



وزارة التعليم العالي والبحث العلمي  
الجامعة التقنية الشمالية  
المعهد التقني / الموصل



# الحقية التعليمية

القسم العلمي: تقنيات البيئة والموارد المائية

اسم المقرر: الاحياء المجهرية الملوثة للمياه

المرحلة / المستوى: الاول

الفصل الدراسي: الاول

السنة الدراسية: 2024 - 2025



## معلومات عامة

اسم المقرر:	الاحياء المجهرية الملوثة للمياه
القسم:	تقنيات البيئة والموارد المائية
الكلية:	المعهد التقني الموصل
المرحلة / المستوى	الاول
الفصل الدراسي:	الاول
عدد الساعات الاسبوعية:	نظري 15   عملي 15
عدد الوحدات الدراسية:	4
رمز المقرر:	WRT0124
نوع المادة	نظري   عملي   كليهما   √
هل يتوفر نظير للمقرر في الاقسام الاخرى	لا يوجد
اسم المقرر النظير	
القسم	
رمز المقرر النظير	
معلومات تدريسي المادة	
اسم مدرس (مدرسي) المقرر:	د.مها محمد طه
اللقب العلمي:	مدرس
سنة الحصول على اللقب	2021
الشهادة :	دكتوراه
سنة الحصول على الشهادة	2021
عدد سنوات الخبرة ( تدريس )	20 سنة

## الوصف العام للمقرر

يهدف هذا المقرر إلى تزويد الطلاب بأسس علم الأحياء المجهرية، وتقديم نظرة عامة على عالم الكائنات الحية الدقيقة. يغطي المقرر دراسة البكتيريا، والفيروسات، والفطريات، والطحالب، والأوليات من حيث تركيبها، ووظائفها، وتصنيفها، ودورة حياتها، وطرق تكاثرها.

يتناول المقرر أيضًا الجوانب التطبيقية للأحياء المجهرية، مثل دورها في البيئة (دورات العناصر، التحلل)، وأهميتها في الصناعة (صناعة الغذاء، الأدوية)، والطب (الأمراض، التشخيص، العلاج). كما يتم التركيز على التقنيات المخبرية الأساسية المستخدمة في زراعة الكائنات الدقيقة، وعزلها، وتحديدتها، وملاحظتها تحت المجهر.

## الاهداف العامة

يهدف مقرر الأحياء المجهرية بشكل عام إلى تزويد الطلاب بالمعرفة والمهارات الأساسية اللازمة لفهم عالم الكائنات الحية الدقيقة وتطبيقاتها المتنوعة. يتضمن ذلك تحقيق الأهداف التالية:

- فهم الخصائص الأساسية للأحياء المجهرية: تمكين الطلاب من التعرف على الأنواع الرئيسية للكائنات الحية الدقيقة (البكتيريا، الفيروسات، الفطريات، الأوليات، الطحالب) وفهم تراكيبها، ووظائفها، وتصنيفها.
- استيعاب العمليات الحيوية للأحياء المجهرية: شرح كيفية نمو الكائنات الدقيقة وتكاثرها، وعمليات الأيض التي تقوم بها، وطرق حصولها على الطاقة.
- اكتساب المهارات المخبرية الأساسية: تدريب الطلاب على التقنيات المعملية الشائعة المستخدمة في دراسة الأحياء المجهرية، مثل طرق الزراعة، العزل، التلوين، والملاحظة المجهرية.
- إدراك الدور البيئي والصناعي للأحياء المجهرية: تسليط الضوء على الأهمية الحيوية للكائنات الدقيقة في الدورات البيوجيوكيميائية (مثل دورة النيتروجين والكربون)، ودورها في معالجة المياه، وإنتاج الغذاء، والمضادات الحيوية، وغيرها من التطبيقات الصناعية.
- فهم العلاقة بين الأحياء المجهرية وصحة الإنسان: تزويد الطلاب بمعرفة عن مسببات الأمراض الميكروبية، وطرق انتقال العدوى، وآليات دفاع الجسم ضدها، بالإضافة إلى مبادئ التشخيص والعلاج والوقاية من الأمراض المعدية.
- تقدير أهمية التعقيم ومكافحة العدوى: فهم المبادئ الأساسية للتعقيم والتطهير، وأساليب مكافحة نمو الكائنات الدقيقة في مختلف البيئات.
- تنمية التفكير النقدي والمهارات التحليلية: تشجيع الطلاب على تحليل المشكلات المتعلقة بالأحياء المجهرية وتطوير حلول مستنيرة بناءً على المعرفة العلمية.

## الأهداف الخاصة

يهدف هذا المقرر إلى تمكين الطلاب من تحقيق الأهداف الخاصة التالية:

- **تحديد الكائنات المجهرية المسببة لتلوث المياه:** أن يكون الطالب قادرًا على تحديد وتصنيف الأنواع الرئيسية من البكتيريا والفيروسات والطفيليات والطحالب التي تشكل ملوثات ميكروبية شائعة في مصادر المياه المختلفة.
- **وصف مصادر وطرق انتقال الملوثات الميكروبية:** أن يشرح الطالب المصادر المحتملة للأحياء المجهرية المسببة للأمراض في المياه (مثل الصرف الصحي، المخلفات الزراعية والصناعية)، وطرق انتقالها إلى البشر والبيئة.
- **تقييم المخاطر الصحية لتلوث المياه:** أن يحلل الطالب التأثيرات الصحية المحتملة للأمراض المنقولة عبر المياه على الأفراد والمجتمعات، وأن يميز بين الأمراض البكتيرية والفيروسية والطفيلية الشائعة المرتبطة بتلوث المياه.
- **تطبيق التقنيات المخبرية للكشف عن الملوثات:** أن يتقن الطالب التقنيات المخبرية الأساسية المستخدمة في الكشف عن الأحياء المجهرية الملوثة للمياه وتعدادها وتحديداتها، بما في ذلك طرق الزراعة، الاختبارات الجزيئية، والتقنيات المناعية.
- **فهم معايير جودة المياه الميكروبيولوجية:** أن يتعرف الطالب على المعايير واللوائح المحلية والدولية لجودة المياه الميكروبيولوجية، وأن يكون قادرًا على تفسير نتائج التحاليل الميكروبيولوجية لتقييم مدى صلاحية المياه للاستخدامات المختلفة.
- **اقتراح حلول للتحكم في تلوث المياه ومعالجتها:** أن يقترح الطالب استراتيجيات فعالة للوقاية من تلوث المياه الميكروبيولوجي ومعالجتها، بما في ذلك طرق معالجة مياه الشرب والصرف الصحي، وأساليب إدارة المصادر الملوثة.
- **تحليل الحالات الدراسية لتفشي الأمراض المنقولة بالمياه:** أن يحلل الطالب حالات تفشي سابقة للأمراض المنقولة عبر المياه، وأن يستخلص الدروس المستفادة منها في مجال المراقبة والوقاية والاستجابة.



## الأهداف السلوكية او نواتج التعلم

- 1- ان يكون الطالب قادرا على تحديد الأنواع الرئيسية من البكتيريا، الفيروسات، الطفيليات، والطحالب التي تسبب تلوث المياه.
- 2- ان يكون الطالب قادرا على وصف الخصائص المورفولوجية والفسولوجية لكل مجموعة من هذه الكائنات الدقيقة الملوثة للمياه
- 3- ان يكون الطالب قادرا على شرح دورة حياة وطرق تكاثر الكائنات الدقيقة الملوثة للمياه الشائعة.
- 4- ان يكون الطالب قادرا على تفسير العلاقة بين التركيب الخلوي للكائنات الدقيقة ووظيفتها في سياق تلوث المياه.
- 5- ان يكون الطالب قادرا على تطبيق تقنيات أخذ عينات المياه بشكل صحيح للتحليل الميكروبيولوجية.
- 6- ان يكون الطالب قادرا على (ختبارات التعداد الميكروبيولوجي للمياه) مثل طريقة العدد الأكثر احتمالا MPN واختبارات الترشيح الغشائي
- 7- ان يكون الطالب قادرا على توظيف لمجهر الضوئي بفعالية لمراقبة وتحديد الكائنات الدقيقة في عينات المياه.

## المتطلبات السابقة

لتحقيق أقصى استفادة من مقرر الأحياء المجهرية، يجب أن يكون الطالب ملماً بعدد من المفاهيم الأساسية التي تُعد متطلبات سابقة وضرورية. هذه المتطلبات تضمن أن الطالب يمتلك القاعدة المعرفية اللازمة لفهم المواضيع المتقدمة التي سيتم تناولها في المقرر. إليك أهم هذه المتطلبات:

- **الكيمياء العامة والعضوية:** يُعد فهم أساسيات الكيمياء، بما في ذلك التفاعلات الكيميائية، أنواع الروابط، المركبات العضوية (مثل الكربوهيدرات، البروتينات، الدهون، والأحماض النووية)، وخصائصها، أمراً بالغ الأهمية. فالكائنات الدقيقة تعتمد على عمليات كيميائية حيوية معقدة في نموها وتكاثرها وأيضها.
- **مقدمة في علم الأحياء:** يجب أن يكون الطالب على دراية بالمفاهيم الأساسية في علم الأحياء، مثل:
  - **الخلية:** تركيب ووظيفة الخلية بدائية النواة وحقيقية النواة، الفروقات بينهما.

- **الوراثة:** أساسيات الحمض النووي (DNA) والحمض النووي الريبوزي (RNA) ، تخليق البروتين، الانقسام الخلوي (الميتوزي والميوزي).
- **التصنيف البيولوجي:** المبادئ الأساسية لتصنيف الكائنات الحية.
- **العمليات الحيوية الأساسية:** مثل التمثيل الغذائي والتنفس الخلوي.
- **مبادئ الكيمياء الحيوية (مرغوب فيه):** على الرغم من أن مقرر الأحياء المجهرية قد يغطي بعض الجوانب الكيميائية الحيوية، إلا أن وجود خلفية مسبقة في مبادئ الكيمياء الحيوية يمكن أن يعزز فهم الطالب للعمليات الأيضية التي تحدث داخل الكائنات الدقيقة بشكل كبير.
- **مهارات المختبر الأساسية:** القدرة على استخدام الأدوات المخبرية الشائعة بأمان وكفاءة، مثل المجهر، الأنابيب المعقمة، وبعض تقنيات التحضير الأساسية، ستكون مفيدة جداً في الجانب العملي للمقرر.

آلية التقييم	الأهداف السلوكية او مخرجات التعليم الأساسية
آلية التقييم	تفصيل الهدف السلوكي او مخرج التعليم
أسئلة الاختيارات المتعددة	<p><b>1</b> فهم دور الكائنات المجهرية في تلوث المياه: أن يصف الطالب الأنواع الرئيسية من الكائنات المجهرية الملوثة للمياه (مثل البكتيريا المسببة للأمراض، الفيروسات، الأوليات، والطحالب السامة)، ومصادرها، وطرق انتقالها في البيئات المائية المختلفة.</p>
أسئلة الإجابات القصيرة	<p><b>2</b> تحديد الأمراض المنقولة عن طريق المياه: أن يربط الطالب بين الكائنات المجهرية المحددة والأمراض التي تسببها للإنسان والحيوان، مع التركيز على دورة حياة مسببات المرض وطرق الوقاية والتحكم.</p>
المناقشات الصفية والتقييم الشفهي	<p><b>3</b> تقييم جودة المياه ميكروبيولوجيًا: أن يتعرف الطالب على المؤشرات الميكروبية المستخدمة لتقييم جودة المياه (مثل بكتيريا القولون الكلبة والبرازية)، وأن يفهم أهمية هذه المؤشرات في الك <sup>المراقبة</sup> الصحية للمياه.</p>
التقييم الشفهي	<p><b>4</b> تطبيق طرق الكشف والتحليل الميكروبيولوجي للمياه: أن يكتسب الطالب المهارات العملية لتحليل عينات المياه ميكروبيولوجيًا، بما في ذلك أخذ العينات، تحضير الأوساط الزرعية، عزل وتحديد الكائنات المجهرية الملوثة باستخدام التقنيات التقليدية والحديثة.</p>

## أساليب التدريس (حدد مجموعة متنوعة من أساليب التدريس لتناسب احتياجات الطلاب ومحتوى المقرر)

مبررات الاختيار	الاسلوب او الطريقة
قياس المعرفة الأساسية ووضع خط أساس: يتيح الاختبار تحديد مدى استيعاب الطلاب للمفاهيم الأساسية قبل التعمق فيها.	1. العصف الذهني
فعالة في نقل كم كبير من المعلومات لعدد كبير من الطلاب، وتوفر هيكلًا واضحًا للمادة.	2.لقاء المحاضرات
تشجيع الطلاب على تبادل الأفكار والآراء حول موضوع معين، مما يعزز الفهم العميق.	3. لمناقشات الجماعية
تشجع الطلبة على البحث والاستنتاج بأنفسهم	4.الاستكشاف
تكليف الطلاب بإنجاز مشاريع عملية مرتبطة بمجال دراستهم.	5.التعليم القائم على المشاريع
توجيه الطلاب لطرح الأسئلة والبحث عن إجابات بأنفسهم .	6. لتعلم القائم على الاستقصاء:

# المحتوى العلمي

م	ع	ن	عدد الساعات الأسبوعية	نظام المقررات	القسم العلمي
4	2	2			تقنيات البيئة والموارد المائية
اسم المقرر باللغة الانكليزية Microorganism polluted water				المستوى الأول / الثاني	اسم المقرر باللغة العربية الاحياء المجهرية الملوثة للمياه
هدف المادة العام : — تهدف مادة الأحياء المجهرية الملوثة للمياه بشكل عام إلى تزويد الطالب بفهم عميق وشامل لدور الكائنات المجهرية في تلوث البيئات المائية المختلفة، وتأثيراتها المحتملة على صحة الإنسان والبيئة.					

أسبوع	المفردات النظرية
1	مقدمه في علم الأحياء المجهرية : التعريف – الأهمية - صفات وأقسام الأحياء المجهرية بصورة عامه العلاقة بين الأحياء المجهرية وأشكال الحياة الأخرى وانتشار الأحياء المجهرية في الطبيعة
2	نظام تسمية وتصنيف الأحياء المجهرية : الطريقة العامة التي سميت بها الأحياء المجهرية باستخدام نظام التسمية الثنائية وأعضاء البكتريا – الأحياء الشائعة لبكتريا تصنيف الأحياء المجهرية بصورة عامة وحسب طبقات التصنيف ( نوع / جنس الى أخرى 00 )
3-4	البكتريا ، تعريفها ، صفاتها الظاهرية – انتشارها في الطبيعة تركيب خلية البكتريا – نمو وتكاثر البكتريا – معدل النمو – منحنى النمو البكتيري 0
5-6	العوامل التي تؤثر على النمو / إنبات البكتريا في ظروف المختبر الاحتياجات المختلفة للإنبات / المزارع النقية أهميتها وطرق عزلها / تقسيم البكتريا
7	السيطرة على الأحياء المجهرية
8	السيطرة على الأحياء المجهرية بالطرق الفيزيائية
9	السيطرة على الأحياء المجهرية بالطرق الكيميائية

10	العفن : انتشاره في الطبيعة / الأهمية - صفاته الظاهرية - التكاثر بعض الاعفان المهمة
-11 12	الخمائر : التعريف - انتشارها في الطبيعة- صفاته الظاهرية- التركيب الخلوي - التكاثر- بعض أنواع المهمة
-13 14	الطحالب والاشنات : تعريفها / انتشارها في الطبيعة / صفاتها تكاثرها / الصفات في الطحالب / الأهمية الاقتصادية للطحالب
15	الابتدائيات : صفاتها / تقسيمها / تركيبها الخلوي - تكاثرها - أهم الأنواع المرضية في المياه

**المصادر : - Brock Biology of Microorganisms" by Michael T. Madigan, Kelly S. Bender, Daniel H. Buckley, W. Matthew Sattley, and David A. Stahl:**

- يُعد هذا الكتاب مرجعاً شاملاً في علم الأحياء الدقيقة، ويحتوي على فصول مخصصة لميكروبيولوجيا البيئة والمياه، وتلوث المياه، ودور الكائنات الدقيقة في معالجة المياه.

• **"Environmental Microbiology" by Ralph Mitchell and Joan E. Spira:**

- يركز هذا الكتاب بشكل خاص على دور الكائنات الدقيقة في البيئة، بما في ذلك الأنظمة المائية وتلوثها، ويوفر نظرة معمقة على الكيمياء الحيوية والبيئة الميكروبية للمياه.

• **"Water Microbiology: Bacterial Pathogens and Waterborne Diseases" by Palmer H. J. and Paul W. J. G.:**

- يركز هذا الكتاب بشكل خاص على مسببات المرض البكتيرية المنقولة عن طريق المياه والأمراض التي تسببها، وهو مصدر ممتاز لفهم الجانب الصحي لتلوث المياه.



