

IDENTIFICATION of microorganisms

Learning outcomes

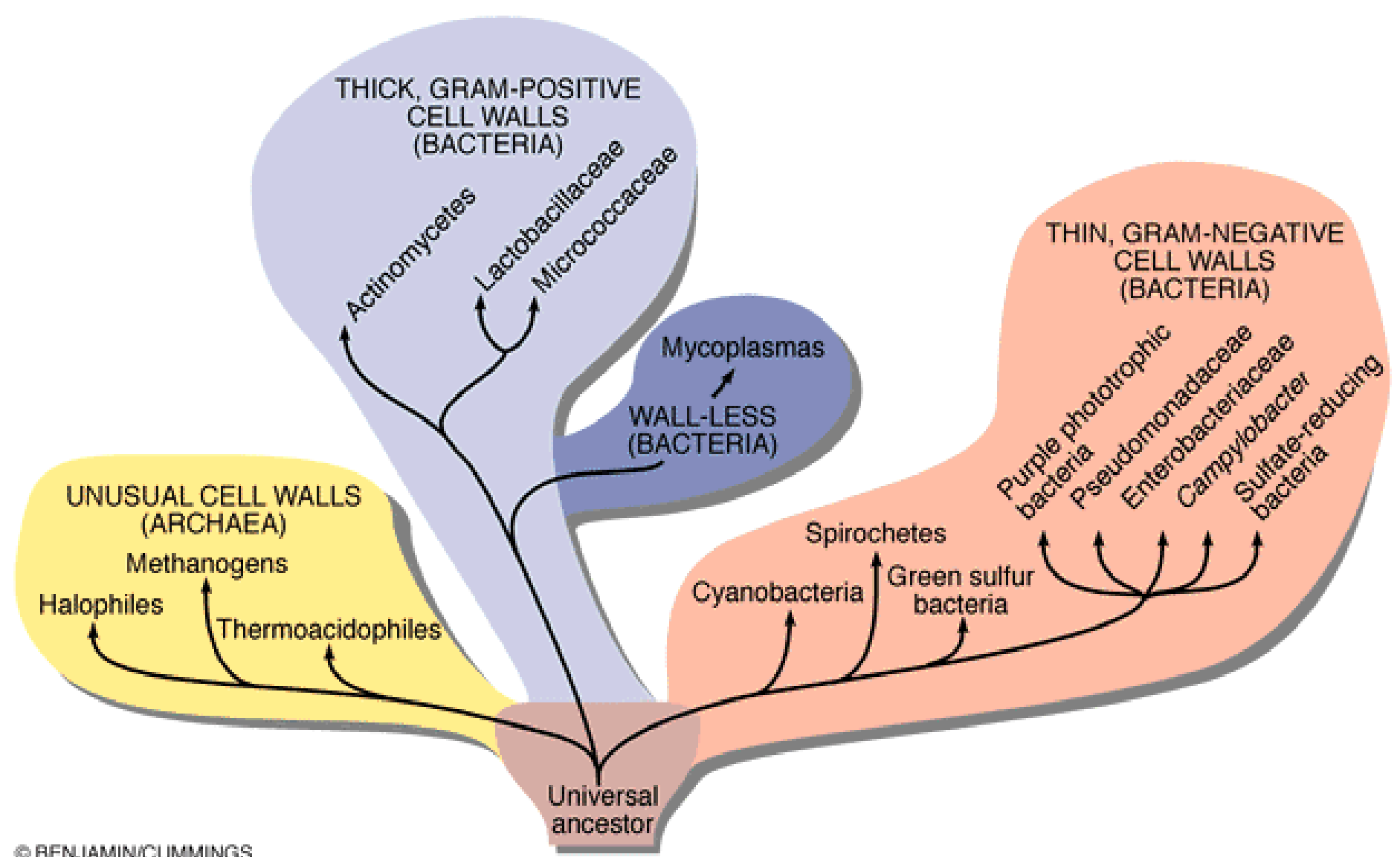
1. Explain the steps for identifying a specific microorganism using the method based on morphological, physiological, physical and chemical characteristics of the microorganism.
2. Explain the steps for identifying a particular microorganism using a method based on the specific nucleotide/protein sequence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



