

# **IDENTIFICATION of microorganisms**

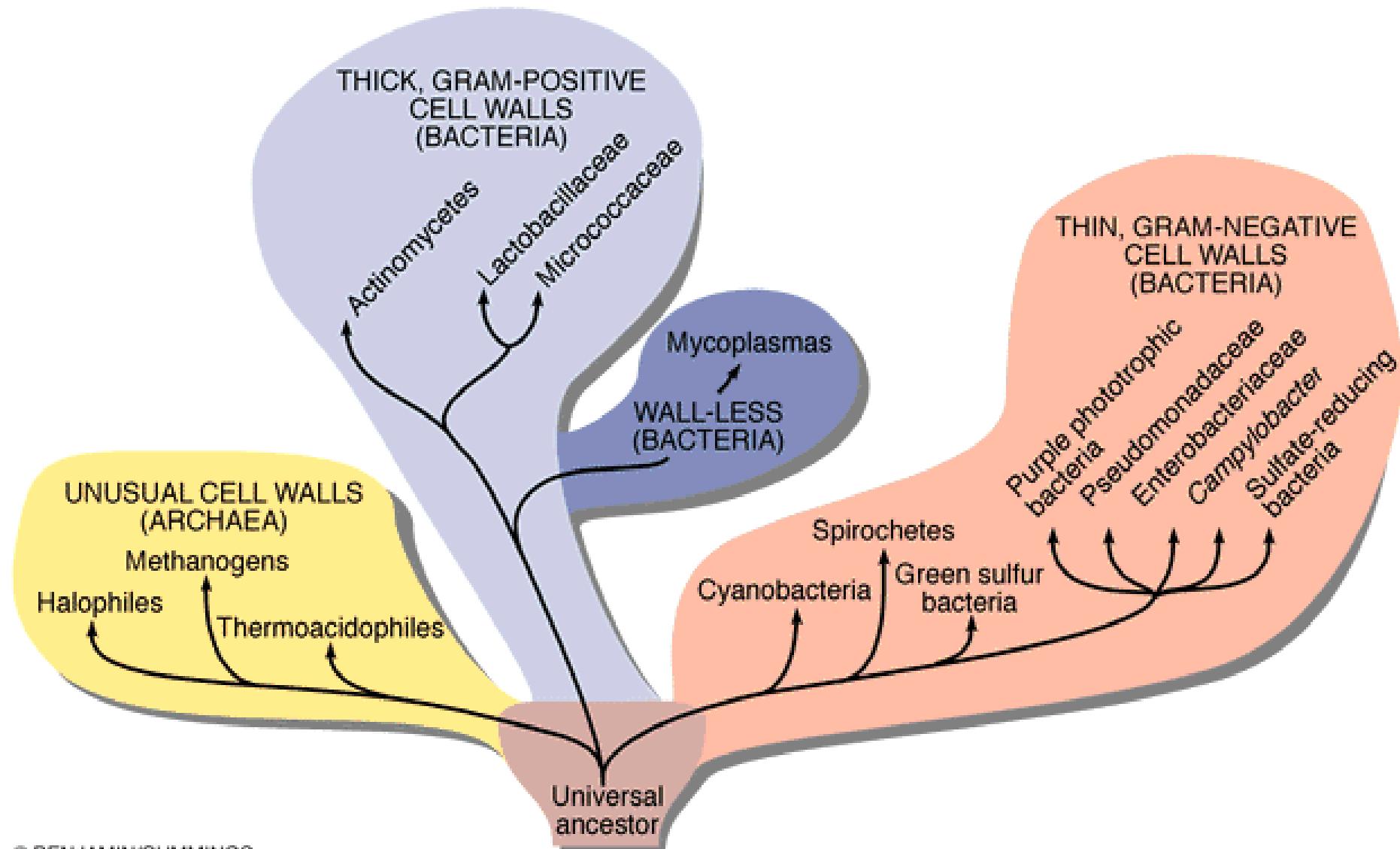
## Learning outcomes

1. Explain the steps for identifying a specific microorganism using the method based on morphological, physiological, physical and chemical characteristics of the microorganism.
2. Explain the steps for identifying a particular microorganism using a method based on the specific nucleotide/protein sequence of the microorganism.