## المفردات العملية

الأسبوع	تفاصيل المفردات
1	مقدمه في علم الأحياء المجهرية : التعريف - الأهمية - صفات وأقسام الأحياء المجهرية بصورة عامه العلاقة بين الأحياء المجهرية وأشكال الحياة الأخرى وانتشار الأحياء المجهرية في الطبيعة
2	نظام تسمية وتصنيف الأحياء المجهرية : الطريقة العامة التي سميت بها الأحياء المجهرية باستخدام نظام التسمية الثنائية وأعضاء البكتريا - الأحياء الشائعة للبكتريا تصنيف الأحياء المجهرية بصورة عامة وحسب طبقات التصنيف ( نوع / جنس الى أخرى 00 )
3-4	البكتريا ، تعريفها ، صفاتها الظاهرية - انتشارها في الطبيعة تركيب خلية البكتريا - نمو وتكاثر البكتريا - معدل النمو - منحنى النمو البكتيري 0
5-6	العوامل التي تؤثر على النمو / إنبات البكتريا في ظروف المختبر / الاحتياجات المختلفة للإنبات / المزارع النقية أهميتها وطرق عزلها / تقسيم البكتريا
7	دراسة الشكل الظاهري لبكتريا ( القطرة المعلقة)
8	التصبيغ البسيط
9	الصبغة المركبة كرام
10	دراسة الشكل الظاهري لعفن
11	دراسة الشكل الظاهري للخمائر
12	دراسة الشكل الظاهري للطحالب
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## المصادر

- **Standard Methods for the Examination of Water and Wastewater"** by American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF):

- يُعتبر هذا الكتاب هو المرجع الذهبي والطبعة الأكثر شمولاً :الأهمية وتفضيلاً للطرق المعيارية المعتمدة عالمياً لتحليل المياه ومياه الصرف الصحي، بما في ذلك جميع التحاليل الميكروبيولوجية للكشف عن البكتيريا الكلية، البكتيريا القولونية، بكتيريا الإشريكية القولونية، الفيروسات، والطفيليات. يحتوي على بروتوكولات مفصلة خطوة بخطوة لكل اختبار.

- يوفر إجراءات دقيقة ومعايرة لضمان موثوقية: لماذا هو أساسي للعملي النتائج في المختبر.

- **"Water Microbiology: A Practical Approach" by W. B. B. M. and W. G. R.:**

- يركز هذا الكتاب بشكل كبير على الجوانب العملية: الأهمية لميكروبيولوجيا المياه، ويقدم توجيهات واضحة حول التقنيات المخبرية، وطرق عزل الكائنات الدقيقة، وكشف مسببات المرضية في عينات المياه.
- مصمم ليكون دليلًا عمليًا للطلاب والباحثين: لماذا هو أساسي للعملي في المختبر.

- **"Manual of Environmental Microbiology" by Christon J. Hurst, Ronald L. Crawford, Gunhild K. Hurst, Alfred L. Mills, Jay V. Garland, and John T. Lisle (Editors):**

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## المحتوى العلمي من الفصل الأول

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الأسبوع الأول	2		<b>Introduction to Microbiology</b>  <b>Branches of ‘ Microbiology</b>  <b>Microorganisms’ as Agents of ‘Disease</b>  <b>Microorganisms, Agriculture, and Digestive ‘Processes</b>	محاضرة ومناقشة	عرض تقديمي، شرح، أسئلة وأجوبة، مناقشة	مهام، واجبات ، اختبار قصير
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# Introduction to Microbiology

**Microbiology** is a specialized area of biology that deals with tiny life forms that are not readily observed without magnification.

Such **microscopic** organisms are collectively referred to as **microorganisms, microbes**, or several other terms, depending upon the purpose.

There are several major groups of microorganisms. They are **archaea, bacteria, fungi, algae, protozoa, helminths** (parasitic worms) and **viruses**.

**Microbiology** is the study of microorganisms.

## Branches of Microbiology

- **Bacteriology**: The study of bacteria.
- **Mycology**: The study of fungi.
- **Protozoology**: The study of protozoa.
- **Phycology/algology**: The study of algae.
- **Parasitology**: The study of parasites.
- **Immunology**: The study of the immune system.
- **Virology**: The study of viruses.
- **Microbial physiology**: The study of how the microbial cell functions biochemically. Includes the study of microbial growth, microbial metabolism and microbial cell structure.

- **Microbial ecology:** The relationship between microorganisms and their environment.
- **Microbial genetics:** The study of how genes are organized and regulated in microbes in relation to their cellular functions. Closely related to the field of molecular biology.
- **Microbial taxonomy:** The naming and classification of microorganisms.
- **Medical microbiology:** The study of the pathogenic microbes and the role of microbes in human illness.
- **Industrial microbiology:** The exploitation of microbes for use in industrial processes. Examples include industrial fermentation and wastewater treatment .
- **Food microbiology:** The study of microorganisms causing food spoilage and foodborne illness. Using microorganisms to produce foods.
- **Soil microbiology:** The study of those microorganisms that are found in soil.
- **Water microbiology (or Aquatic microbiology):** The study of those microorganisms that are found in water.
- **Aeromicrobiology (or Air microbiology):** The study of airborne microorganisms.

## **The Impact of Microorganisms on Humans**

Through the years microbiologists have had great success in discovering **how microorganisms work**, and **application of this knowledge** has greatly increased the beneficial effects of microorganisms and curtailed many of their harmful effects.

Microbiology has thus greatly advanced human health and welfare. Besides understanding microorganisms as agents of disease, microbiology has made great advances in understanding the role of microorganisms in food and agriculture, and in exploiting microbial activities for producing valuable human products, generating energy, and cleaning up the environment.

### **Microorganisms as Agents of Disease**

At the beginning of the twentieth century, the major causes of death in humans were **infectious diseases** caused by microorganisms called **pathogens**. Today, however, infectious diseases are much less deadly, at least in developed countries.

Control of infectious disease has come from an increased understanding of disease processes, improved sanitary and public health practices, and the use of antimicrobial agents, such as antibiotics. Although many infectious diseases can now be controlled, microorganisms can still be a major threat, particularly in developing countries.

In the latter, microbial diseases are still the major causes of death, and millions still die yearly from other microbial diseases such as **malaria, tuberculosis, cholera, African sleeping sickness, measles, pneumonia and other respiratory diseases, and diarrheal syndromes.**

In addition to these, humans worldwide are under threat from diseases that could emerge suddenly, such as **bird or swine flu**, or **Ebola hemorrhagic fever**, which are primarily animal diseases that under certain circumstances can be transmitted to humans and spread quickly through a population.

### **Microorganisms, Agriculture, and Digestive Processes**

Agriculture benefits from the cycling of nutrients by microorganisms. For example:

1. A number of major crop plants are legumes. Legumes live in close association with bacteria that form structures called nodules on their roots. In the root nodules, these bacteria convert atmospheric nitrogen ( $N_2$ ) into ammonia ( $NH_3$ ) that the plants use as a nitrogen source for growth (**Figure 1**).

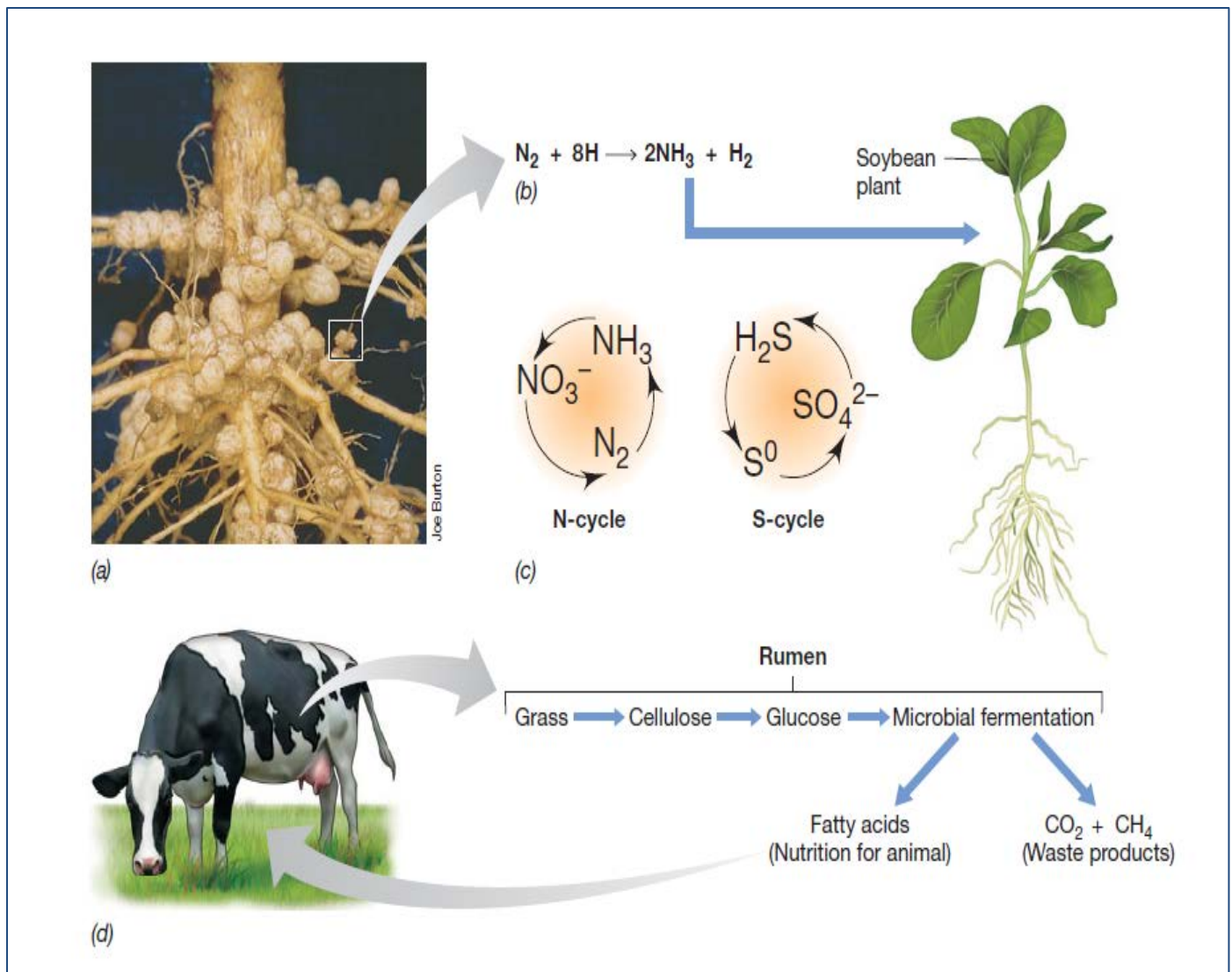
2. Other bacteria cycle sulfur compounds, oxidizing toxic sulfur species such as hydrogen sulfide ( $H_2S$ ) into sulfate ( $SO_4^{2-}$ ), which is an essential plant nutrient (Figure 1 c).



3. Also of major agricultural importance are the microorganisms that inhabit ruminant animals, such as cattle and sheep. These important domesticated animals have a characteristic digestive vessel called the rumen in which large populations of microorganisms digest and ferment cellulose, the major component of plant cell walls, at neutral pH (Figure 1 d). Without these **symbiotic** microorganisms, cattle and sheep could not thrive on cellulose-rich (but otherwise nutrient-poor) food, such as grass and hay. Many domesticated and wild herbivorous mammals—including deer, bison, camels, giraffes, and goats— are also ruminants.

*The ruminant digestive system contrasts sharply with that of humans and most other animals. In humans, food enters a highly acidic stomach where major digestive processes are chemical rather than microbial. In the human digestive tract, large microbial populations occur only in the colon (large intestine), a structure that comes after the stomach and small intestine and which lacks significant numbers of cellulose-degrading bacteria. However, other parts of the human body can be loaded with bacteria.*

*In addition to the large intestine, the skin and oral cavity contain a significant normal microbial flora, most of which benefits the host or at least does no harm. In addition to benefiting plants and animals, microorganisms can also, of course, have negative effects on them.*



**Figure 1:** Microorganisms in modern agriculture.

(a, b) Root nodules on this soybean plant contain bacteria that fix molecular nitrogen ( $\text{N}_2$ ) for use by the plant.

(c) The nitrogen and sulfur cycles, key nutrient cycles in nature.

(d) Ruminant animals. Microorganisms in the rumen of the cow convert cellulose from grass into fatty acids that can be used by the animal.

## **Microorganisms, Food, Energy, and the Environment**

### **1.Food**

Microorganisms play important roles in the **food industry**, including in the areas of **spoilage, safety, and production**. After plants and animals are produced for human consumption, the products must be delivered to consumers in a wholesome form. Food spoilage alone results in huge economic losses each year.

Indeed, the **canning, frozen food, and dried-food industries** were founded as means to preserve foods that would otherwise easily undergo microbial spoilage. Food safety requires constant monitoring of food products to ensure they are **free of pathogenic microorganisms** and to **track disease outbreaks to identify the source(s)**.

However, not all microorganisms in foods have harmful effects on food products or those who eat them. For example:

1. Many dairy products depend on the activities of microorganisms, including the fermentations that yield cheeses, yogurt, and buttermilk.

2. Sauerkraut, pickles, and some sausages are also products of microbial fermentations.

3. Moreover, baked goods rely on the fermentative activities of yeast, which generate carbon dioxide (CO<sub>2</sub>) to raise the dough

## 2. Energy

Some microorganisms produce biofuels. Natural gas (methane) is a product of the anaerobic degradation of organic matter by methanogenic microorganisms .

Ethyl alcohol (ethanol), which is produced by the microbial fermentation of glucose from feedstocks such as sugarcane or cornstarch, is a major motor fuel in some countries .

## 3. The Environment

Microorganisms are used to clean up human pollution, a process called microbial bioremediation, and to produce commercially valuable products by industrial microbiology and biotechnology. For example: microorganisms can be used to **consume spilled oil, solvents, pesticides, and other environmentally toxic pollutants.**

Bioremediation accelerates cleanup in either of two ways:

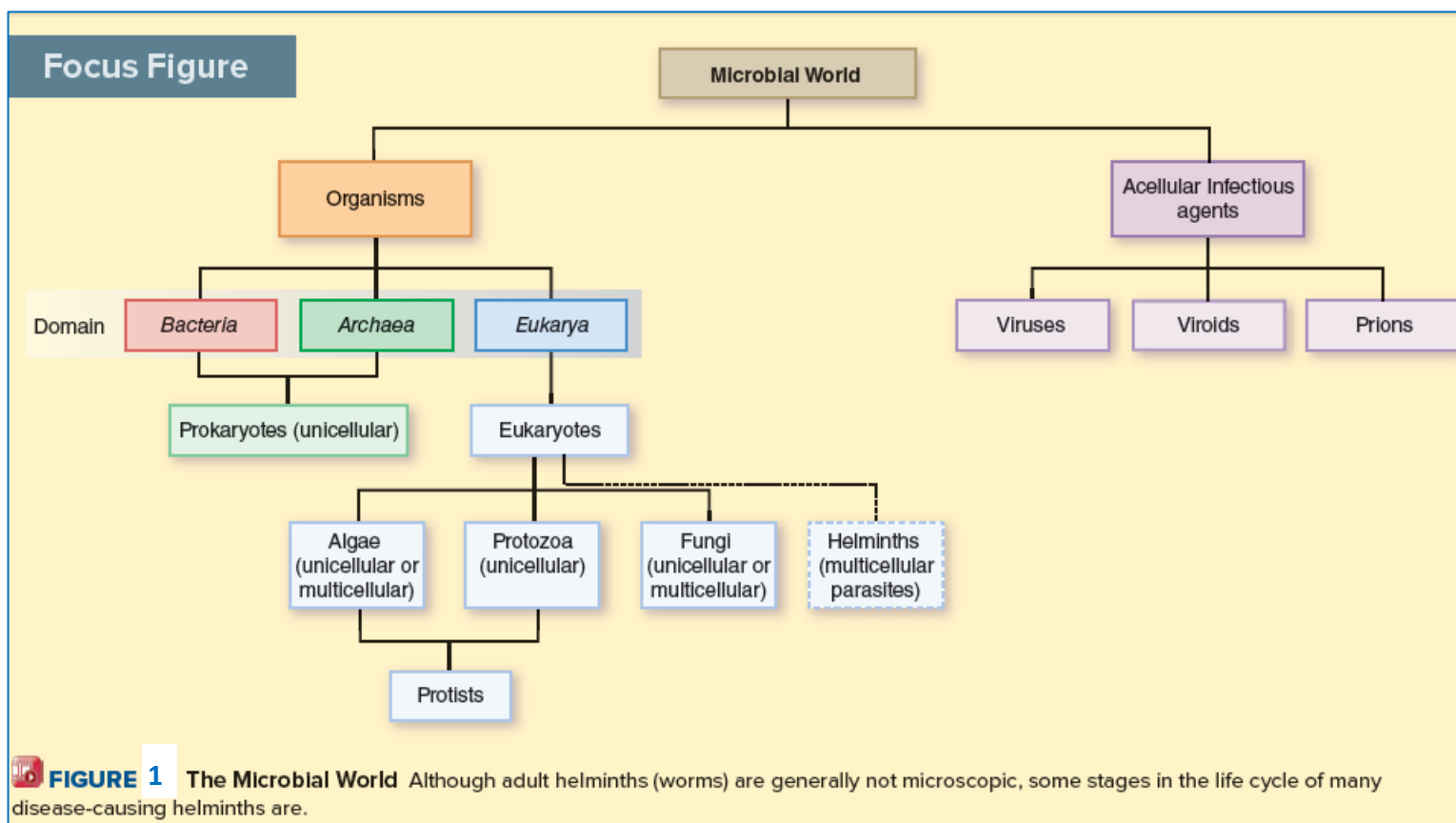
(1) by introducing specific microorganisms to a polluted environment.

or (2) by adding nutrients that stimulate preexisting microorganisms to degrade the pollutants.