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

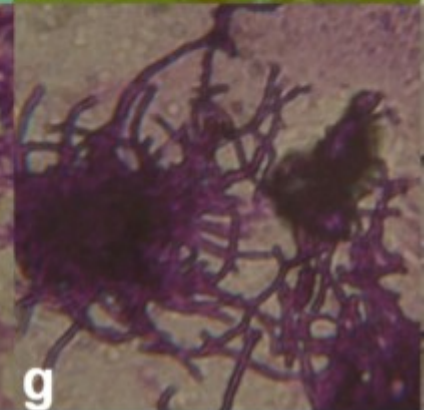
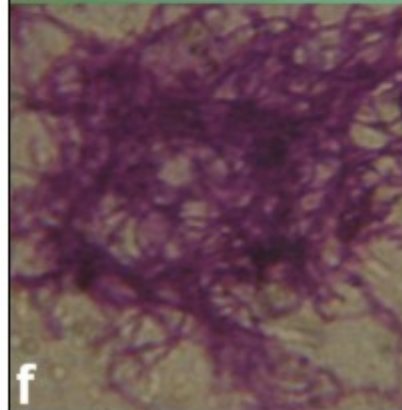
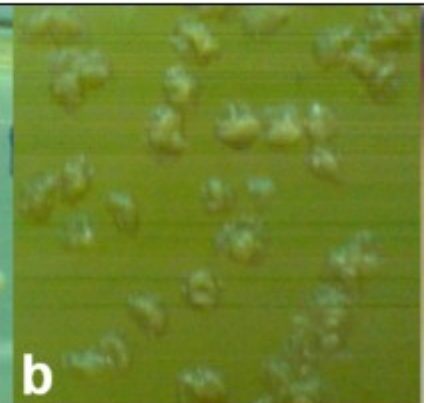
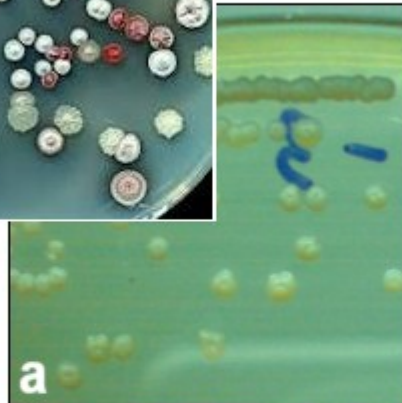
Bergey's Manual: Bacteria are classified into four divisions/phyla; Each division is divided into groups according to phenotypes:

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

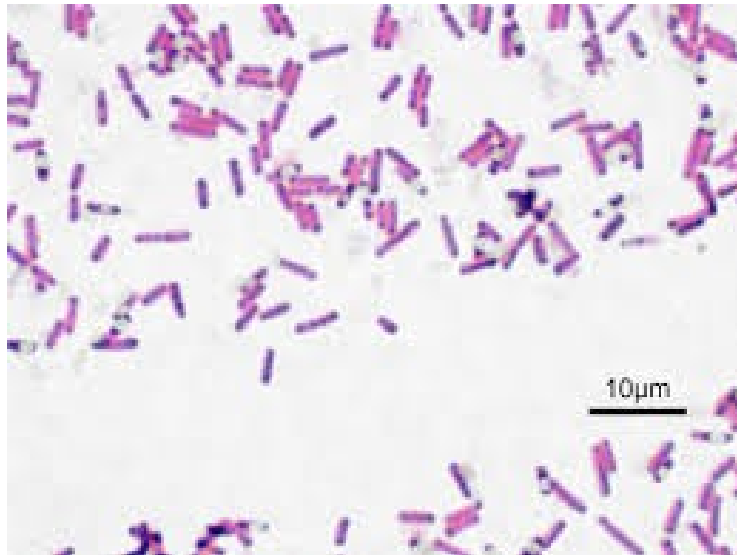
Bergey's Manual of Determinative Bacteriology (current editions): 5 volumes; bacterial phyla organized according to evolutionary relationships between 16S rDNA sequences:

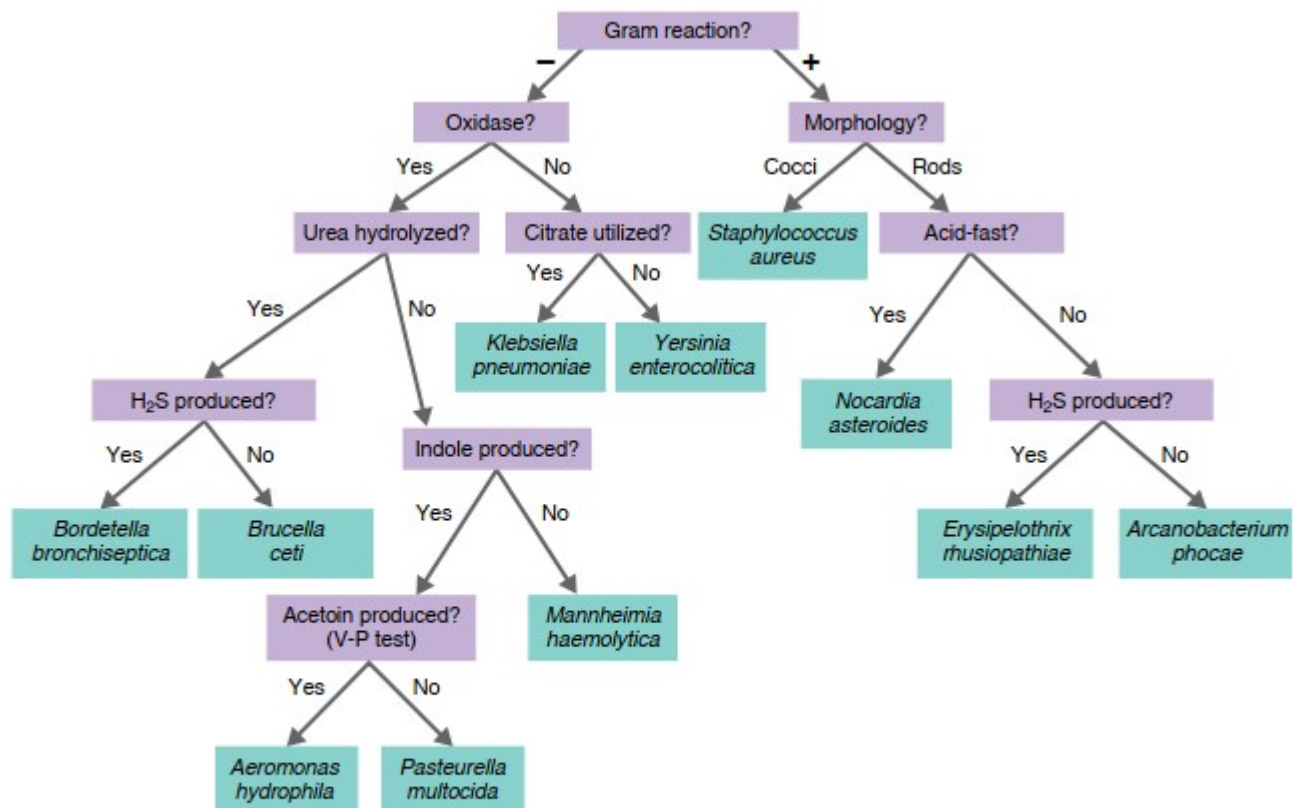
- **Volume 1 (2001):** Archaea, Actinomycetes, & photoautotrophs.
- **Volume 2 (2005):** Proteobacteria, includes:
 - » 2A: Basic methods
 - » 2B: Class Gammaproteobacteria
 - » 2C: Other Proteobacteria classes
- **Volume 3 (2009):** Firmicutes
- **Volume 4 (2011):** Bacteroidetes, Spirochaetes, Tenericutes (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.

