifying bacteria, based on molecular data (DNA/Protein) using bioinformatics software in the form of NCBI properly and correctly.
2. Students are able to understand the role of nitrifying bacteria that can support soil fertility and the health of aquatic ecosystems properly and correctly.
3. Students are able to create phylogenetic trees using bioinformatics tools such as MEGA to identify the kinship relationships and taxonomic potential of local bacterial species, including nitrifying bacteria, correctly.
4. Students are able to develop critical thinking and scientific argumentative skills through case studies of water pollution and biological solutions with appropriate molecular data-based approaches.

# ACTIVITY

## A. Introduction

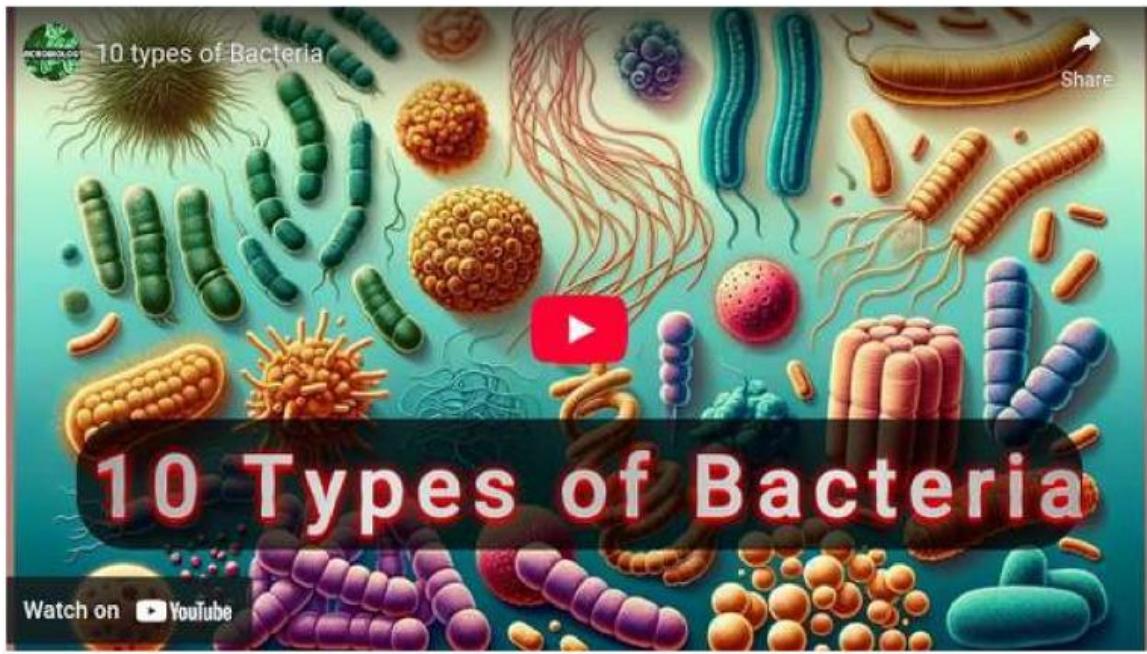
Answer the following questions!

Question	Answer
What do you know about bacteria?	
What types of bacteria do you know?	
What are the roles of bacteria that you know?	
In your opinion, can bacteria affect the environment?	
What is a phylogenetic tree?	
What do you know about NCBI?	
What do you know about MEGA?	

Now, let's watch the following video about the role of bacteria.