In both cases the goal is to accelerate metabolism of the pollutant.

## Members of the Microbial World

Considering that small size is the only shared feature of all microbes, the group is tremendously diverse ( **figure 1** ). If you look at the macroscopic world around you—the plants and animals—you should be impressed by the assortment of what you see. That range, however, is dwarfed by the huge variety of microbes! The extent of the diversity makes sense considering that microbes have inhabited this planet for billions of years and have evolved to thrive in every conceivable environment—from the hydrothermal vents at the bottom of the ocean, to the icy tops of the highest mountains.



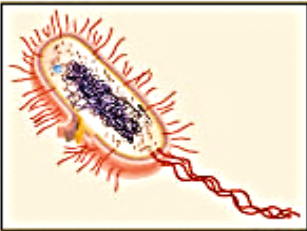

Living organisms are all composed of cells with one of two basic structures— **prokaryotic** ( *pro* means “prior to” and *karyote* means “nucleus”) and **eukaryotic** ( *eu* means “true”). Prokaryotic cells typically do not have a membrane-bound nucleus or any other membrane-bound organelles. Instead, the genetic material is located in a region called the nucleoid. In contrast, the genetic material in eukaryotic cells is contained within a membrane-bound nucleus. Eukaryotic cells often have a variety of other organelles as well, and the cells are more complex than prokaryotic cells. Organisms that consist of a prokaryotic cell are called **prokaryotes** , whereas those composed of one or more eukaryotic cells are called **eukaryotes**.

Prokaryotes fall into two very different groups—bacteria and archaea—as different from each other as they are from eukaryotes. Because of the fundamental differences between bacteria and archaea as well as differences between those two groups and eukaryotic cells, all living organisms are now classified into three different **domains** — *Bacteria*, *Archaea*, and *Eukarya* (sometimes spelled *Eucarya*). Members of the *Bacteria* and *Archaea* are prokaryotes, whereas members of the *Eukarya* are eukaryotes. The names of the domains are italicized, with the first letter capitalized; the members of the domains are referred to as bacteria, archaea, and eukarya, respectively.

**Table 1** compares **some features** of members of **prokaryotes & eukaryotes**.

**Figure 2** compares **some features** of members of the three domains.

**TABLE 1** Principal Differences Between Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic	Eukaryotic
		
Size of cell	Typically 0.2–2.0 $\mu\text{m}$ in diameter	Typically 10–100 $\mu\text{m}$ in diameter
Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear membrane and nucleoli
Membrane-enclosed organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria, and chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors present
Cytoplasm	No cytoskeleton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA)	Single circular chromosome; lacks histones	Multiple linear chromosomes with histones arrangement
Cell division	Binary fission	Mitosis
Sexual reproduction	No meiosis; transfer of DNA fragments only	Involves meiosis

**Bacteria**

- Prokaryotic cell structure, including a nucleoid; no membrane-bound organelles.
- Ribosomal RNA nucleotide sequences that are unique to this group.
- Unicellular.
- Cell wall contains peptidoglycan.
- Typical cell sizes range from 0.3–2  $\mu\text{m}$

**Archaea**

- Prokaryotic cell structure, including a nucleoid; no membrane-bound organelles.
- Ribosomal RNA nucleotide sequences that are unique to this group.
- Unicellular.
- No peptidoglycan.
- Typical cell sizes range from 0.3–2  $\mu\text{m}$

**Eukarya**

- Eukaryotic cell structure, including a nucleus and other membrane-bound organelles.
- Ribosomal RNA nucleotide sequences that are unique to this group.
- Unicellular or multicellular.
- No peptidoglycan.
- Typical cell sizes range from 5–50  $\mu\text{m}$ .

**FIGURE 2** Characteristics of Members of the Three Domains



## Bacteria

Bacteria (singular: bacterium) are single-celled prokaryotes. Most bacteria have specific shapes, commonly cylindrical (rod-shaped), spherical (round), or spiral.

They typically have rigid cell walls that contain peptidoglycan, a compound unique to bacteria. Many of the bacteria can move using flagella (singular: flagellum), appendages that extend from the cell.

Bacteria typically multiply by binary fission, a process in which one cell enlarges and then divides. This forms two cells, each equivalent to the original.

Many bacteria obtain energy from foods similar to what humans eat, but others can gain energy from seemingly unlikely sources such as hydrogen sulfide (a gas that smells like rotten eggs). Still others are photosynthetic, meaning they make cellular material using the radiant energy of sunlight.

Bacteria are abundant in soil, water, and air, including sites that have extreme temperatures, pH, or salinity.

Bacteria are also major inhabitants of our skin, mouth, and intestines.

Most bacteria are beneficial, but some cause serious diseases.

## Archaea

**Like bacteria**, archaea (singular: archaeon) are single-celled prokaryotes. They have similar shapes, sizes, and appearances to bacteria. In addition, they also multiply by binary fission, move primarily by means of flagella, and have rigid cell walls. Like bacteria, different groups of archaea use different energy sources; some are photosynthetic, harvesting the energy of sunlight to make cellular material.

**Archaea differ from bacteria** in several of their structural and functional components. For example, the archaeal cell wall does not contain peptidoglycan, whereas the bacterial wall does. Archaea also have characteristic nucleotide sequences in their ribosomal RNA (a molecule involved in protein synthesis) that differ significantly from those of bacteria. The discovery of the differences in ribosomal RNA sequences helped provide the basis for separating the two groups of prokaryotes into different domains.

**An interesting feature of many archaea** is their ability to grow in extreme environments in which most other organisms cannot survive. Some, for example, can grow in salt concentrations 10 times higher than that of seawater. These organisms grow in such habitats as the Great Salt Lake and the Dead Sea. Others grow best at extremely high temperatures. One archaeon can grow at a temperature of 121° C! (100° C is the temperature at which water boils at sea level). Although the archaea that grow in extreme environments are the most intensively studied, many others are common in moderate environments. They are widely distributed in soils, the oceans, marshes, as well as in the intestinal tract. Some are part of microbial communities found to be associated with severe cases of periodontitis, a destructive inflammation of the gums.

## Eukarya

Eukarya are eukaryotes; those studied by microbiologists include fungi, algae, protozoa, and helminths (worms) ( **table 2** ). Algae and protozoa are also referred to as **protists** .

TABLE 2 Eukaryotic Organisms Studied by Microbiologists	
Organism	Characteristics
Fungi	Use organic material for energy. Size range from microscopic (yeasts) to macroscopic (molds and mushrooms).
Algae	Use sunlight for energy. Size range from microscopic (single-celled algae) to macroscopic (multicellular algae).
Protozoa	Use organic material for energy. Single-celled microscopic organisms.
Helminths	Use organic material for energy. Adult worms are typically macroscopic and often quite large, but their eggs and larval forms are microscopic.

## **Fungi**

**Fungi** (singular: fungus) are a diverse group of eukaryotes, ranging from single celled yeasts that can reproduce by budding to multicellular filamentous molds. The microscopic filaments of molds, called hyphae (singular: hypha), can branch as well as twist and turn to form a visible mat. When you see moldy foods, you are looking at the mat, sometimes along with structures that give rise to a reproductive form called conidia (also referred to as spores). The conidia easily become airborne, allowing the fungus to spread. Some fungi make macroscopic reproductive structures that we call mushrooms.

Fungi gain their energy from degrading organic materials. Unlike animals, however, which ingest their foods, fungi secrete enzymes to degrade the organic material, and then take in the nutrients that are released. Fungi are found in most places where organic materials, including dead plants and animals, are present.

They absorb nutrients from their environment, including the organic molecules they use as sources of carbon and energy.

Because of their metabolic capabilities, many fungi play beneficial roles, including making bread rise, producing antibiotics, and decomposing dead organisms.

Some fungi associate with plant roots to form mycorrhizae. Mycorrhizal fungi transfer nutrients to the roots, improving growth of the plants, especially in poor soils.

Other fungi cause plant diseases (e.g., rusts, powdery mildews, and smuts) and diseases in humans and other animals.

## Algae

**Algae** (singular: alga) are a diverse group of photosynthetic eukaryotes. Some are single-celled, whereas others are multicellular, such as seaweed.

All algae contain chloroplasts, which have chlorophyll, a green pigment. Some also contain other pigments that give them characteristic colors. The pigments absorb the energy of light, which is used in photosynthesis.

Algae are usually found near the surface of either salt or fresh water or in moist terrestrial habitats.

Their cell walls are rigid, but the chemical composition of the wall is quite distinct from that of bacteria and archaea. Many algae move by means of flagella, which are structurally far more complex and unrelated to flagella of prokaryotes.

## Protozoa

**Protozoa** (singular: protozoan) are a diverse group of microscopic, single-celled eukaryotes that live in both aquatic and terrestrial environments. Although microscopic, they are very complex organisms and generally much larger than prokaryotes.

Unlike algae and fungi, protozoa do not have a rigid cell wall. Most protozoa are motile and ingest organic material as food sources.

## Helminths

Parasitic **helminths** are worms that live at the expense of a host. They are an important cause of disease, particularly in developing countries.

The adult worms are generally macroscopic, meaning they can be seen with the unaided eye, and some of them are quite large, so technically they are not microorganisms.

Microbiologists study them, however, because they cause disease and because diagnosis often involves identifying their eggs and larval forms, which are microscopic. Helminths include roundworms, tapeworms, and flukes.

## Acellular Infectious Agents

Viruses, viroids, and prions are **acellular infectious agents**, meaning that they are not composed of cells. They cannot reproduce independently and are considered non-living. By definition, an organism must be composed of one or more cells, so these acellular infectious agents are not microorganisms. Their distinguishing features are listed in **table 3**.

<b>TABLE 3      Acellular Infectious Agents</b>	
<b>Agent</b>	<b>Characteristic</b>
<b>Viruses</b>	Consist of either DNA or RNA, surrounded by a protein coat. Obligate intracellular agents that use the machinery and nutrients of host cells to replicate.
<b>Viroids</b>	Consist only of RNA; no protein coat. Obligate intracellular agents that use the machinery and nutrients of host cells to replicate.
<b>Prions</b>	Consist only of protein; no DNA or RNA. Misfolded versions of normal cellular proteins that cause the normal versions to misfold.

### Viruses

**Viruses** consist of nucleic acid packaged within a protein coat. To multiply, viruses infect living cells—referred to as **hosts**—and then use the machinery and nutrients of those cells to replicate. Outside the hosts, however, viruses are inactive. **Thus, viruses are obligate intracellular agents, meaning that they cannot replicate outside of a host.**

All forms of life, including bacteria, archaea, and eukarya, can be infected by viruses but of different types. Although viruses frequently kill the cells in which they replicate, some types can remain within the host cell without causing obvious ill effects. As the host cells multiply, they copy the viral genetic information, passing it along to their progeny.

## Viroids

**Viroids** are simpler than viruses, consisting of only a single, short piece of ribonucleic acid (RNA). Like viruses, they are obligate intracellular agents. Viroids cause a number of plant diseases, and some scientists speculate that they may cause diseases in humans, although no evidence for this yet exists.

## Prions

**Prions** are infectious proteins that cause diseases called spongiform encephalopathies, a name that reflects the sponge-like appearance of the brain tissue ( *encephalo* means “brain” and *patho* means “disease”). Perhaps the most widely recognized example is bovine spongiform encephalopathy (BSE), commonly called mad cow disease. *Prions are simply misfolded versions of normal cellular proteins found in the brain. When the misfolded version comes into contact with the normal cellular protein, it forces the normal protein to also misfold. These misfolded versions bind together within the cell to form thread-like structures called fibrils. The fibril-filled cells are not able to function and eventually die, forming spaces in the brain that lead to the characteristic sponge-like appearance.*

Prions are more resistant to degradation by cellular enzymes than are their normal counterparts.

Prions are also resistant to the usual sterilization procedures that destroy viruses and bacteria.

# THE PROKARYOTIC CELL

The members of the prokaryotic world make up a vast heterogeneous group of very small unicellular organisms. **Prokaryotes** include **bacteria** and **archaea**.

Although **bacteria** and **archaea** look similar, their **chemical composition** is different.

The thousands of species of **bacteria** are **differentiated** by many **factors** including:

**morphology (shape)**, **chemical composition** (often detected by staining reactions), **nutritional requirements**, **biochemical activities**, and **sources of energy** (sunlight or chemicals).

*It is estimated that 99% of the bacteria in nature exist in biofilms.*

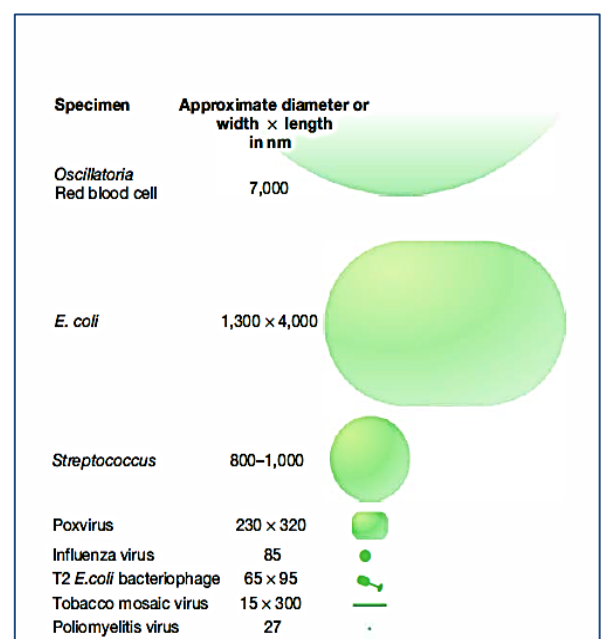
## The Size, Shape, and Arrangement of Bacterial Cells

Bacteria come in a great **many sizes** and **several shapes**.

**Size:** Most bacteria range from:

**0.2 to 2.0  $\mu\text{m}$  in diameter** and from  
**2 to 8  $\mu\text{m}$  in length** (figure 1):

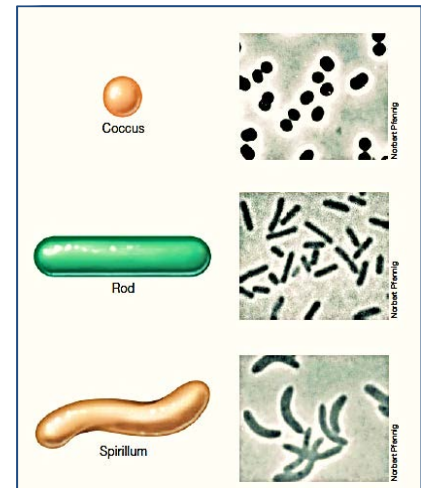
**Figure 1:** Sizes of Bacteria Relative to a Red Blood Cell and Viruses.  
Recall that 1,000 nm = 1  $\mu\text{m}$ .  
Thus *E. coli* is 1.3 X 4 $\mu\text{m}$ .





**Shape:** They have a few basic shapes (figure 2):

- spherical **coccus** (plural: **cocci**, meaning berries).
- rod-shaped **bacillus** (plural: **bacilli**, meaning little staffs).
- and **spiral**.



**Figure 2:** Basic shapes of bacteria.

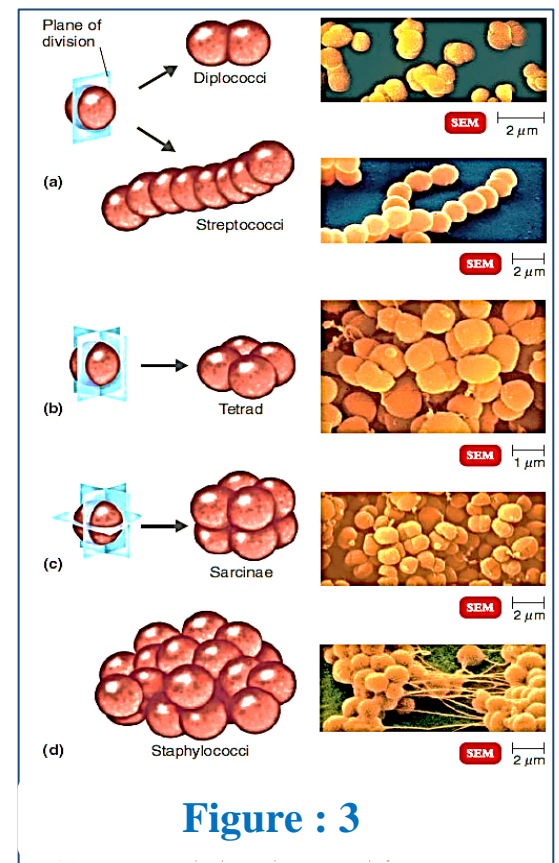
### Arrangement of Bacterial Cells:

#### • Cocci:

**Cocci** are usually **round** but can be **oval**, **elongated**, or **flattened on one side**.

When cocci divide to reproduce, the cells can remain attached to one another.