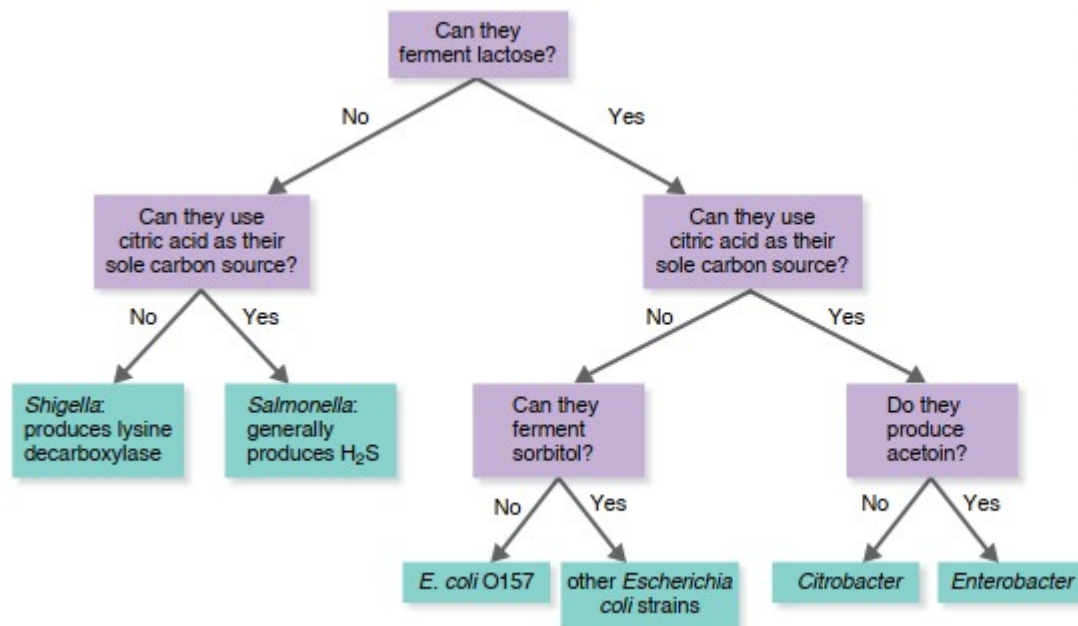
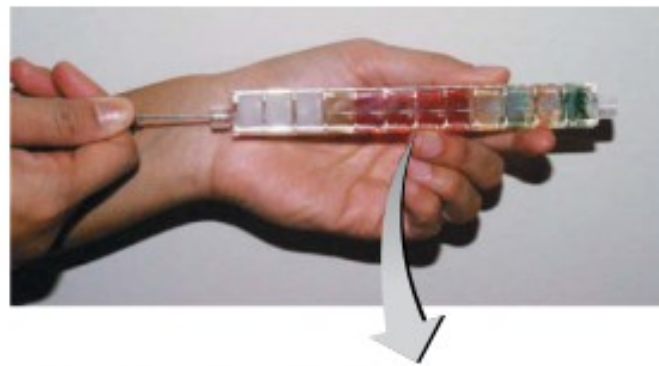