**L.O.1:** Explain the steps for identifying a specific microorganism using the method based on morphological, physiological, physical and chemical characteristics of the microorganism.

- ✓ Bergey's Manual of Determinative Bacteriology, (1980-1984) 4 divisions/phyla.
  - Volume 1: All Gram-negative bacteria (of medical and industrial importance)
  - Volume 2: All Gram-positive bacteria.
  - Volume 3: All remaining bacteria, some Gram-negative bacteria and archaea.
  - Volume 4: Actinomycetes and other bacteria.

# Bergey's manual of Systematic Bacteriology



=> Does not provide a clear evolutionary history of bacteria.

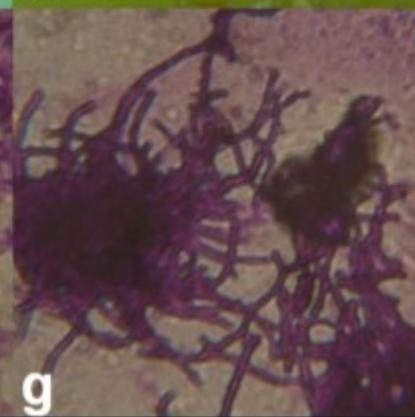
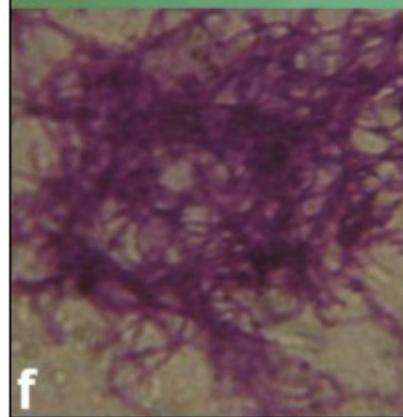
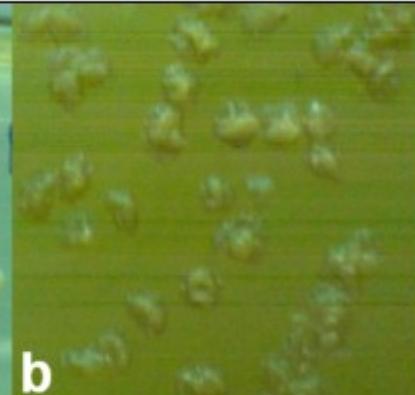
**Bergey's Manual:** Bacteria are classified into four divisions/phyla; Each division is divided into groups according to phenotypes:

- Gram stain reaction
- Cell shape
- Cell arrangement
- Oxygen requirement
- Movement ability
- Nutritional and metabolic characteristics

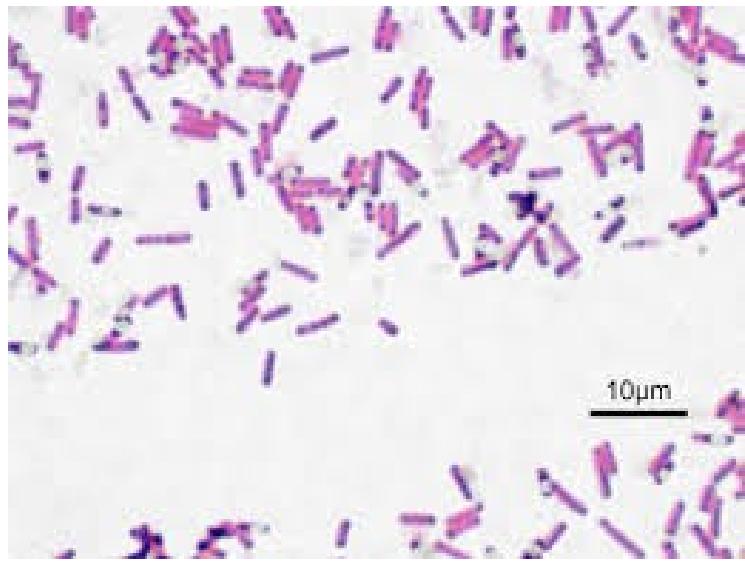
Bergey's Manual of Determinative Bacteriology (current editions): 5 volumes; bacterial phyla organized according to evolutionary relationships between 16S rDNA sequences:

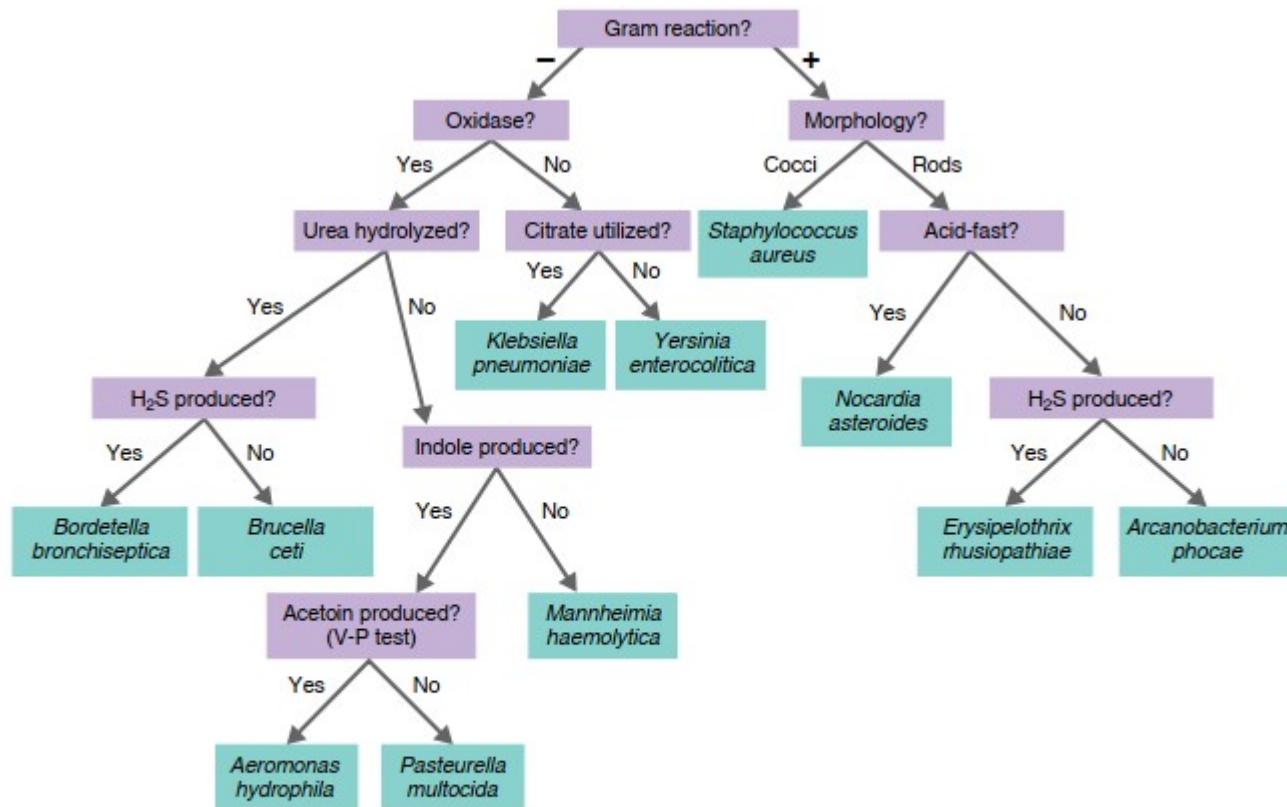
- **Volume 1 (2001):** Archaea, Actinomycetes, & photoautotrophs.
- **Volume 2 (2005):** Proteobacteria, includes:
  - » 2A: Basic methods
  - » 2B: Class Gammaproteobacteria
  - » 2C: Other Proteobacteria classes
- **Volume 3 (2009):** Firmicutes
- **Volume 4 (2011):** Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, & Planctomycetes.
- **Volume 5 (2012):** Ngành Actinobacteria.