- Cocci that remain in pairs after dividing are called **diplococci** (figure 3 a).
- Those that divide and remain attached in **chainlike** patterns are called **streptococci** (figure 3 a).
- Those that divide in two planes and remain in groups of four are known as **tetrads** (figure 3 b).
- Those that divide in three planes and remain attached in **cubelike** groups of eight are called **sarcinae** (figure 3 c).
- Those that divide in multiple planes and form **grapelike clusters** or broad sheets are called **staphylococci** (figure 3 d).



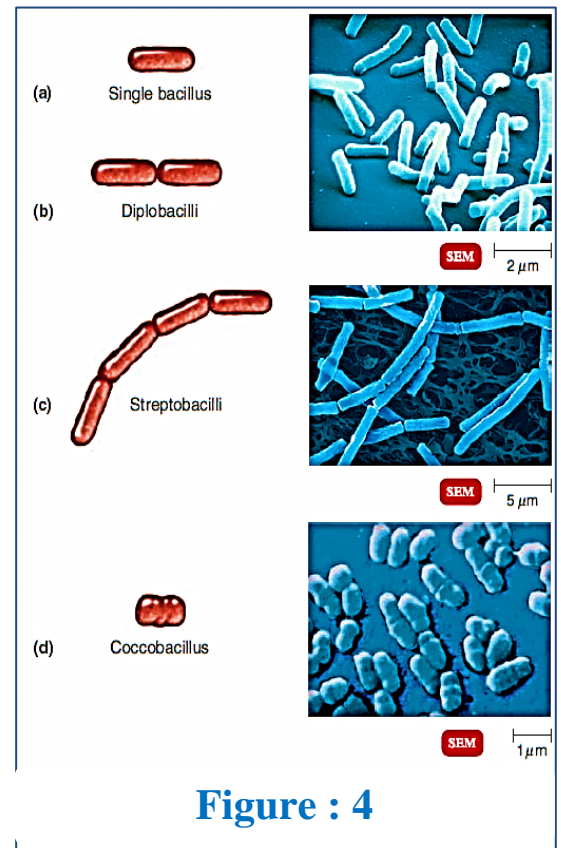
**Figure : 3**

These group characteristics are frequently helpful in **identifying** certain cocci.

- **Bacilli :**

**Bacilli** divide only across their short axis, so there are fewer groupings of bacilli than of cocci.

- Most bacilli appear as **single rods** (figure 4 a).
- **Diplobacilli** appear in pairs after division (figure 4 b).
- **Streptobacilli** occur in chains (figure 4 c).
- Some bacilli look like straws.
- Others have tapered ends, like cigars.
- Still others are oval and look so much like cocci that they are called **coccobacilli** (figure 4 d).



**“Bacillus” has two meanings in microbiology.** As we have just used it, bacillus refers to a bacterial shape. When capitalized and italicized, it refers to a specific genus. For example, the bacterium *Bacillus anthracis* is the causative agent of anthrax. Bacillus cells often form long, twisted chains of cells.

## • Spiral :

**Spiral** bacteria have one or more twists; they are **never straight**.

- Bacteria that look like curved rods are called **vibrios** (Figure 5 a).
- Others, called **spirilla**, have a helical shape, like a corkscrew, and fairly rigid bodies (Figure 5 b).
- Yet another group of spirals are helical and flexible; they are called **spirochetes** (Figure 5 c).

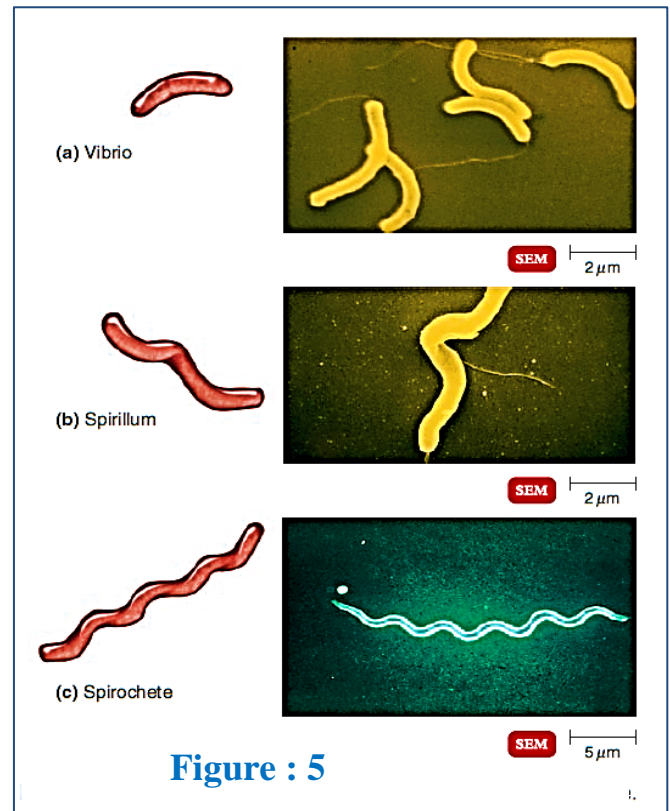


Figure : 5

\*\* In addition to the three basic shapes, there are :

- **star-shaped** cells (genus **Stella**; figure 6 a);
- **rectangular**, flat cells (halophilic archaea) of the genus **Haloarcula** (figure 6 b);
- and **triangular** cells.

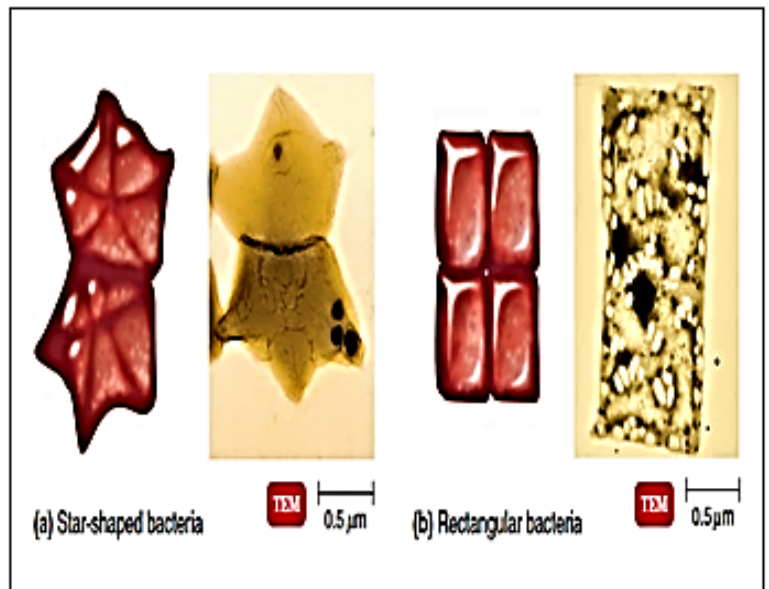


Figure : 6 : Star-shaped and rectangular prokaryotes.  
(a) *Stella* (star-shaped).  
(b) *Haloarcula*, a genus of halophilic archaea (rectangular cells).

\*\* The shape of a bacterium is determined by **heredity**. Genetically, most bacteria are **monomorphic**; that is, **they maintain a single shape**.

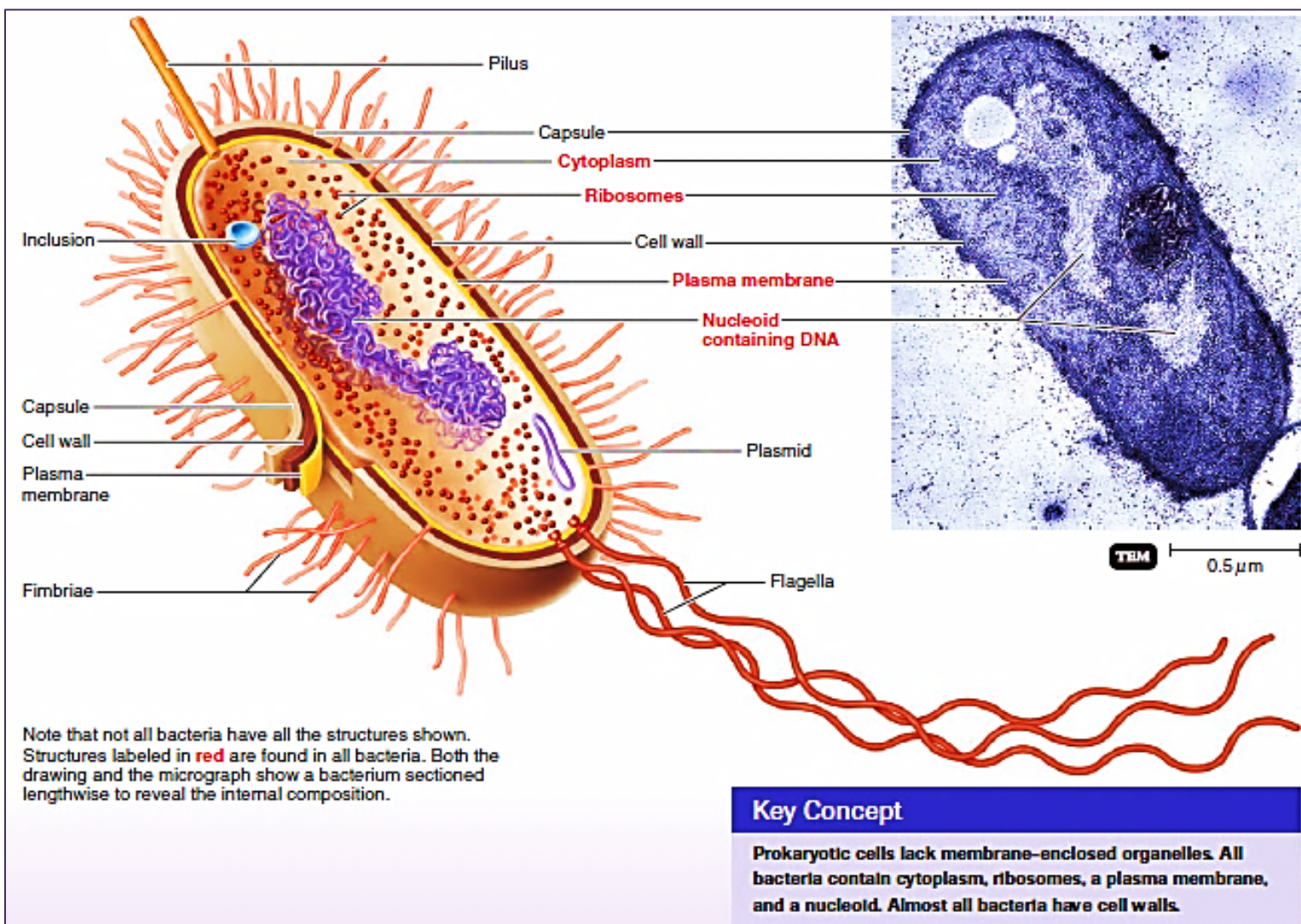
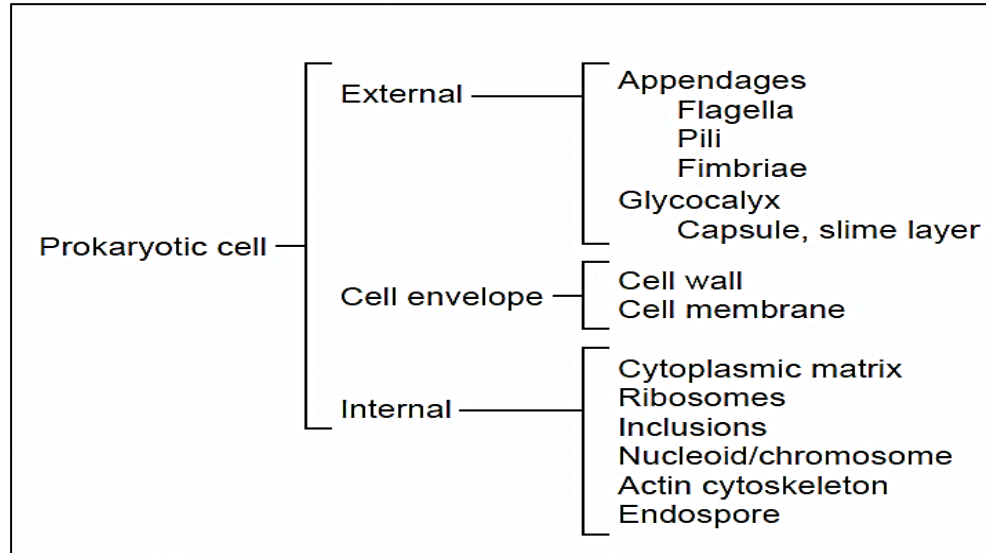
\*\* However, a number of environmental conditions can alter that shape. If the shape is altered, identification becomes difficult.

\*\*Moreover, some bacteria, such as **Rhizobium** and **Corynebacterium**, are genetically **pleomorphic**, which means **they can have many shapes, not just one**.



## Prokaryotic Cell Structure

The general cellular organization of a prokaryotic cell can be represented with this flowchart and figure 7 :



## External Cell Structures

### A. Appendages

Prokaryotes often bear accessory **appendages** sprouting from their surfaces. **Appendages can be divided into two major groups:**

- Those that provide **motility** (**flagella** and **axial filaments**).
- Those that provide **attachments** or **channels** (**fimbriae** and **pili**).

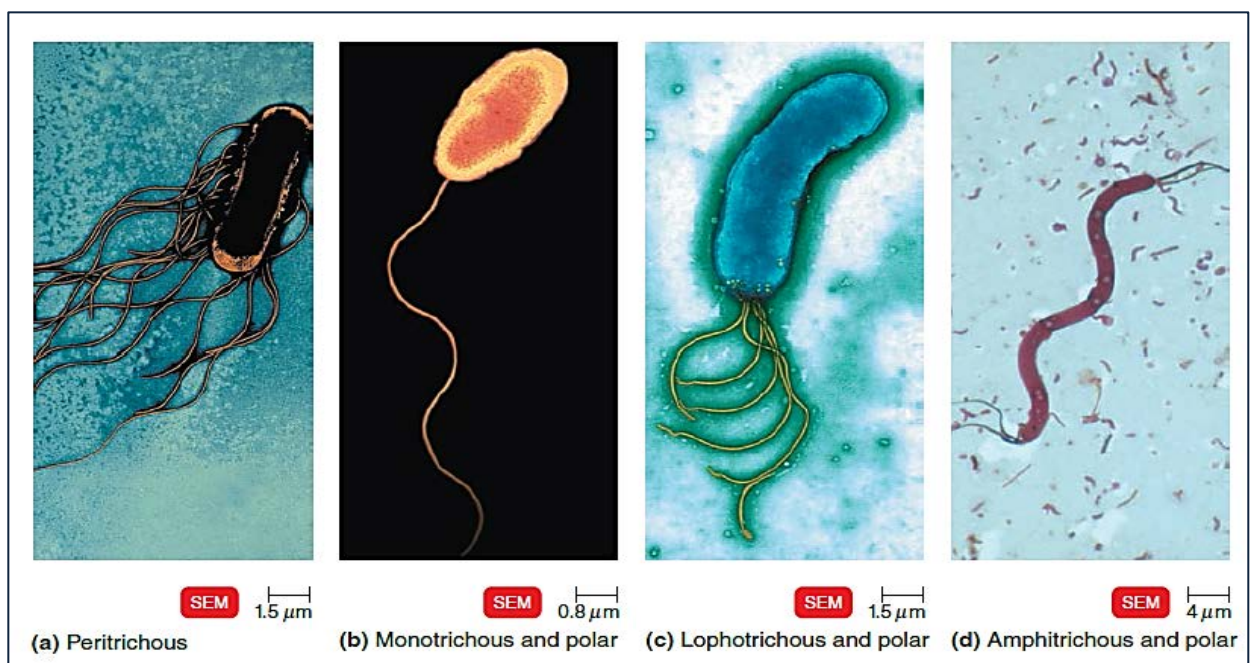
#### 1. Flagella

Some prokaryotic cells have **flagella** (singular: **flagellum**), which are **semi-rigid, helical structure and long filamentous that propel bacteria.**

One can generalize that **all spirilla**, about **half of the bacilli**, and a **small number of cocci** are **flagellated**.

- Bacteria that lack flagella are referred to as **atrichous** .

- Flagella vary both in **number** and **arrangement** according to two general patterns (figure 8):
  1. In a **peritrichous** arrangement, flagella are dispersed randomly over the surface of the cell. A *Proteus* cell in the swarming stage may have more than 1000 peritrichous flagella.
  2. In a **polar** arrangement, the flagella are attached at one or both ends of the cell. Three subtypes of this pattern are:
    - **Monotrichous** with a single flagellum. ( e.g. the predatory bacterium *Bdellovibrio* ).
    - **Lophotrichous** with small bunches or tufts of flagella emerging from the same site (*Vibrio fischeri* ).
    - **Amphitrichous** with flagella at both poles of the cell (*Aquaspirillum*).