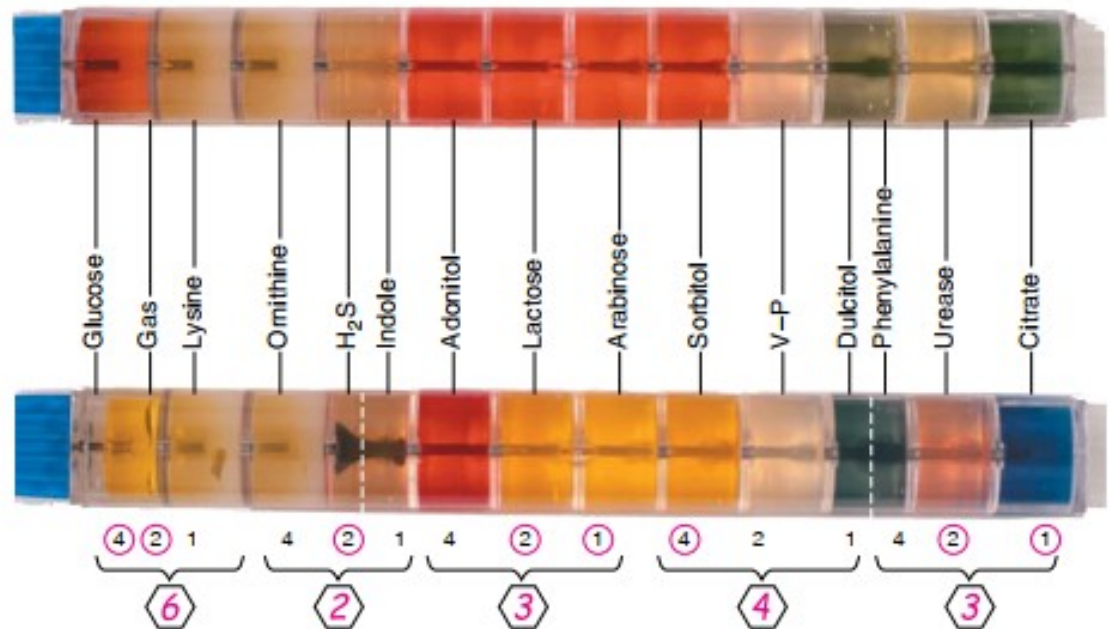
Figure 10.8 The use of metabolic characteristics to identify selected genera of enteric bacteria.

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

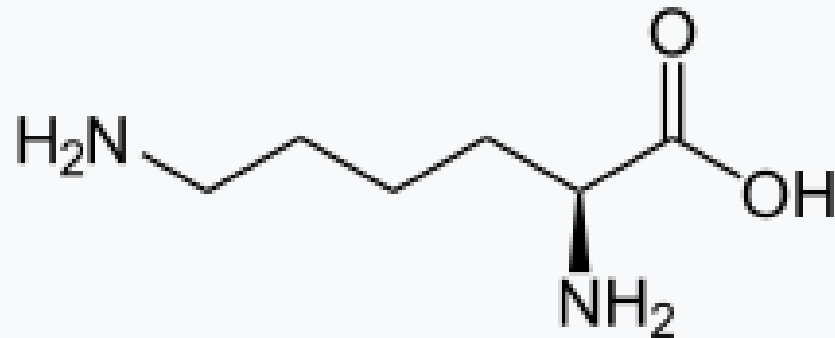
Figure 10.9 One type of rapid identification method for bacteria: Enteropluri test from BD Diagnostics. This example shows results for a typical strain of *C. freundii*; however, other strains may produce different test results, which are listed in the Atypical Test Results column.

Q How can one species have two different code numbers?