– Actinomycetes:



– Firmicutes: *Bacillus*

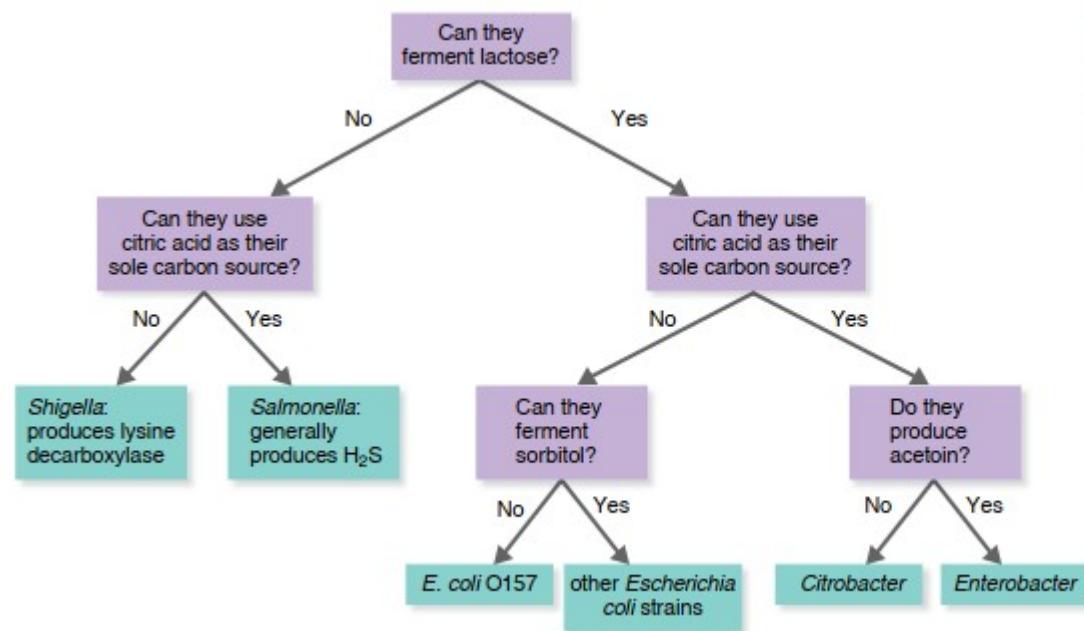




**Biochemical tests used to identify selected species of human pathogens isolated from marine mammals.**

- Q Assume you isolated a gram-negative rod that is oxidase-positive, is indole-negative, and does not produce urease or acetoin. What is the bacterium?

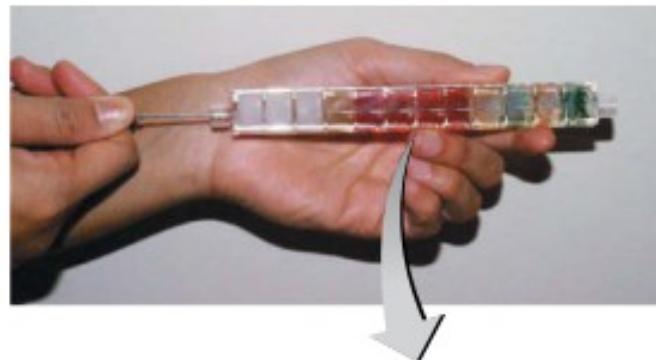
All members of the family *Enterobacteriaceae* do not contain oxidase, including members of the genera *Escherichia*, *Enterobacter*, *Shigella*, *Citrobacter*, and *Salmonella*. *Escherichia*, *Enterobacter*, and *Citrobacter* can ferment lactose to produce acid and gas, which distinguishes them from *Salmonella* and *Shigella*. Other biochemical tests can differentiate species of different genera.