**Figure 8:** Arrangements of bacterial flagella. (a) Peritrichous. (b)–(d) Polar.

## Flagellum Description

- The flagella cannot be seen with the light microscope.
- Special stains can increase thickness to visualise flagellum .
- Detailed structure of the flagella can be seen with the Electron Microscope.

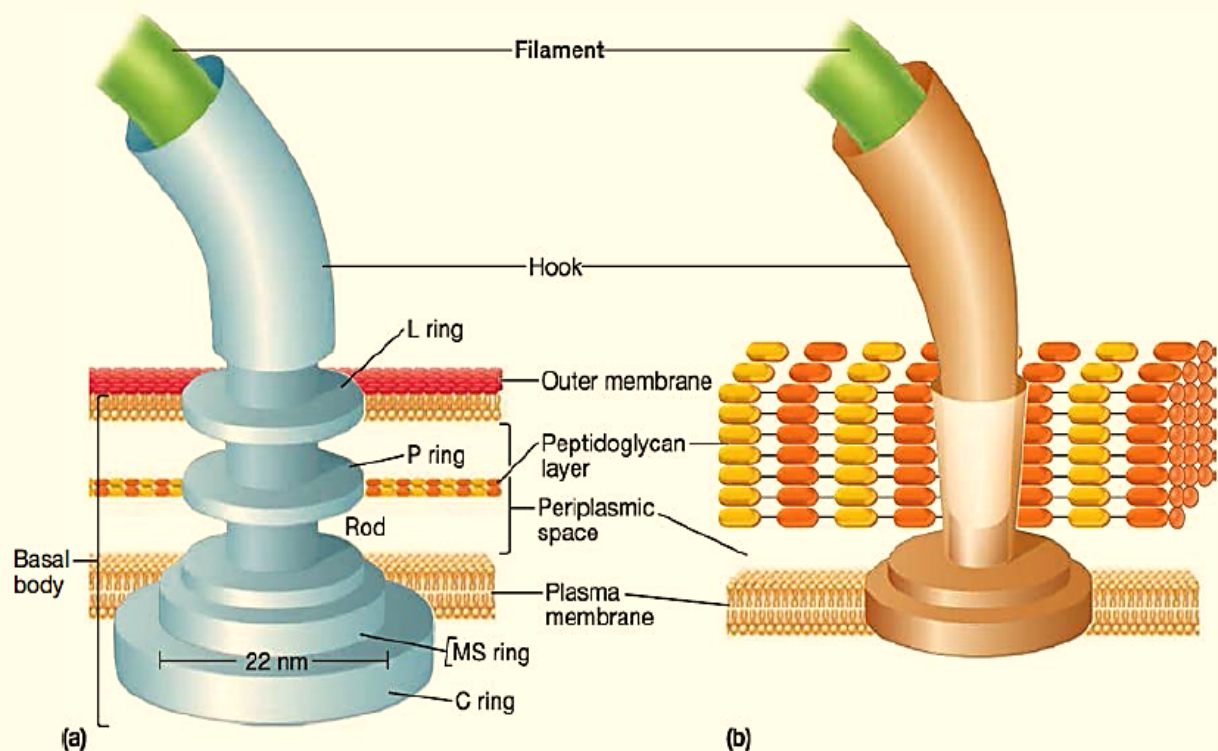
The bacterial flagellum when viewed under high magnification displays three distinct parts:

**the filament, the hook (sheath), and the basal body.**

1. The **filament**, is constant in diameter (*about 20 nm across and up to 20  $\mu$ m long*) and contains the globular (roughly spherical) protein **flagellin** arranged in several chains that intertwine and form a helix around a hollow core. In most bacteria, filaments are not covered by a membrane or sheath, as in eukaryotic cells.
2. The **hook** a curved protein structure, contiguous with the proximal end of the filament.
3. The **basal body ( flagellar motor)**, a structure which anchors the hook in the cell envelope and incorporates the energy-converting apparatus. The basal body is composed of a small central rod inserted into a series of rings.



- **Gram-negative bacteria:** basal body contain rings; the outer pair of rings is anchored to various portions of the cell wall (the outer **L** and **P** rings associate with the **L**ipopolysaccharide and **P**eptidoglycan layers, respectively), and the inner pair of rings called the **MS** ( **M**embrane/**S**upra membrane) and **C** rings(**C**ytoplasmic), are located within the cytoplasmic membrane and the cytoplasm, respectively (figure 9 a).
- **Gram positive bacteria** have only two basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan ( figure 9 b).



**Figure 3.44 The Ultrastructure of Bacterial Flagella.** Flagellar basal bodies and hooks in (a) typical Gram-negative and (b) typical Gram-positive bacteria.

## Function of Flagella

The flagellum enables the prokaryotic cell to swim through an aqueous medium. Each prokaryotic flagellum moves the cell by rotating from the basal body.

The rotation of a flagellum is either **clockwise** or **counterclockwise** around its long axis. (*Eukaryotic flagella, by contrast, undulate in a wavelike motion*). The movement of a prokaryotic flagellum results from rotation of its basal body and is similar to the movement of the shaft of an electric motor. As the flagella rotate, they form a bundle that pushes against the surrounding liquid and propels the bacterium.

**Anti-clockwise (Counterclockwise)** rotation of the helical filament propels the cell through the aqueous environment, known as a **“run”** or **“swim”**: movements of a cell in a single direction for some time.

**The clockwise** rotation causes the cell to stop and re-orientate, known as a **“tumble”**.

Bacteria move with a series of **“runs”** punctuated by **“tumbles.”** Both runs and tumbles occur in response to stimuli (Figure 1).

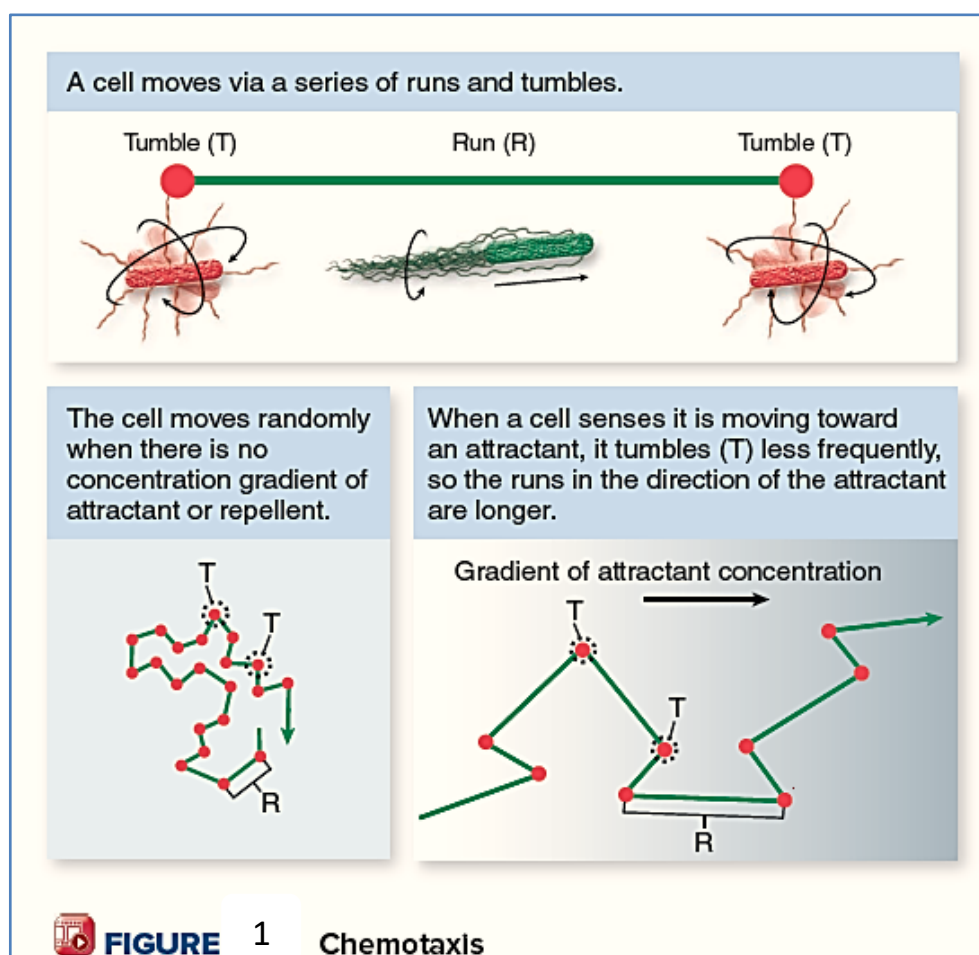
If more than one flagellum is present, the flagella align and rotate together as a bundle.

Some species of bacteria endowed with many flagella, **Proteus** for example, can “swarm,” or show rapid wavelike movement across a solid culture medium.

### Advantage of motility:

One advantage of motility is that it **enables a bacterium to move toward a favorable environment or away from an adverse one**. The movement of a bacterium toward or away from a particular stimulus is called **taxis**. **Examples of taxis:**

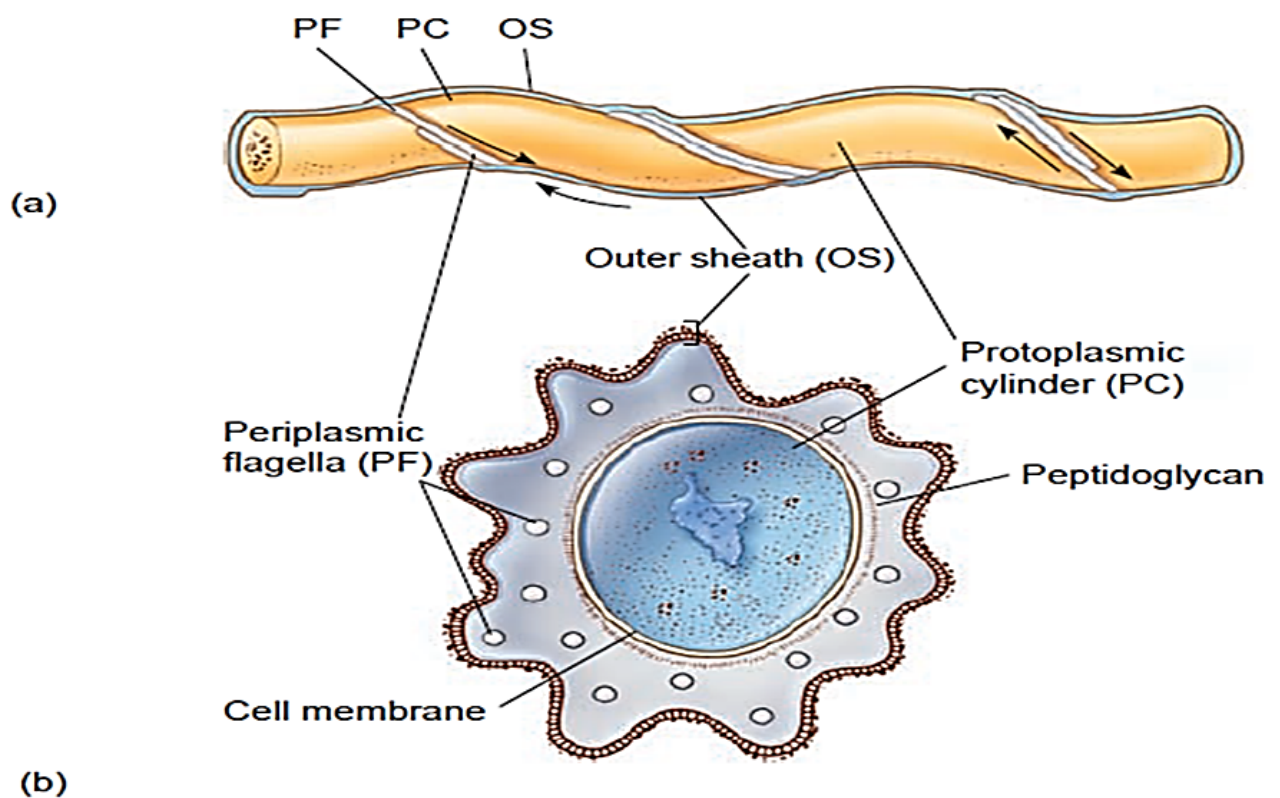
- Phototaxis – Light.
- Thermotaxis – Heat.
- Magnetotaxis -Magnetic field.
- Chemotaxis – Chemical.



## 2. Axial Filaments (Periplasmic Flagella)

An **axial filament** is a type of **internal flagellum** that is enclosed in the space between the outer sheath and the cell wall peptidoglycan.

Spirochetes are a group of bacteria that have unique structure and motility. One of the best-known spirochetes is *Treponema pallidum*, the causative agent of syphilis. Spirochetes move by means of **axial filaments**, or **endoflagella** or **periplasmic flagella**, bundles of fibrils that arise at the ends of the cell beneath an outer sheath and spiral around the cell (Figure 2).



**Figure 2** The orientation of periplasmic flagella on the spirochete cell. (a) Longitudinal section. (b) Cross section. Contraction of the filaments imparts a spinning and undulating pattern of locomotion.

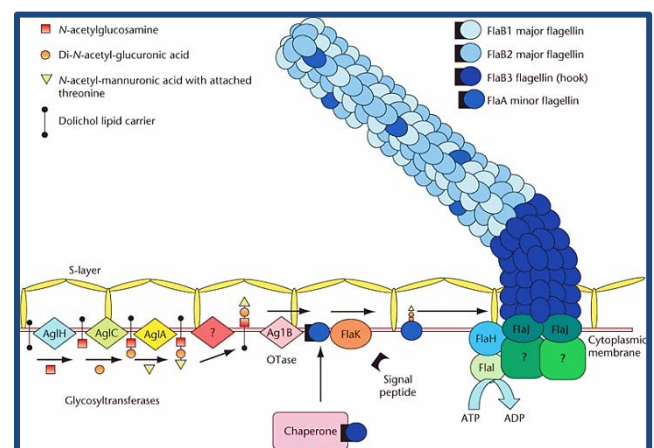
## • Archaeal Flagella

Archaea use flagella to move though at a slower speed than bacteria. An archaeal flagellum (Figure 3) is superficially similar to a bacterial flagellum: it consists of a **basal body**, **hook**, and **filament**, each composed of protein. The flagellum extends outside the cell and is not covered by a membrane.

However, scientists have discovered **many differences between archaeal and bacterial flagella**:

- Archaeal flagella are 10–14 nm in diameter, which is about half the thickness of bacterial flagella.
- The proteins making up archaeal flagella share common amino acid sequences across archaeal species. These are very different from the amino acid sequences common to bacterial flagella.
- Sugar molecules are attached to the filaments of many archaeal flagella, a condition that is rare in bacteria.

- Archaeal flagella are powered with energy stored in molecules of ATP, whereas the flow of hydrogen ions across the membrane powers bacterial flagella.



**Figure 3** : Schematic speculative representation of the archaeal flagellum.

## External Cell Structures

### A. Appendages

#### Fimbriae and Pili

Many gram-negative bacteria contain **hairlike appendages** that are shorter and thinner than flagella. These structures are divided into two types, **fimbriae** (s. **fimbria** ) and **pili** (s. **pilus** ), having very different functions. (*Some microbiologists use the two terms interchangeably to refer to all such structures, but we distinguish between them.*).

The structures termed **fimbria** and **pilus** both refer to bacterial surface appendages that are **involved in interactions with other cells** but **do not provide locomotion, except for some specialized pili**.

#### 1. Fimbriae

**Fimbriae** are small, bristle like fibers emerging from the surface of many bacterial cells (figure 1-a). They are slender tubes composed of helically arranged protein subunits *and are about 3 to 10 nm in diameter and up to several micrometers long*. A cell may be covered with up to 1,000 fimbriae, but they are only visible in an electron microscope due to their small size .



## Function:

Fimbriae have an inherent tendency to **stick to surfaces**. Some pathogens can colonize and infect host tissues because of a **tight adhesion** between their fimbriae and epithelial cells (figure 4- b ).