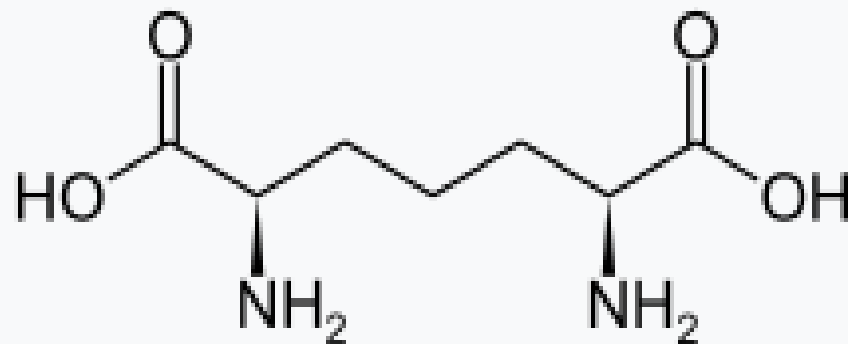
✓ ***Related scientific reports :***

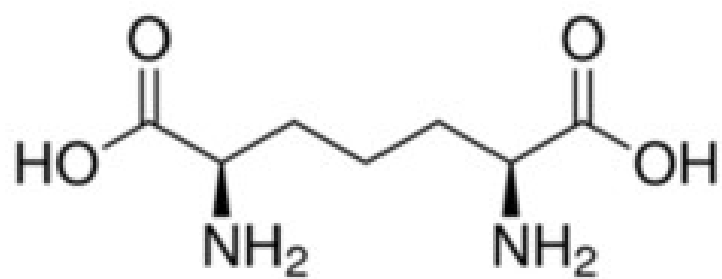
- » Colony morphology: colony shape, mycelium color, spore-forming ability, spore color, ability to diffuse color into the environment, ...
- » Cell morphology: vegetative cell shape, spore shape, ...
- » Chemical composition of cell wall: peptidoglycan, glucosamine, alanine, glutamic acid, glycine,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

Lysine

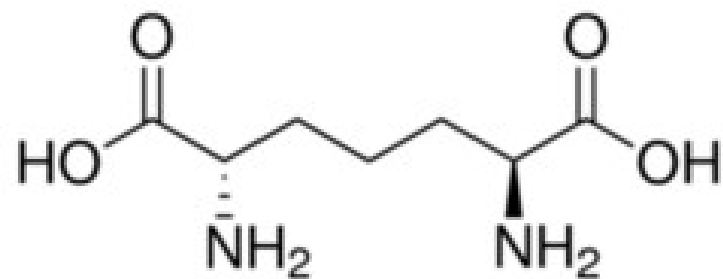


Diaminopimelic acid





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

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

☐ Exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude
 Optional

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to
 Optional

☐ Sequences from type material

Entrez Query
 Optional

[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 |cl|Query_188307
Description None
Molecule type nucleic acid
Query Length 471

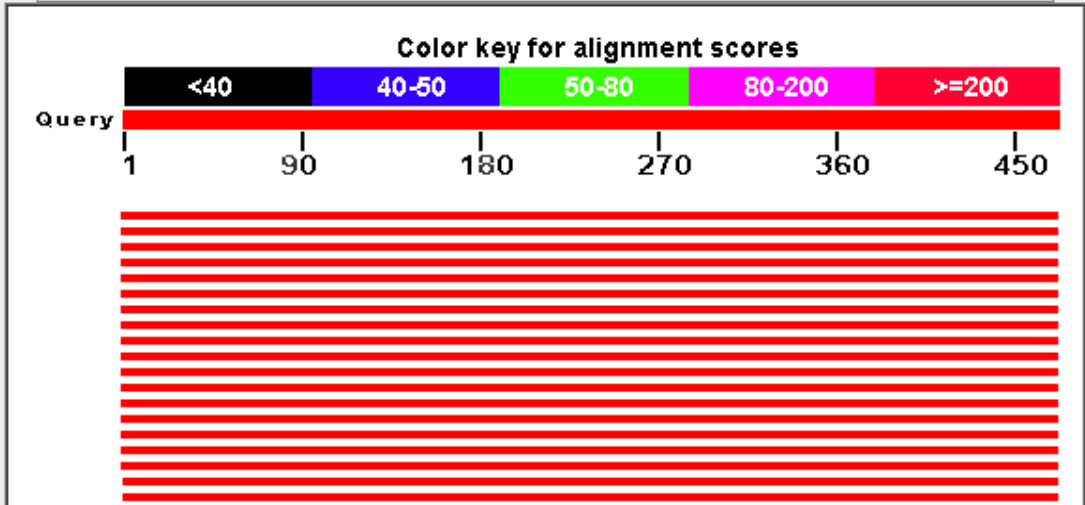
Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.3.1+ [Citation](#)