### a. Video Types of Bacteria

<https://youtu.be/AY0Rs-hI8tU?si=DstR2UPLit5tf5VY>



### b. Video of Nitrifying Bacteria

[https://youtu.be/AYkkpI\\_G6iE?si=ce13atcluwBeVWPr](https://youtu.be/AYkkpI_G6iE?si=ce13atcluwBeVWPr)



## B. Investigation

A researcher observed the water in the Pontang River, Serang Regency, Banten. From the observations, it was found that there was a lot of trash causing an increase in organic nitrogen content in the water. This condition triggered eutrophication, which is the excessive growth of algae and aquatic plants that reduces oxygen and water quality. Further investigation showed that the nitrification process was not working properly. The nitrification process is carried out by **Nitrosomonas sp** bacteria, which convert ammonia into nitrite, and **Nitrobacter sp** bacteria, which convert nitrite into nitrate. However, at the site in question, the numbers of both bacteria were too low. As a result, ammonia accumulated and caused fish mortality.

A researcher conducted an experiment by isolating bacteria from the river water. From the isolation results, seven bacteria were obtained that needed to be identified. Six of them have been successfully identified as nitrifying bacteria. However, the researcher had difficulty identifying the remaining bacteria. To determine whether the unidentified bacterium also plays a role in nitrification, the researcher compared the DNA sequence of the bacterium with the six known nitrifying bacteria. The DNA data was obtained from the NCBI database and analyzed using MEGA software to construct a phylogenetic tree to examine the relationships between species.

### Modification of the article :

Rarassari, M.A., Yonarta, D., Wijayanti, M., Aulia, D., Dwinanti, S.H. (2024). DNA Barcoding and Water Quality Analysis of Nitrifying Bacteria in Lebak Lebung Swamp, South Sumatera. International Journal of Design & Nature and Ecodynamics. 19(2), 563 - 569.



To find out the results of the experiment, let's help the researcher complete the experiment.



## Let's help researchers by following these steps.

Based on the existing sequence data, the relationship between the Bacterial Samples and Nitrifying Bacteria can be proven by creating a phylogenetic tree.

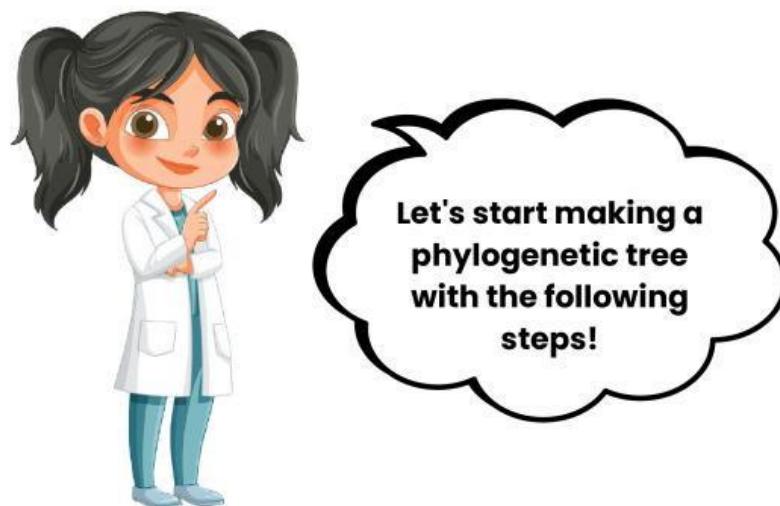
### Bacterial Sample Sequences

Can be accessed via the Drive link or via the following QR code: <https://shorturl.at/0vPQr>



### Nitrifying Bacteria

- Burkholderia sp. strain 172 1492R 16S ribosomal RNA gene partial sequence
- Burkholderia cepacia strain N8 16S ribosomal RNA gene partial sequence
- Nitrosococcus sp. clone 4 355 16S ribosomal RNA gene partial sequence
- Nitrosococcus sp. clone 113 16S ribosomal RNA gene partial sequence
- Nitrobacter sp. clone Sb-05-27 16S ribosomal RNA gene partial sequence
- Nitrosomonas sp. clone Pad-87 16S ribosomal RNA gene partial sequence



## How to create a phylogenetic tree using NCBI and MEGA

### Steps at NCBI

- Go to the NCBI (*National Center for Biotechnology Information*) website, and select the **Nucleotide** option.