For example:

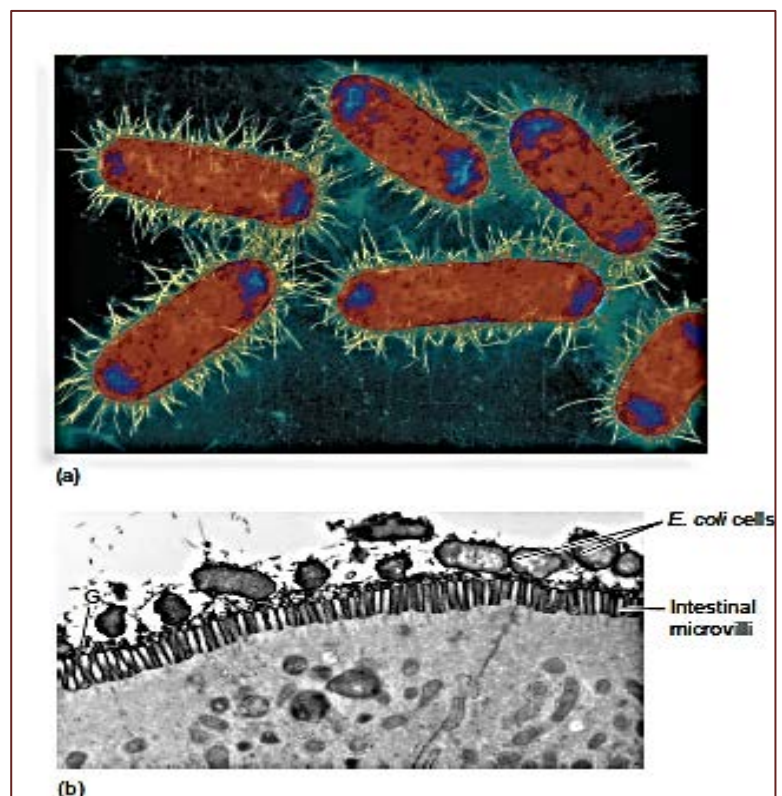
- *Escherichia coli* colonizes the intestine by this means.
- *Salmonella* species causing salmonellosis.
- *Bordetella pertussis* causing whooping cough.

Mutant forms of these pathogens that lack fimbriae are unable to cause infections.

### Figure 4 : Form and function of bacterial fimbriae.

(a) Several cells of pathogenic *Escherichia coli* covered with numerous stiff fibers called fimbriae.

(b) A row of *E. coli* cells tightly adheres by their fimbriae to the surface of intestinal cells. This is how the bacterium clings and gains access to the inside of cells during an infection.



## 2. Pili

A **pilus** is an elongate, rigid tubular structure made of a special protein, *pilin* .

Pili are similar to fimbriae, but are **typically longer** and **only one or a few (about 10) pili** are present on the surface of a cell.

### The most important classes of pili:

**Many classes of pili are known**, distinguished by their **structure** and **function**.

- **Type IV pili:**

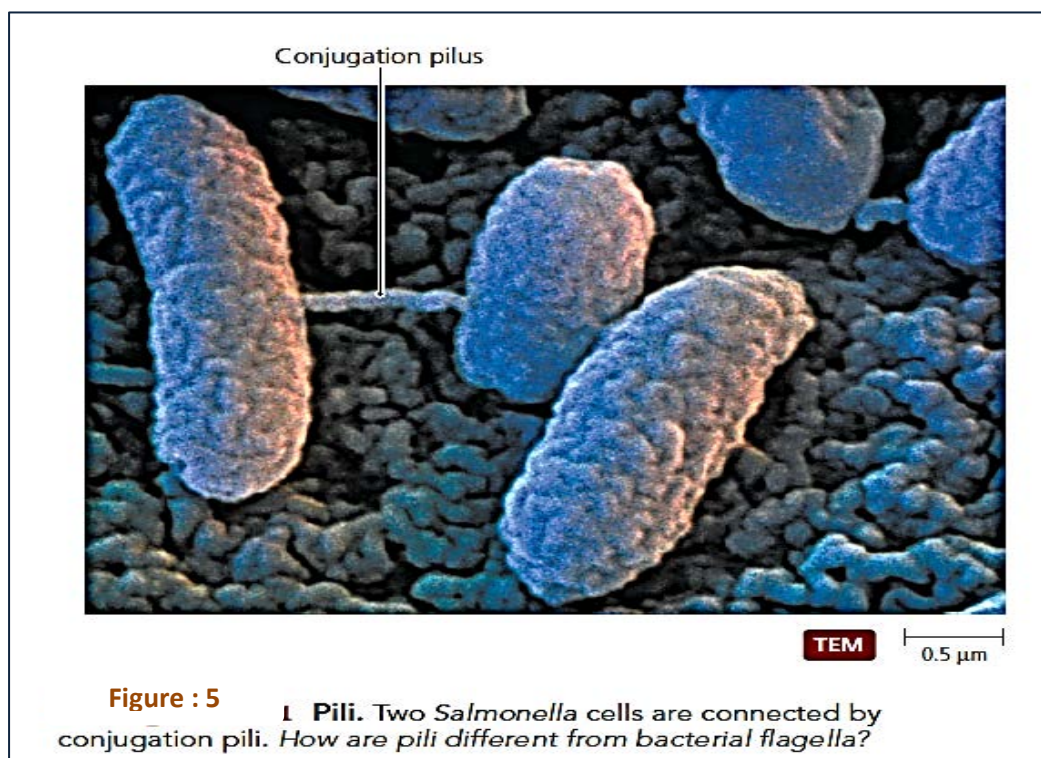
One important class of pili, called type **IV pili**, assist cells in **adhesion** but also allow for an **unusual form of cell motility** called **twitching motility**. Twitching motility is a **type of gliding motility**, movement along a solid surface. In twitching motility, extension of pili followed by their retraction drags the cell along a solid surface, with energy supplied by ATP. Certain species of **Pseudomonas** and **Moraxella** are well known for their twitching motility.



- **Conjugation pili or Sex pili:**

True pili have been found only on gram-negative bacteria, where they are utilized primarily in a “mating” process between cells called **conjugation**, which involves a transfer of DNA from one cell to another (figure 5 ). A pilus from a donor cell unites with a recipient cell, thereby providing a connection for making the transfer.

- **Sex pili** are genetically determined by conjugative plasmids and are required for conjugation.
- Some bacterial viruses attach specifically to receptors on sex pili at the start of their multiplication cycle.



## B. The Bacterial Surface Coating or Glycocalyx

Some bacterial cells have a gelatinous, sticky substance that surrounds the outside of the cell. This substance is known as a **glycocalyx** (plural: *glycocalyces*), which literally means “sugar cup.”

The **glycocalyx** develops as a coating of macromolecules to **protect the cell** and, in some cases, **help it adhere to its environment**.

Glycocalyces differ among bacteria in **thickness, organization, and chemical composition**.

The glycocalyx may be composed of **polysaccharides, polypeptides, or both**. These chemicals are produced inside the cell and are extruded onto the cell's surface.

The glycocalyx may be **thick** or **thin** and **rigid** or **flexible**, depending on **their chemistry** and **degree of hydration**. Traditionally:

1. If the layer is organized in a **tight matrix** that excludes small particles, such as India ink, it is called a **capsule**.

By contrast, if the layer is **more easily deformed**, it will not exclude particles and is more difficult to see; this form is called a **slime layer**.

2. In addition, **capsules** typically **adhere firmly to the cell wall**, and **some** are even covalently **linked to peptidoglycan** (figure 1 - a).

By contrast, **slime layers** are **loosely attached** and **can be lost from the cell surface** (figure 1 - b).

## Specialized Functions of the Glycocalyx:

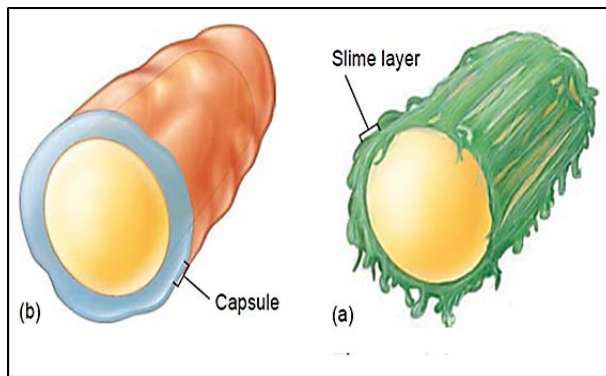
Although capsules are not required for growth and reproduction in laboratory cultures, they confer several advantages when prokaryotes grow in their normal habitats. A capsule is bound more tightly to the cell than a slime layer is, and it has a thicker, gummy consistency that gives a prominently sticky (mucoid) character to the colonies of most encapsulated bacteria (figure 2 - a ).

1. They help pathogenic bacteria **resist phagocytosis** by host phagocytes .

*(Phagocytes are a natural body defense that can engulf and destroy foreign cells, which helps to prevent infection).* A capsular coating blocks the mechanisms that phagocytes use to attach to and engulf bacteria. By escaping phagocytosis, the bacteria are free to multiply and infect body tissues. **Encapsulated bacteria that mutate to nonencapsulated forms usually lose their pathogenicity.**

- *Streptococcus pneumoniae* (a cause of pneumonia) provides a dramatic example. When it lacks a capsule, it is destroyed easily and does not cause disease.

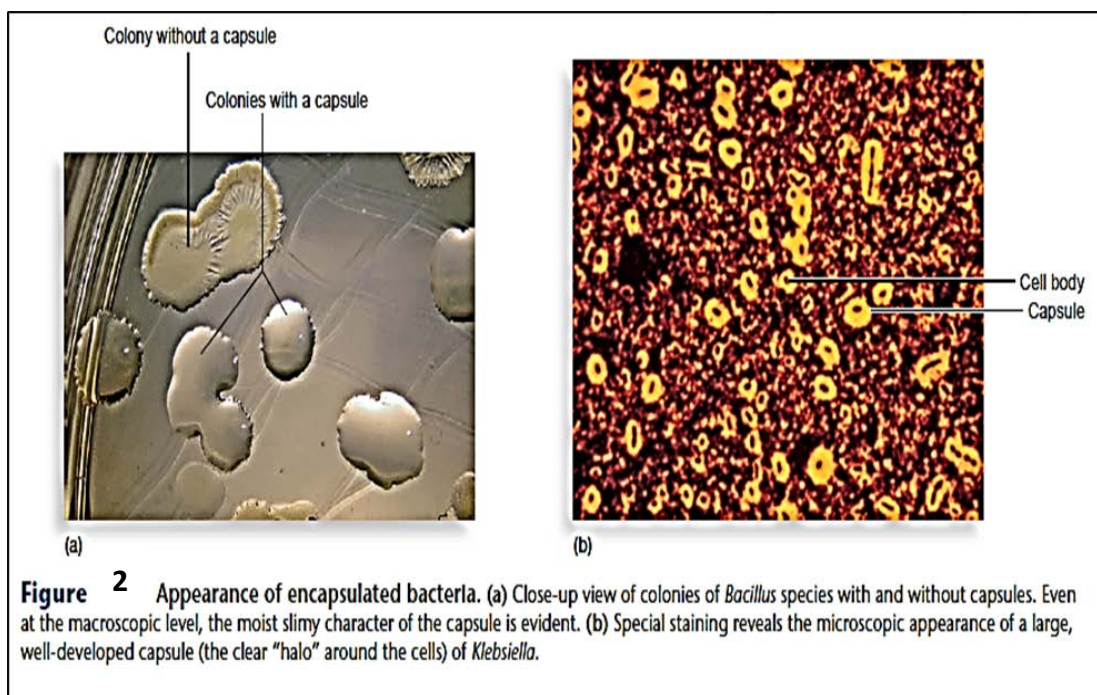
2. Capsules contain a great deal of water and can **protect against desiccation.**
3. They **exclude viruses** and **most hydrophobic toxic materials** such as detergents.
4. The glycocalyx also aids in **attachment to solid surfaces**, including tissue surfaces in plant and animal hosts ( figure 3 ).
5. Other types of glycocalyxes can be important in **formation of biofilms.**



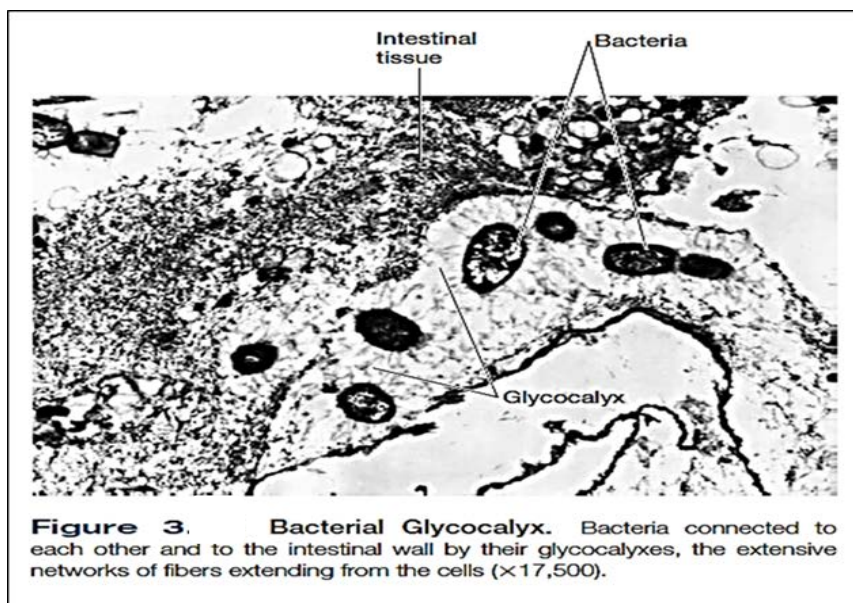
**Figure 1 :** Types of glycocalyxes seen through cutaway views of cells.

(a) The **slime layer** is a loose structure that is easily washed off.

(b) The **capsule** is a thick, structured layer that is not readily removed.



**Figure 2** Appearance of encapsulated bacteria. (a) Close-up view of colonies of *Bacillus* species with and without capsules. Even at the macroscopic level, the moist slimy character of the capsule is evident. (b) Special staining reveals the microscopic appearance of a large, well-developed capsule (the clear "halo" around the cells) of *Klebsiella*.



**Figure 3.** Bacterial Glycocalyx. Bacteria connected to each other and to the intestinal wall by their glycocalyxes, the extensive networks of fibers extending from the cells ( $\times 17,500$ ).



## **Biofilm :**

In nature, microorganisms can live suspended in an aqueous environment, but **most attach to surfaces** and live in polymer-encased communities called **biofilms** .

Biofilms cause the slipperiness of rocks in a stream bed, the slimy “gunk” that coats kitchen drains, the scum that gradually accumulates in toilet bowls, and the dental plaque that forms on teeth. So, biofilms can develop **on living tissues (e.g. oral cavity)**, and (on or in) non-living things such as **on catheters, in water pipes , and on ships’ hulls**.

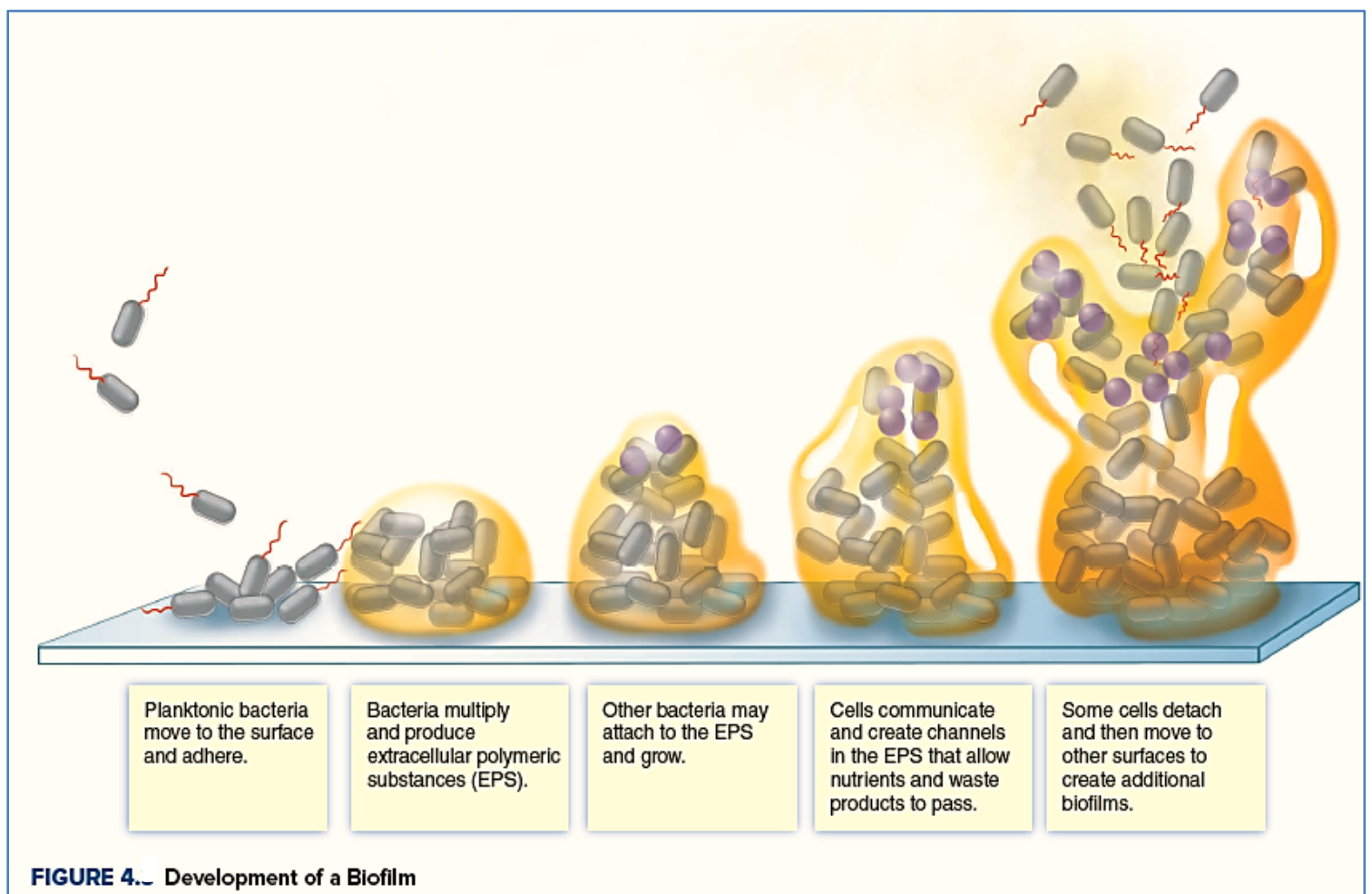
**Biofilm formation begins** when planktonic (free-floating) cells move to a surface and adhere. They then multiply and release polysaccharides, DNA, and other hydrophilic polymers to which unrelated cells may attach and grow ( **figure 4** ). The mesh-like accumulation of these polymers, referred to as **extracellular polymeric substances (EPS)**, gives a biofilm its characteristic slimy appearance.