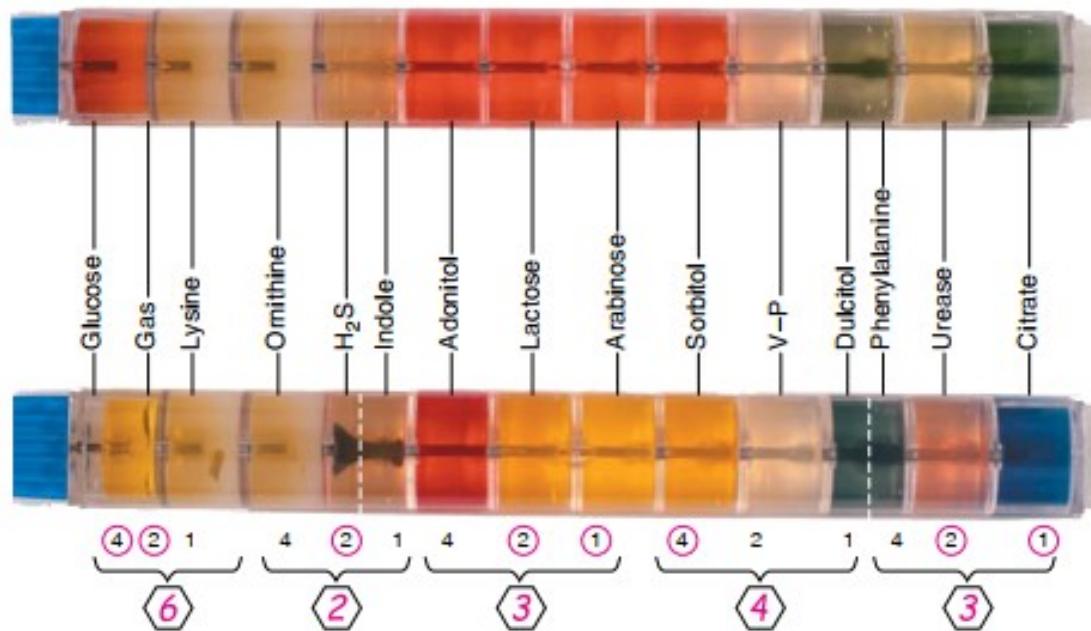
**Figure 10.8** The use of metabolic characteristics to identify selected genera of enteric bacteria.

**Q** Assume you have a gram-negative bacterium that produces acid from lactose and cannot use citric acid as its sole carbon source. What is the bacterium?

- 1 One tube containing media for 15 biochemical tests is inoculated with an unknown enteric bacterium.



- 2 After incubation, the tube is observed for results.



- 3 The value for each positive test is circled, and the numbers from each group of tests are added to give the code number.

- 4 Comparing the resultant code number with a computerized listing shows that the organism in the tube is *Citrobacter freundii*.

Code Number	Microorganism	Atypical Test Results
62342	<i>Citrobacter freundii</i>	Citrate
62343	<i>Citrobacter freundii</i>	None

**Figure 10.9** One type of rapid identification method for bacteria: EnteroPluri test from

**BD Diagnostics.** This example shows results for a typical strain of *C. freundii*; however, other strains may produce different test results, which are listed in the Atypical Test Results column.

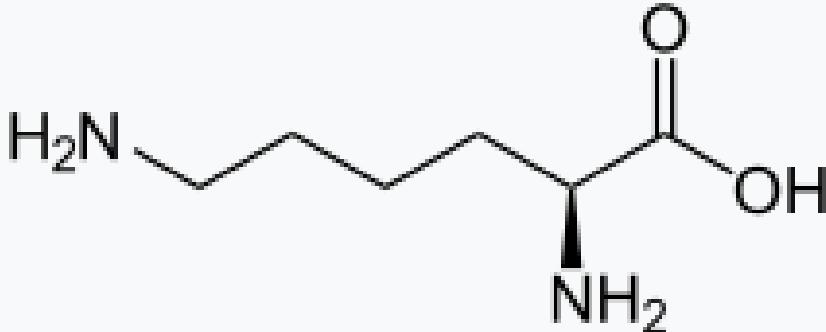
**Q** How can one species have two different code numbers?



