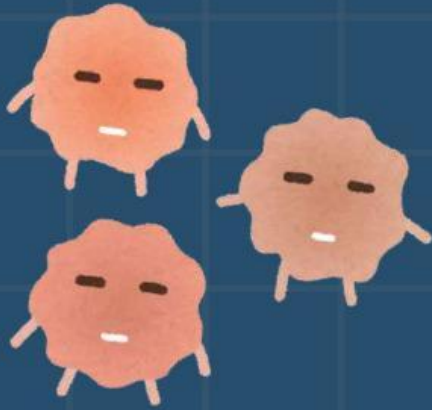




# Student Worksheet



## Identification Probiotic of Local Fish



# STUDENT WORKSHEET

**Theme** : **Bacteria**  
**Sub-theme** : **Diversity and Role of Bacteria**  
**Class** : **X**  
**Semester** : .....  
**Group** : .....  
**Group Members** : 1.....  
2.....  
3.....  
4.....  
5.....

## Learning Outcomes

- Students are able to explain the characteristics and roles of probiotic bacteria, especially in freshwater fish such as Ikan Mas Sinyonya, as part of the local biodiversity of Banten.
- Students are able to interpret the results of biochemical tests (Gram, catalase, proteolytic) to identify the potential of bacteria as probiotics.
- Students are able to use simple Python to analyze genetic data (simulation) and replace the MEGA function in creating phylogenetic trees or comparing DNA similarities.

# ACTIVITY

## A. Introduction

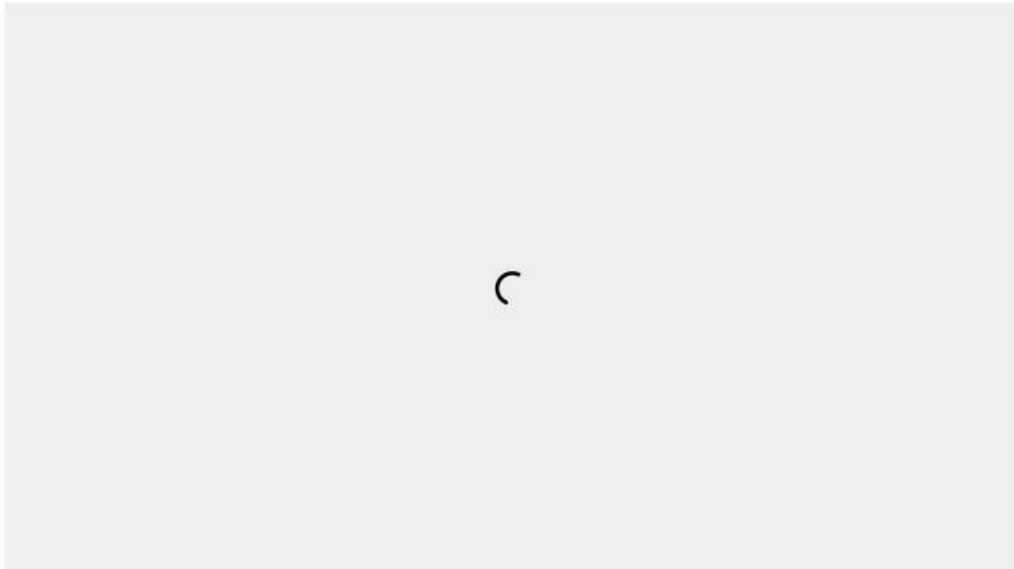
Answer the following questions!

| Question                                      | Expected Answer |
|-----------------------------------------------|-----------------|
| What do you know about bacteria?              |                 |
| What types of bacteria do you know?           |                 |
| What roles do bacteria play that you know of? |                 |
| What are probiotic bacteria?                  |                 |
| What do you know about NCBI?                  |                 |
| What do you know about MEGA?                  |                 |
| What do you know about Google Colab?          |                 |