Surprisingly, **biofilms are not random mixtures of microbes** in a layer of EPS, but instead **have characteristic formations with channels through which nutrients and wastes pass**. Cells communicate with one another by synthesizing and responding to **chemical signals**—an exchange important in establishing structure.

Biofilms are more than just an unsightly annoyance. Dental plaque leads to tooth decay and gum disease. Even troublesome, persistent ear infections and the complications of cystic fibrosis are due to biofilms. In fact, the majority of bacterial infections seem to involve biofilms. **Treatment of these infections is difficult because microbes within the biofilm often resist the effects of antibiotics as well as the body’s defenses.**

Biofilms are also **important in industry**, where their accumulations in pipes, drains, and cooling water towers can interfere with processes as well as damage equipment. The conditions within the biofilm protect the microbes, so the bacteria in a biofilm may be hundreds of times more resistant to disinfectants than are their planktonic (free-floating) counterparts.

Although **biofilms** can be damaging, they also **can be beneficial**. Many bioremediation efforts, which use microbes to degrade harmful chemicals, are enhanced by biofilms. So as some industries are exploring ways to destroy biofilms, others, such as wastewater treatment facilities, are looking for ways to encourage their development.



# The Cell Envelope

## 1. The Cell Wall

The **cell wall** of the bacterial cell is a **complex, semirigid** structure **responsible for the shape of the cell**. The cell wall **surrounds the underlying, fragile plasma (cytoplasmic) membrane and protects it and the interior of the cell from adverse changes in the outside environment**. **Almost** all prokaryotes have cell walls.

**The major functions of the cell wall are:**

1. To prevent bacterial cells from rupturing when the water pressure inside the cell is greater than that outside the cell.
2. It also helps maintain the shape of a bacterium.
3. Serves as a point of anchorage for flagella.
4. Clinically, the cell wall is important because it contributes to the ability of some species to cause disease and is the site of action of some antibiotics.
5. In addition, the chemical composition of the cell wall is used to differentiate major types of bacteria.

Although the cells of some eukaryotes, including plants, algae, and fungi, have cell walls, their walls differ chemically from those of prokaryotes, are simpler in structure, and rigid.

## Composition and Characteristics

The bacterial cell wall is composed of a macromolecular network called **peptidoglycan** (also known as **murein**), which is present either alone or in combination with other substances.

Peptidoglycan consists of a repeating **disaccharide attached by polypeptides to form a lattice** that surrounds and protects the entire cell.

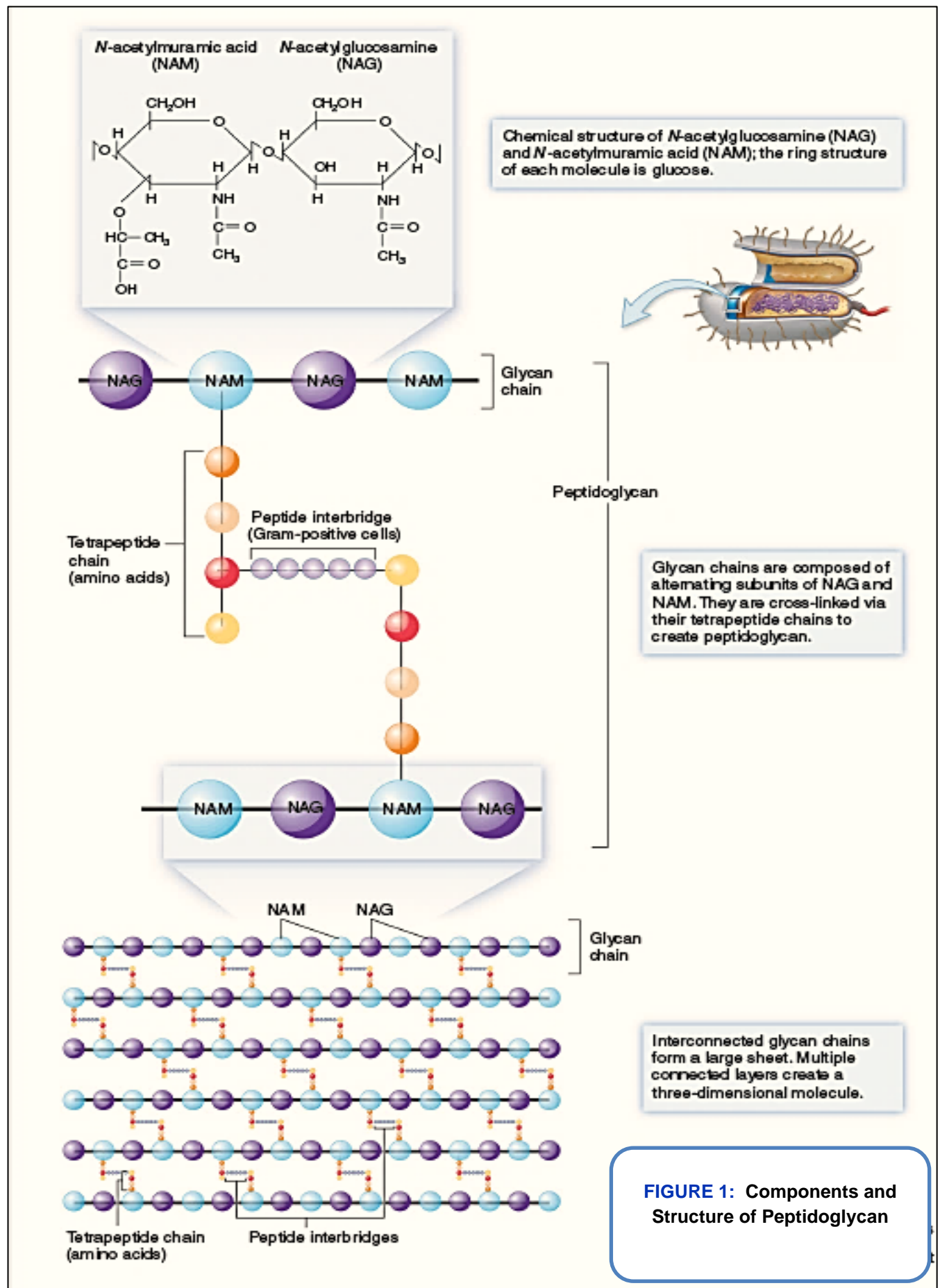
The **disaccharide portion** is made up of monosaccharides called **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)** (figure 1). They bound by  **$\beta$ -1,4-glycosidic bonds**.

Alternating NAM and NAG molecules are linked in rows of 10 to 65 sugars to form a carbohydrate “**backbone**” (the **glycan portion** of peptidoglycan).

Adjacent rows are linked by **polypeptides** (the **peptide portion** of peptidoglycan). Although the structure of the polypeptide link varies, it always includes **tetrapeptide side chains, which consist of four amino acids attached to NAMs in the backbone**. The amino acids occur in an alternating pattern of D and L forms. *This is unique because the amino acids found in other proteins are L forms.* The amino acids, including **L-alanine, D-alanine, D-glutamic acid**, and **either lysine or the structurally similar amino acid analog, diaminopimelic acid (DAP)** (Figure 3 ).

Parallel tetrapeptide side chains may be **directly bonded to each other** or linked by **a peptide cross-bridge**, consisting of a **short chain of amino acids** (Figure 3 ).

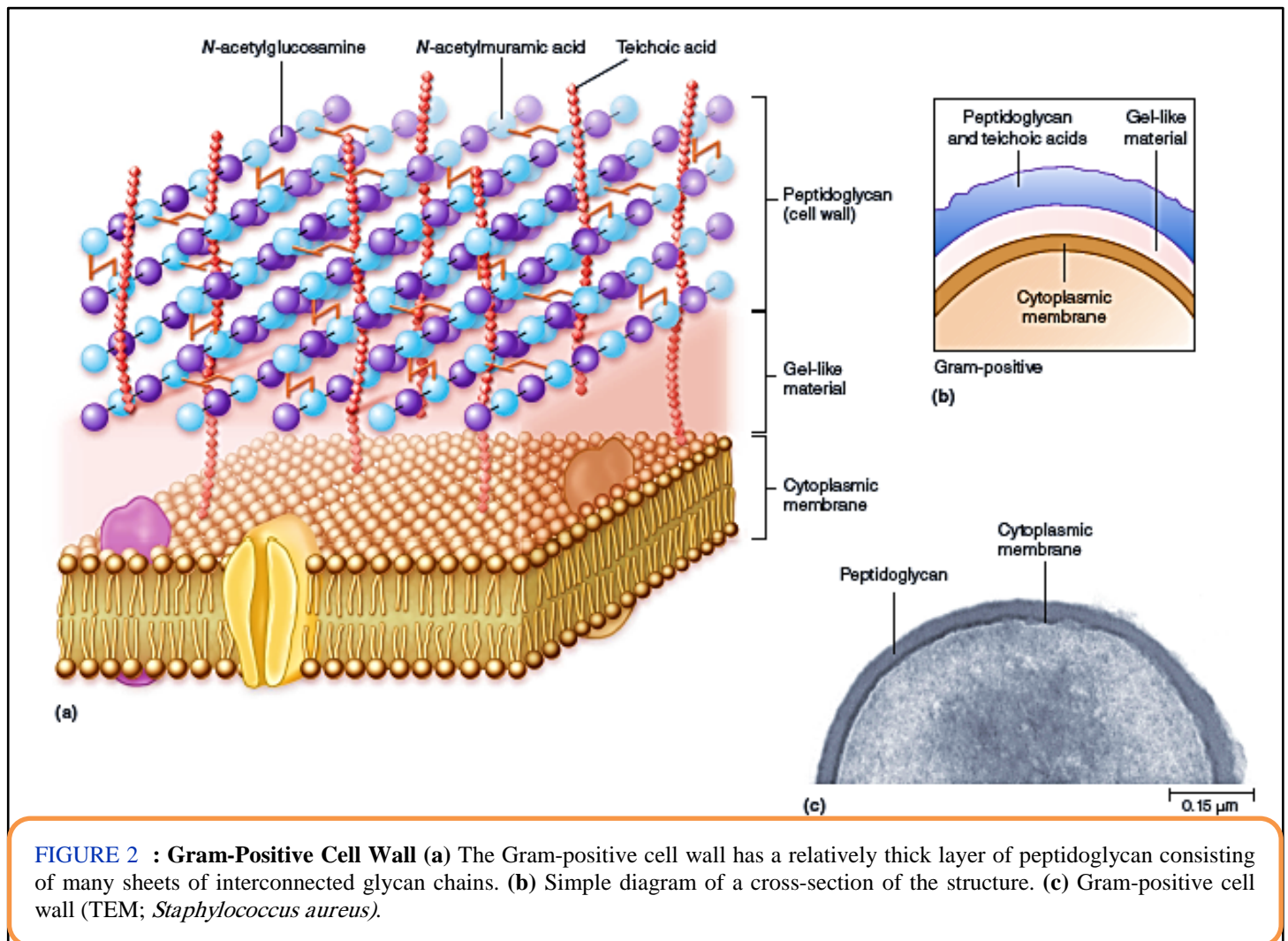




## Gram-Positive Cell Walls

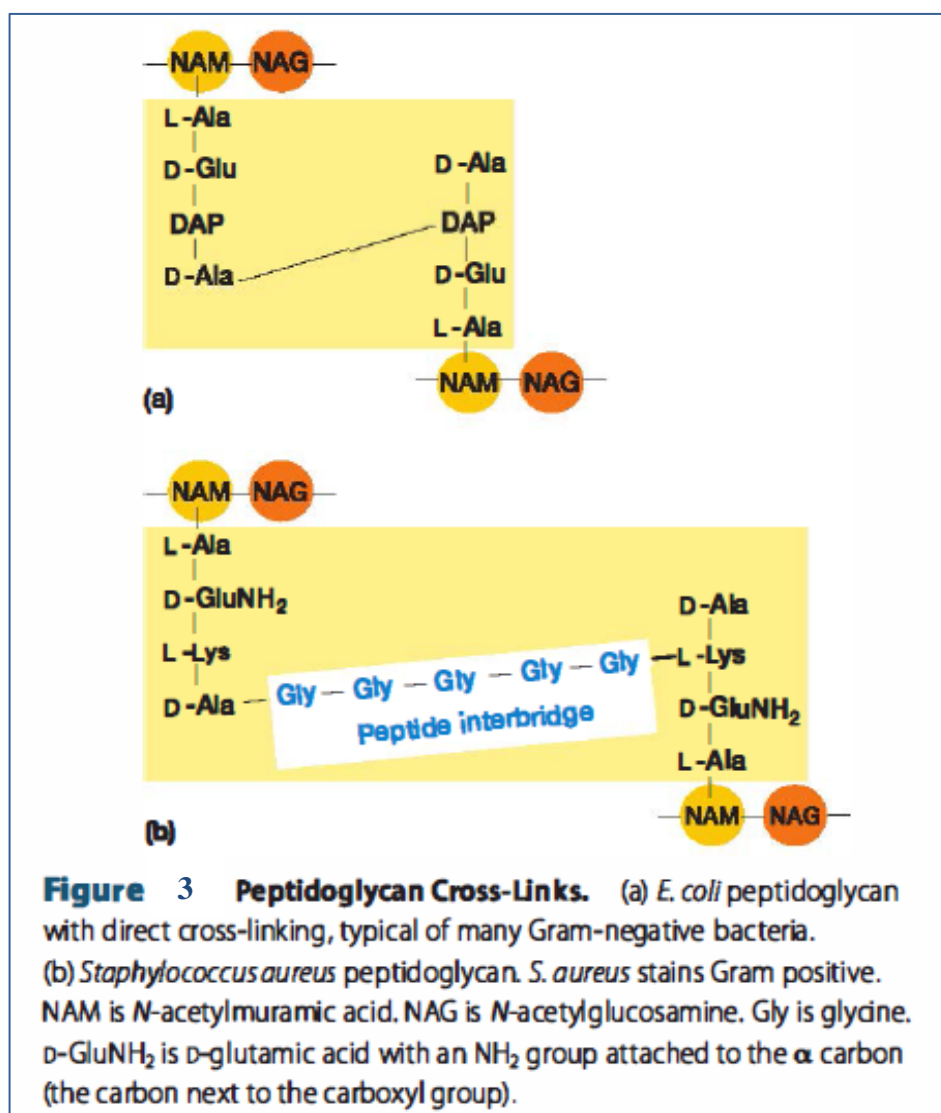
In most **gram-positive bacteria**, the cell wall consists of **many layers** of **peptidoglycan**, forming a **thick, rigid structure** (Figure 2 ).

By contrast, **gram-negative cell walls** contain only a **thin layer** of **peptidoglycan** (Figure 4). The most differences between **gram-positive** & **gram-negative** bacteria illustrated in table 1) pp. 11.



**FIGURE 2 : Gram-Positive Cell Wall** (a) The Gram-positive cell wall has a relatively thick layer of peptidoglycan consisting of many sheets of interconnected glycan chains. (b) Simple diagram of a cross-section of the structure. (c) Gram-positive cell wall (TEM; *Staphylococcus aureus*).

In gram-positive bacteria, cross-linkage may occur through a short peptide interbridge, the kinds and numbers of amino acids in the interbridge varying from species to species. For example, in the gram-positive *Staphylococcus aureus*, the interbridge peptide is composed of **five glycine** residues, a common interbridge amino acid (Figure 3 ).



In addition, the cell walls of gram-positive bacteria contain **teichoic acids**, which consist primarily of an alcohol (such as glycerol or ribitol) and phosphate.

There are two classes of teichoic acids: **lipoteichoic acid**, which spans the peptidoglycan layer and is linked to the plasma membrane, and **wall teichoic acid**, which is linked to the peptidoglycan layer.