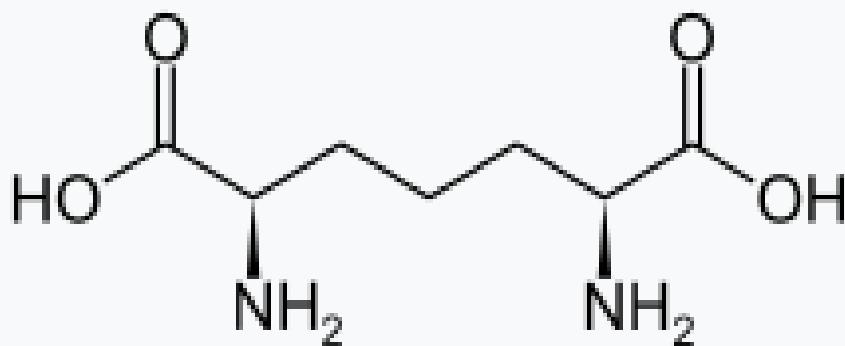
### ***Related scientific reports :***

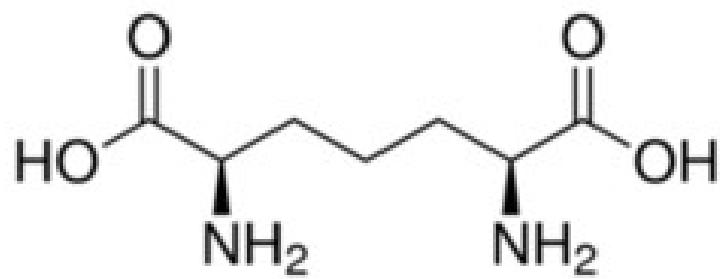
- » Colony morphology: colony shape, mycelium color, spore-forming ability, spore color, ability to diffuse color into the environment, ...
- » Cell morphology: vegetative cell shape, spore shape, ...
- » Chemical composition of cell wall: peptidoglycan, glucosamine, alanine, glutamic acid, glycine, arabinose, galactose, LL- $\alpha$ ,  $\epsilon$  (epsilon)-diaminopimelic acid (L-DAP); meso- $\alpha$ ,  $\epsilon$  -diaminopimelic acid (meso-DAP), ...
- » Culture characteristics.

**Lysine**

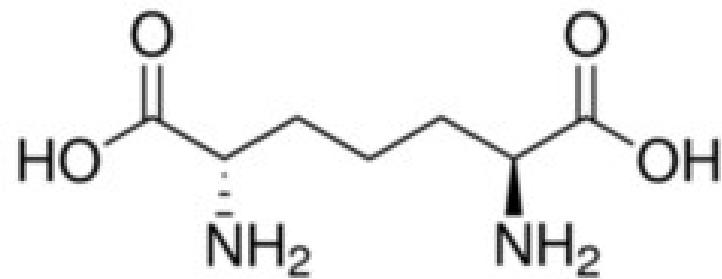


**Diaminopimelic acid**





meso-DAP



L-DAP

**L.O.2:** Explain the steps for identifying a particular microorganism using a method based on the specific nucleotide/protein sequence of the microorganism.

- ❖ **Specific gene sequences:** 16S rDNA, 23S rDNA, 18S rDNA, ITS (Internal transcribed spacer), 25S rDNA, rpoB, ...
  - ✓ Primer design & PCR
  - ✓ PCR fragment sequencing
  - ✓ Search for sequence similarity in databases (Homology, BLASTN, BLASTP).
  - ✓ Phylogenetic tree construction:
    - » Multiple sequence alignment.
    - » Choose evolutionary models and methods to construct the phylogenetic tree.
    - » Determine the reliability of the phylogenetic tree.

# BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST)

Standard Nucleotide BLAST

**blastn** **blastp** **blastx** **tblastn** **tblastx**

Enter Query Sequence BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#) [Query subrange](#)

From   
To

Or, upload file Chọn tệp Không có tệp nào được chọn

Job Title  Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

**Database**  Human genomic + transcript  Mouse genomic + transcript  Others (nr etc.): Nucleotide collection (nr/nt)  [?](#)

**Organism** Optional Enter organism name or id--completions will be suggested  Exclude  [?](#)

**Exclude** Optional Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Models (XM/XP)  Uncultured/environmental sample sequences

**Limit to** Optional  Sequences from type material

**Entrez Query** Optional  [YouTube](#) [Create custom database](#)

Enter an Entrez query to limit search [?](#)

# BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST)

Program Selection

Optimize for

- Highly similar sequences (megablast)
- More dissimilar sequences (discontiguous megablast)
- Somewhat similar sequences (blastn)

Choose a BLAST algorithm 

**BLAST**

Search database **Nucleotide collection (nr/nt)** using **Megablast (Optimize for highly similar sequences)**

Show results in a new window

 [Algorithm parameters](#)

## BLAST Results

[Edit and Resubmit](#) [Save Search Strategies](#) [► Formatting options](#) [► Download](#)

[YouTube](#) [How to read this page](#) [Blast report description](#)

### Nucleotide Sequence (471 letters)

RID [E0GSF923014](#) (Expires on 03-10 22:36 pm)

Query ID [Idl|Query\\_188307](#)

Description None

Molecule type nucleic acid

Query Length 471

Database Name nr

Description Nucleotide collection (nt)

Program BLASTN 2.3.1+ [► Citation](#)

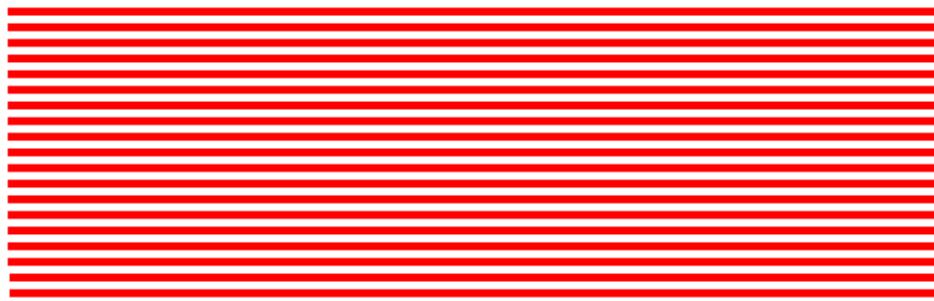
Other reports: [► Search Summary](#) [\[Taxonomy reports\]](#) [\[Distance tree of results\]](#)

### Graphic Summary

#### Distribution of 111 Blast Hits on the Query Sequence [?](#)

Mouse-over to show define and scores, click to show alignments

##### Color key for alignment scores



## Descriptions

### Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	<a href="#">Streptomyces flaveus strain X418 16S ribosomal RNA gene, partial sequence</a>	870	870	100%	0.0	100%	JX293177.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. NEAU-P17 16S ribosomal RNA gene, partial sequence</a>	870	870	100%	0.0	100%	HQ916736.1
<input type="checkbox"/>	<a href="#">Streptomyces flaveus strain S13 16S ribosomal RNA gene, partial sequence</a>	870	870	100%	0.0	100%	HQ850408.1
<input type="checkbox"/>	<a href="#">Streptomyces flaveus strain T111 16S ribosomal RNA gene, partial sequence</a>	870	870	100%	0.0	100%	GU084180.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. SXY10 16S ribosomal RNA gene, partial sequence</a>	870	870	100%	0.0	100%	GU045527.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. FH-2006 16S ribosomal RNA gene, partial sequence</a>	870	870	100%	0.0	100%	DQ989584.1
<input type="checkbox"/>	<a href="#">Streptomyces lavendulae DNA for 16S rRNA, strain IFO 13709</a>	867	867	100%	0.0	99%	D85115.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. DP_00001 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	KM274097.1
<input type="checkbox"/>	<a href="#">Streptomyces avidinii strain IHBA 9994 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	KR085928.1
<input type="checkbox"/>	<a href="#">Streptomyces subutilus strain IHBA 9229 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	KR085841.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. CKS2 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	KP715851.1
<input type="checkbox"/>	<a href="#">Streptomyces flaveus strain S10714 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	KF956565.1
<input type="checkbox"/>	<a href="#">Streptomyces spororaveus strain JQZST-6 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	KF973242.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. HJ1-2 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	FJ626862.1
<input type="checkbox"/>	<a href="#">Streptomyces subutilus strain HND39 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	EU030437.1
<input type="checkbox"/>	<a href="#">Streptomyces nojiriensis strain B-33 16S ribosomal RNA gene, partial sequence</a>	865	865	100%	0.0	99%	EF063481.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. So54 partial 16S rRNA gene, strain So54</a>	865	865	100%	0.0	99%	AJ308576.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. DP_00015 16S ribosomal RNA gene, partial sequence</a>	863	863	99%	0.0	99%	KM274100.1
<input type="checkbox"/>	<a href="#">Streptomyces sp. A24 gene for 16S ribosomal RNA, partial sequence</a>	863	863	99%	0.0	99%	AB856298.1