The screenshot shows the NCBI homepage on a Google search results page. The search query "NCBI" is entered in the search bar. The results list the "National Center for Biotechnology Information" website at the top. Below it, there are links for "PubMed", "Nucleotide", "Nucleotide BLAST", and "BLAST". The "Nucleotide" link is highlighted with a black arrow pointing to it.

**Pusat Nasional untuk  
Informasi Bioteknologi  
(National Center for  
Biotechnology  
Information)**

Perusahaan I  
Pusat Nasional Informasi Bioteknologi adalah bagian dari  
Perpustakaan Kedokteran Nasional Amerika Serikat,  
sebuah cabang dari Institut Kesehatan Nasional. NCBI  
terletak di Bethesda, Maryland dan didirikan pada tahun  
1988 melalui undang-undang yang disponsori oleh Senator  
Claude Pepper.

Source: Wikipedia

- Copy one by one the names of the Nitrifying bacteria found in the LKPD.
- Click the species name that appears on the screen.
- After displaying a new page, on the left side under the species name there is the word "FASTA" click the word Fasta After displaying a new page, on the right side there is the word "send to" with a (v) sign, click the icon. Click "File" and click "Create File". The nucleotide sequence of the specimen has been downloaded in file form.
- Do the steps above, until you get all the results of the nitrifying bacteria

## Steps in MEGA

- Open the MEGA application.
- Klik Ikon "Align" → "Edit/Build Alignment".
- Select "Create a new alignment" → click "OK".
- Select the data type "DNA" click OK.
- Click "Edit" → "Insert Sequence From File" NCBI → click Open. Enter the data of 6 bacteria from
- Enter the "bacterial sample" sequence data in the same way.
- Delete blank lines by right-clicking → Delete.
- Press Ctrl + A to block all sequences click the "Align" icon (muscle image)
- → select "Align DNA".
- Click OK on the Muscle Alignment Options bar and wait until the process is complete.
- Save the results by clicking "Data" → "Export Alignment" → "Mega Format".
- Name the aligned result file and click "Save"
- Enter the file name in the "Input title of the data" bar, click OK.
- Click the "Data" menu, select "Phylogenetic Analysis" (5th option).
- When the confirmation appears again, select No.
- Go back to the MEGA home menu, click the "Phylogeny" icon, select "Construct/Test Neighbor-Joining".
- On the "Analysis Preferences" screen, click OK.
- Enlarge the screen by clicking the box icon in the right corner.
- A phylogenetic tree will appear, enable "Branch Lengths" to
- displays the branch length.
- Click on the "Phylogenetic Tree" icon to see the distance between branches clearly.



- Klik ikon "Image" di menu bar → pilih format penyimpanan (misal: PNG).
- Pada "Image Export Option", beri nama file → klik "Gambar file", pilih lokasi penyimpanan → klik OK.



**Congratulations... You  
have successfully  
helped the researcher  
complete his research.**

## Now, let's answer the questions below!

Based on the results of the phylogenetic tree analysis that you have created, answer the questions below:

Question	Answer
Are the bacteria in the research sample closely related to nitrifying bacteria? Explain your reasons!	
Based on the results of your phylogenetic tree analysis, are the bacteria in the research sample indicated as nitrifying bacteria? Explain your reasons!	
According to the results of your analysis, what genus do the research sample bacteria belong to?	
How important do you think the role of nitrifying bacteria is in bioremediation and environmental quality restoration?	

**Please answer the questions below again!**

Question	Answer
What do you know about bacteria?	
What types of bacteria do you know?	
What are the roles of bacteria that you know?	
In your opinion, can bacteria affect the environment?	
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# CONGRATULATIONS YOU HAVE COMPLETED THIS LEARNING COURSE!



**Let's see your answers after doing the activities in this lesson. If your answers have changed, become more detailed and better than before, congratulations! Your understanding has increased. However, if your answers after this activity remain the same, don't worry, please study again and consult with your teacher.**