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

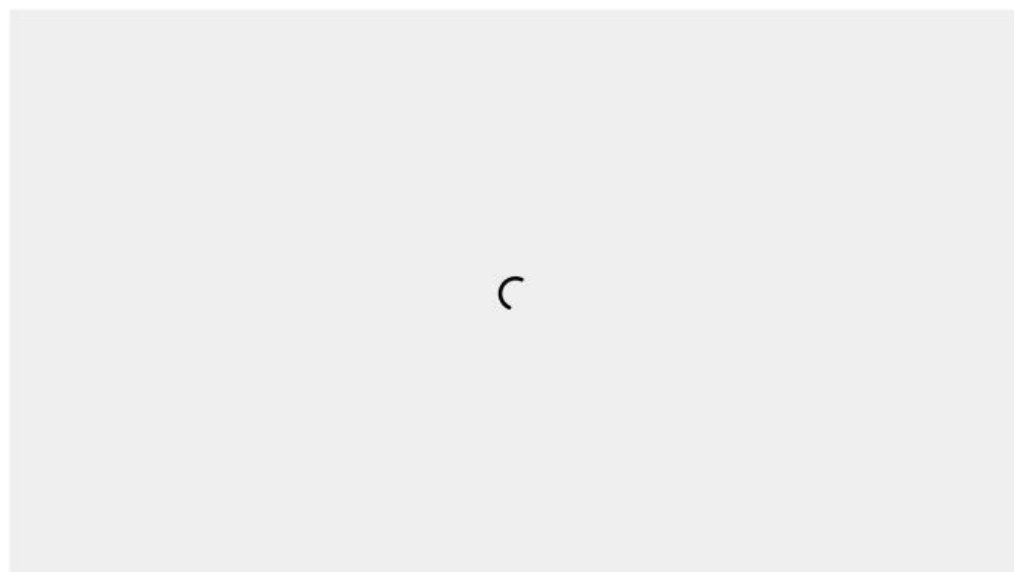
**A. The Role of Probiotic Bacteria**

[https://youtu.be/ZggHg\\_Si6fYsi=aIem3pMGYzZu\\_JND](https://youtu.be/ZggHg_Si6fYsi=aIem3pMGYzZu_JND)



**B. The Role of Probiotic Bacteria**

<https://www.youtube.com/watch?v=ZSSWQVPLUMw>





## B. Investigation

A young researcher is observing his synony fish, an endemic species that lives in the waters of Pandeglang, Banten. This fish is not only a local cultural icon, but also has high economic value and is an important source of protein for the local community. The researcher is curious: why can this fish thrive even though its aquatic environment is not always stable?

From observations and laboratory experiments, it was discovered that the intestines of the sinyonya carp contain various types of naturally occurring bacteria. One of these is strongly suspected to be a probiotic bacterium—a beneficial microbe that helps the fish digest food, boosts its immune system, and fights harmful disease-causing bacteria.

However, a big question arose: “What type of bacteria were actually found? Are they really probiotic bacteria such as *Lactobacillus* sp.?”

To answer this, the researchers used modern technology: biological data analysis with Python. Although they were unable to perform DNA sequencing directly, the researchers utilized genetic data already available in public databases such as NCBI (National Center for Biotechnology Information).

*To find out the results of the experiment, let's help the researcher complete his research!*





## Case Study: Researchers Discover New Bacteria in the Intestines of Sinyonya Carp

Researchers have isolated a colony of bacteria from the intestines of fish. Biochemical test results:

| Test        | Result                            |
|-------------|-----------------------------------|
| Gram        | Positive (+)                      |
| Catalase    | Negative (-)                      |
| Proteolytic | Positive (+) (clear zone present) |

These characteristics are very similar to lactic acid bacteria (LAB) such as *Lactobacillus*, which are known as probiotics. To confirm this, researchers compared the 16S rRNA gene sequence of the bacteria with data from the NCBI.



## How to create a phylogenetic tree using NCBI and Google Colab (Python)

### Step 1: Open Google Colab

- Open your browser (Chrome, Firefox, etc.).
- Go to: <https://colab.research.google.com>
- Click "File" → "New notebook".
- Change the notebook title to: "Simulation of the Phylogenetic Tree of Goldfish Bacteria"

## Step 2: Prepare Simulation Data (Percentage of Genetic Similarity)

Since we are not performing actual alignment, we will create simulated data based on the genetic similarity of the 16S rRNA sequence, which can be obtained from NCBI and BLAST.

**Note:** *This data is an educational simulation, not actual analysis results. However, it is very suitable for learning purposes.*

```
python
1 # Langkah 2: Masukkan data kesamaan genetik (%)
2 # Data ini mewakili hasil perbandingan sekuens 16S rRNA antara sampel dan bakteri referensi
3
4 kesamaan_genetik = {
5     "Lactobacillus acidophilus": 98.5,
6     "Enterococcus faecium": 90.2,
7     "Bacillus subtilis": 78.0,
8     "Escherichia coli": 65.4,
9     "Staphylococcus epidermidis": 62.1,
10    "Clostridium perfringens": 60.3
11 }
12
13 print("Data Kesamaan Genetik (Simulasi):")
14 for bakteri, nilai in kesamaan_genetik.items():
15     print(f"{bakteri}: {nilai}%")
```

Run this cell by clicking the play button  or pressing **Ctrl+Enter**.