## Alignments

[Download](#) ▾ [GenBank](#) [Graphics](#)
▼ Next ▲ Previous ▲ Descriptions

Streptomyces flaveus strain X418 16S ribosomal RNA gene, partial sequence

Sequence ID: [gb|UX293177.1](#) Length: 1503 Number of Matches: 1Range 1: 29 to 499 [GenBank](#) [Graphics](#)
▼ Next Match ▲ Previous Match
**Related Information**

Score	Expect	Identities	Gaps	Strand
870 bits(471)	0.0	471/471(100%)	0/471(0%)	Plus/Plus

Query 1	TGGCTCAGGACCAACGCTGGCGGTGCTAACACATGCAAGTCGAACGATGAAGCCCTT 	60
Sbjct 29	TGGCTCAGGACCAACGCTGGCGGTGCTAACACATGCAAGTCGAACGATGAAGCCCTT 	88
Query 61	CGGGGTGGATTAGTGGCGAACGGGTGAGTAACACACGTGGGCAATCTGCCCTTCACTCTGGG 	120
Sbjct 89	CGGGGTGGATTAGTGGCGAACGGGTGAGTAACACACGTGGGCAATCTGCCCTTCACTCTGGG 	148
Query 121	ACAAGCCCTGGAAACGGGTCTAATACCGGATACCACCTCCTGCCTGCATGGCGGGGTT 	180
Sbjct 149	ACAAGCCCTGGAAACGGGTCTAATACCGGATACCACCTCCTGCCTGCATGGCGGGGTT 	208
Query 181	GAAAGCTCCGGCGGTGAAGGATGAGCCCCGGCTATCAGCTTGTGGTGGGTAATGGC 	240
Sbjct 209	GAAAGCTCCGGCGGTGAAGGATGAGCCCCGGCTATCAGCTTGTGGTGGGTAATGGC 	268
Query 241	CTACCAAGGCACGACGGTAGCCGGCTGAGAGGGCGACCGGCCACACTGGGACTGAGA 	300
Sbjct 269	CTACCAAGGCACGACGGTAGCCGGCTGAGAGGGCGACCGGCCACACTGGGACTGAGA 	328
Query 301	CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGCACAATGGCGAAAGCCT 	360
Sbjct 329	CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGCACAATGGCGAAAGCCT 	388
Query 361	GATGCAGCGACCCCGCGTGAGGGATGACGGCTTGGTTGTAAACCTTTCAAGCAGGG 	420
Sbjct 389	GATGCAGCGACCCCGCGTGAGGGATGACGGCTTGGTTGTAAACCTTTCAAGCAGGG 	448
Query 421	AAGAAGCGAAAGTACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC 	471
Sbjct 449	AAGAAGCGAAAGTACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC 	499

[Download](#) ▾ [GenBank](#) [Graphics](#)
▼ Next ▲ Previous ▲ Descriptions

Streptomyces sp. NEAU-P17 16S ribosomal RNA gene, partial sequence

Sequence ID: [gb|HQ916736.1](#) Length: 1517 Number of Matches: 1
▼ Previous ▲ Next ▲ Descriptions