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



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"/>	Streptomyces flaveus strain X418 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	100%	JX293177.1
<input type="checkbox"/>	Streptomyces sp. NEAU-P17 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	100%	HQ916736.1
<input type="checkbox"/>	Streptomyces flaveus strain S13 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	100%	HQ850408.1
<input type="checkbox"/>	Streptomyces flaveus strain T111 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	100%	GU084180.1
<input type="checkbox"/>	Streptomyces sp. SXY10 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	100%	GU045527.1
<input type="checkbox"/>	Streptomyces sp. FH-2006 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	100%	DQ989584.1
<input type="checkbox"/>	Streptomyces lavendulae DNA for 16S rRNA, strain IFO 13709	867	867	100%	0.0	99%	D85115.1
<input type="checkbox"/>	Streptomyces sp. DP_00001 16S ribosomal RNA gene, partial sequence	865	865	100%	0.0	99%	KM274097.1
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<input type="checkbox"/>	Streptomyces flaveus strain S10714 16S ribosomal RNA gene, partial sequence	865	865	100%	0.0	99%	KF956565.1
<input type="checkbox"/>	Streptomyces spororaveus strain JQZST-6 16S ribosomal RNA gene, partial sequence	865	865	100%	0.0	99%	KF973242.1
<input type="checkbox"/>	Streptomyces sp. HJ1-2 16S ribosomal RNA gene, partial sequence	865	865	100%	0.0	99%	FJ626862.1
<input type="checkbox"/>	Streptomyces subutilus strain HND39 16S ribosomal RNA gene, partial sequence	865	865	100%	0.0	99%	EU030437.1
<input type="checkbox"/>	Streptomyces nojiriensis strain B-33 16S ribosomal RNA gene, partial sequence	865	865	100%	0.0	99%	EF063481.1
<input type="checkbox"/>	Streptomyces sp. So54 partial 16S rRNA gene, strain So54	865	865	100%	0.0	99%	AJ308576.1
<input type="checkbox"/>	Streptomyces sp. DP_00015 16S ribosomal RNA gene, partial sequence	863	863	99%	0.0	99%	KM274100.1
<input type="checkbox"/>	Streptomyces sp. A24 gene for 16S ribosomal RNA, partial sequence	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|JX293177.1](#) Length: 1503 Number of Matches: 1

Related Information

Range 1: 29 to 499 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
870 bits(471)	0.0	471/471(100%)	0/471(0%)	Plus/Plus
Query 1	TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCTT	60		
Sbjct 29	TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCTT	88		
Query 61	CGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGG	120		
Sbjct 89	CGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGG	148		
Query 121	ACAAGCCCTGGAAACGGGGTCTAATACCGGATACCACTCCTGCCTGCATGGGCGGGGGTT	180		
Sbjct 149	ACAAGCCCTGGAAACGGGGTCTAATACCGGATACCACTCCTGCCTGCATGGGCGGGGGTT	208		
Query 181	GAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCTATCAGCTTGTTGGTGGGGTAATGGC	240		
Sbjct 209	GAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCTATCAGCTTGTTGGTGGGGTAATGGC	268		
Query 241	CTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGA	300		
Sbjct 269	CTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGA	328		
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Sbjct 329	CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCT	388		
Query 361	GATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG	420		
Sbjct 389	GATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG	448		
Query 421	AAGAAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC	471		
Sbjct 449	AAGAAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC	499		

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

Sequence ID: [gb|HQ916736.1](#) Length: 1517 Number of Matches: 1