## Step 3: Visualization with Bar Charts

```
python
1 import matplotlib.pyplot as plt
2
3 # Ambil nama dan nilai dari dictionary
4 bakteri = list(kesamaan_genetik.keys())
5 nilai = list(kesamaan_genetik.values())
6
7 # Buat grafik batang
8 plt.figure(figsize=(10, 6))
9 plt.bar(bakteri, nilai, color=['green', 'skyblue', 'orange', 'gray', 'pink', 'red'])
10 plt.title("Kesamaan Genetik Sampel Bakteri Usus Ikan Mas Sinyonya")
11 plt.xlabel("Nama Bakteri")
12 plt.ylabel("Kesamaan Genetik (%)")
13 plt.xticks(rotation=45, ha='right') # agar nama tidak tumpang tindih
14 plt.ylim(50, 100)
15 plt.grid(axis='y', alpha=0.3)
16 plt.tight_layout() # agar grafik tidak terpotong
17 plt.show()
```

Run this cell → A bar chart will appear showing the similarity comparison.



## Step 4: Simple “Phylogenetic Tree” Simulation with Line Graphs (Hierarchy)

Since we did not perform alignment, we cannot create an actual phylogenetic tree. But we can create a visual simulation that resembles a tree, based on sequence similarity.

```
# Urutkan dari yang paling mirip
sorted_bakteri = sorted(kesamaan_genetik.items(), key=lambda x: x[1], reverse=True)

# Ambil nama dan nilai yang sudah diurutkan
nama_urut = [item[0] for item in sorted_bakteri]
nilai_urut = [item[1] for item in sorted_bakteri]

# Buat grafik garis hierarki sederhana
plt.figure(figsize=(10, 6))
plt.plot(nilai_urut, range(len(nilai_urut)), 'o-', color='blue', linewidth=2, markersize=8)

# Tambahkan label
for i, (nama, nilai) in enumerate(sorted_bakteri):
    plt.text(nilai - 2, i, nama, va='center', ha='right', fontsize=9)

plt.title("Simulasi Hubungan Kekerabatan Bakteri")
plt.xlabel("Kesamaan Genetik (%)")
plt.yticks([]) # hilangkan angka sumbu Y
plt.xlim(55, 100)
plt.grid(axis='x', alpha=0.3)
plt.tight_layout()
plt.show()
```

## Step 5: Automatic Analysis & Conclusions

```
python
1 # Cari bakteri dengan kesamaan tertinggi
2 bakteri_terdekat = max(kesamaan_genetik, key=kesamaan_genetik.get)
3 nilai_tertinggi = kesamaan_genetik[bakteri_terdekat]
4
5 print("🔍 HASIL ANALISIS:")
6 print(f"Bakteri dengan kesamaan genetik tertinggi: {bakteri_terdekat}")
7 print(f"Kesamaan: {nilai_tertinggi}%")
8 print()
9
10 # Beri kesimpulan berdasarkan hasil
11 if "Lactobacillus" in bakteri_terdekat or nilai_tertinggi > 95:
12     print("✅ KESIMPULAN: Bakteri sampel kemungkinan besar termasuk genus Lactobacillus.")
13     print("Ini mendukung bahwa bakteri ini adalah bakteri probiotik alami dari usus ikan.")
14 elif "Enterococcus" in bakteri_terdekat:
15     print("⚠️ KESIMPULAN: Bakteri sampel mirip dengan Enterococcus. Beberapa spesies bersifat probiotik, namun perlu u")
16 else:
17     print("❌ KESIMPULAN: Bakteri sampel tidak mirip dengan bakteri probiotik utama.")
18     print("Kemungkinan bukan bakteri probiotik yang diharapkan.")
```

## Step 5: Automatic Analysis & Conclusions

- Click File → Download → Download .ipynb (to save the code).
- To export the graph: Right-click on the graph → Save image as.
- You can also click Share → Copy link to share.



**Please answer the questions below.**

| Question                                      | Expected Answer |
|-----------------------------------------------|-----------------|
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**Congratulations!**  
**You have completed**  
**today's lesson!**