The functions of **teichoic acids** are:

1. Because of their negative charge (from the phosphate groups), teichoic acids may bind and regulate the movement of cations (positive ions) into and out of the cell.
2. They may also assume a role in cell growth, preventing extensive wall breakdown and possible cell lysis.
3. Finally, teichoic acids provide much of the wall's antigenic specificity and thus make it possible to identify gram-positive bacteria by certain laboratory tests.

*Similarly, the cell walls of gram positive streptococci are covered with various polysaccharides that allow them to be grouped into medically significant types.*

## Gram-Negative Cell Walls

The cell walls of gram-negative bacteria consist of **one** or **a very few layers** of peptidoglycan and an **outer membrane** (Figure 4 ).

In gram-negative bacteria, peptidoglycan cross-linkage occurs by peptide bond formation from the amino group of DAP of one glycan chain to the carboxyl group of the terminal D-alanine on the adjacent glycan chain.

The peptidoglycan is bonded to lipoproteins (lipids covalently linked to proteins) in the outer membrane and is in the **periplasm**.

**The periplasm** : a gel-like fluid between the outer membrane and the plasma membrane. The periplasm contains a high concentration of **degradative enzymes** and **transport proteins**.

Gram-negative cell walls **do not contain** teichoic acids.

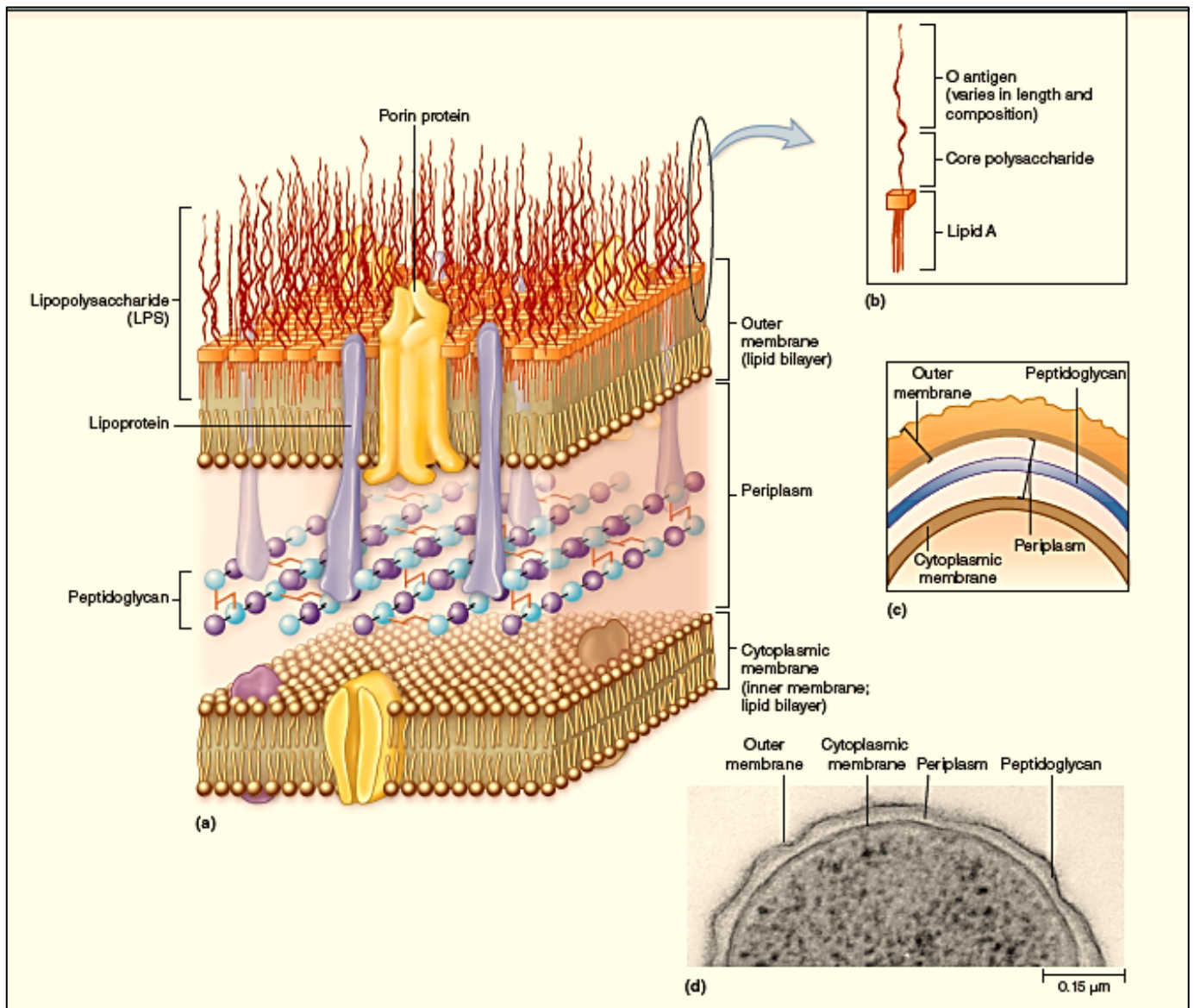
**Because the cell walls of gram-negative bacteria contain only a small amount of peptidoglycan, they are more susceptible to mechanical breakage.**

The **outer membrane** of the gram-negative cell consists of **lipopolysaccharides (LPS), lipoproteins, and phospholipids** (Figure 4).

The **lipopolysaccharide (LPS)** of the outer membrane is a large complex molecule that contains lipids and carbohydrates and consists of three components: (1) **lipid A**, (2) **a core polysaccharide**, and (3) **an O polysaccharide**(Figure 4 b).

1. **Lipid A** is the lipid portion of the LPS and is embedded in the top layer of the outer membrane. When gram-negative bacteria die, they release lipid A, which functions as an endotoxin . Lipid A is responsible for the symptoms associated with infections by gram-negative bacteria such as fever, dilation of blood vessels, shock, and blood clotting.
2. The **core polysaccharide** is attached to lipid A and contains unusual sugars. Its role is structural—to provide stability.
3. The **O polysaccharide** extends outward from the core polysaccharide and is composed of sugar molecules. The O polysaccharide functions as an antigen and is useful for distinguishing species of gram negative bacteria. For example, the foodborne pathogen *E. coli* O157:H7 is distinguished from other serovars by certain laboratory tests that test for these specific antigens. This role is comparable to that of teichoic acids in gram-positive cells.





**FIGURE 4: Gram-Negative Cell Wall** (a) The Gram-negative cell wall has a thin layer of peptidoglycan made up of only one or two sheets of interconnected glycan chains. The outer membrane is a typical phospholipid bilayer, except the outer layer is lipopolysaccharide. Porins span the membrane to allow specific molecules to pass. Periplasm fills the region between the two membranes. (b) Structure of lipopolysaccharide. The lipid A portion is responsible for the symptoms associated with endotoxin. The sugars in the O antigen vary among bacterial species. (c) Simple diagram of a cross-section of the structure. (d) Gram-negative cell wall (TEM; *Myxococcus xanthus*)

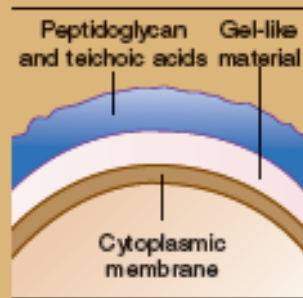
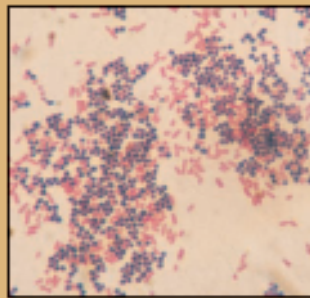
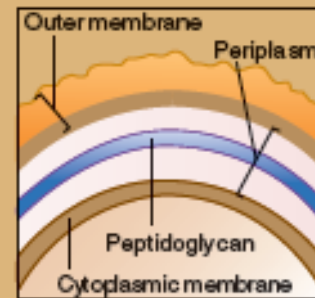
### **The outer membrane has several specialized functions:**

1. Its strong negative charge is an important factor in evading phagocytosis and the actions of complement (lyses cells and promotes phagocytosis), two components of the defenses of the host.
2. The outer membrane also provides a barrier to certain antibiotics (for example, penicillin), digestive enzymes such as lysozyme, detergents, heavy metals, bile salts, and certain dyes.

However, the outer membrane does not provide a barrier to all substances in the environment because nutrients must pass through to sustain the metabolism of the cell.

Part of the permeability of the outer membrane is due to proteins in the membrane, called **porins**, that form channels. Porins permit the passage of molecules such as nucleotides, disaccharides, peptides, amino acids, vitamin B12, and iron.



**TABLE 1****Comparison of Features of Gram-Positive and Gram-Negative Bacteria****Gram-Positive****Gram-Negative**

<b>Color of Gram-Stained Cell</b>	Purple	Pink
<b>Representative Genera</b>	<i>Bacillus</i> , <i>Staphylococcus</i> , <i>Streptococcus</i>	<i>Escherichia</i> , <i>Neisseria</i> , <i>Pseudomonas</i>
<b>Distinguishing Structures/Components</b>		
Peptidoglycan	Thick layer	Thin layer
Teichoic acids	Present	Absent
Outer membrane	Absent	Present
Lipopolysaccharide (endotoxin)	Absent	Present
Porin proteins	Absent (unnecessary because there is no outer membrane)	Present; allow molecules to pass through outer membrane
<b>General Characteristics</b>		
Sensitivity to penicillin	Generally more susceptible (with notable exceptions)	Generally less susceptible (with notable exceptions)
Sensitivity to lysozyme	Yes	No

## Antibacterial Substances That Target Peptidoglycan

Peptidoglycan can be destroyed by certain agents. One such agent is the enzyme **lysozyme**, a protein that **cleaves the  $\beta$ -1,4-glycosidic bonds between N-acetylglucosamine and N-acetylmuramic acid** in peptidoglycan, thereby weakening the wall; water can then enter the cell and cause lysis. *Lysozyme is found in animal secretions including tears, saliva, and other body fluids, and functions as a major line of defense against bacterial infection.*

The important antibiotic penicillin also targets peptidoglycan, but in a different way from that of lysozyme. Whereas lysozyme destroys preexisting peptidoglycan, **penicillin instead prevents its biosynthesis, leading eventually to osmotic lysis.** *Penicillin interferes with the final linking of the peptidoglycan rows by peptide cross-bridges (Figure 1a). As a result, the cell wall is greatly weakened and the cell undergoes lysis, destruction caused by rupture of the plasma membrane and the loss of cytoplasm.*

## Atypical Cell Walls

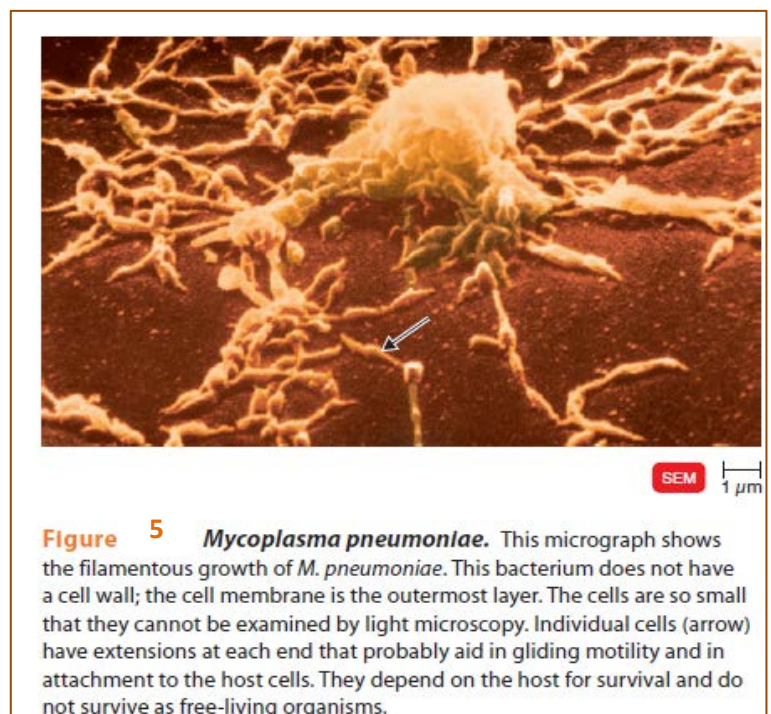
### 1. The cells have no walls

Among bacteria, certain types of cells have no walls or have very little wall material. These include members of the genus *Mycoplasma* and related organisms.

**Mycoplasmas** (figure 5 ) are the smallest known bacteria that can grow and reproduce outside living host cells. Their plasma membranes are unique among bacteria in having lipids called sterols, which are thought to help protect them from lysis (rupture).

**Mycoplasmas** lack a target for cell wall–inhibiting antimicrobial agents (eg, penicillins and cephalosporins) and are therefore resistant to these drugs.

The difference between L forms and mycoplasmas is that when the murein is allowed to reform, L forms revert to their original bacteria shape, but mycoplasmas never do.



## 2. Acid-Fast Cell Walls

The acid-fast stain is used to identify **all bacteria of the genus *Mycobacterium*** and **pathogenic species of *Nocardia***. These bacteria contain high concentrations (60%) of a hydrophobic waxy lipid (**mycolic acid**) **in their cell wall** that prevents the uptake of dyes, including those used in the Gram stain.

**The mycolic acid** forms a layer outside of a thin layer of **peptidoglycan**. The mycolic acid and peptidoglycan are held together by a polysaccharide.

Acid-fast bacteria can be stained with carbolfuchsin (Figure 6), which penetrates bacteria more effectively when heated. The carbolfuchsin penetrates the cell wall, binds to the cytoplasm, and resists removal by washing with acid-alcohol. Acid-fast bacteria retain the red color of carbolfuchsin because it's more soluble in the cell wall's mycolic acid than in the acid-alcohol.

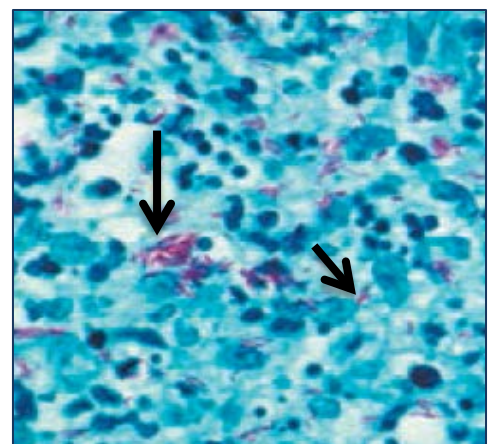


Figure 6 : *Mycobacterium*

## **Protoplasts, Spheroplasts, and L Forms**

Removal of the bacterial wall may be accomplished by hydrolysis with **lysozyme** or by **blocking peptidoglycan synthesis** with an antibiotic such as penicillin. In osmotically protective media, such treatments liberate **protoplasts from gram positive cells** and **spheroplasts** (which retain outer membrane and entrapped peptidoglycan) **from gram-negative cells**.

If such cells are able to grow and divide, they are called **L forms**.

- L forms are produced more readily with penicillin than with lysozyme, *suggesting the need for residual peptidoglycan*.
- Some bacterial species produce L forms spontaneously. The spontaneous or antibiotic-induced formation of L forms in the host may produce chronic infections, the organisms persisting by becoming sequestered in protective regions of the body. Because L-form infections are relatively resistant to antibiotic treatment, they present special problems in chemotherapy. Their reversion to the bacillary form can produce relapses of the overt infection.

## Archaeal Cell Walls

*Before they were distinguished as a unique domain of life, archaeal species were characterized as being either Gram positive or Gram negative based on their response to Gram staining. Thus, like all cells (even eukaryotic cells), they will stain either purple or pink when Gram stained. However, their staining reaction does not correlate reliably with a particular cell wall structure as it does for bacteria.*

**Archaeal cell walls exhibit considerable variety in terms of their chemical makeup. Furthermore, their cell walls lack peptidoglycan.**

a. The most common type of archaeal cell wall is an **S-layer** composed of either **glycoprotein** or **protein** (figure 7 a). The layer may be as thick as 20 to 40 nm. Some methanogens (Methanococcus), salt-loving archaea (Halobacterium), and extreme thermophiles (Thermoproteus, and Pyrodictium) have S-layer cell walls.

b & c. Other archaea have **additional layers of material outside the S-layer**:

b. For instance, Methanospirillum has a **protein sheath external to the S-layer** (figure 7 b).

c. Another methanogen, Methanosarcina, has a **polysaccharide layer covering the S-layer** (figure 7 c). This material, called **methanochondroitin**, is similar to the chondroitin sulfate of animal connective tissue.

d. In some archaea, the S-layer is the outermost layer and is separated from the plasma membrane by a **peptidoglycan-like molecule called pseudomurein** (figure 7 d).

Pseudomurein differs from peptidoglycan in that it has

- L-amino acids instead of D-amino acids in its cross-links,
- N-acetylalosaminuronic acid instead of N-acetylmuramic acid,
- and  $\beta(1-3)$  glycosidic bonds instead of  $\beta(1-4)$  glycosidic bonds.

These differences mean that lysozyme, penicillin, and other chemicals that affect bacterial cell wall structure and synthesis have no effect on archaeal cell walls.

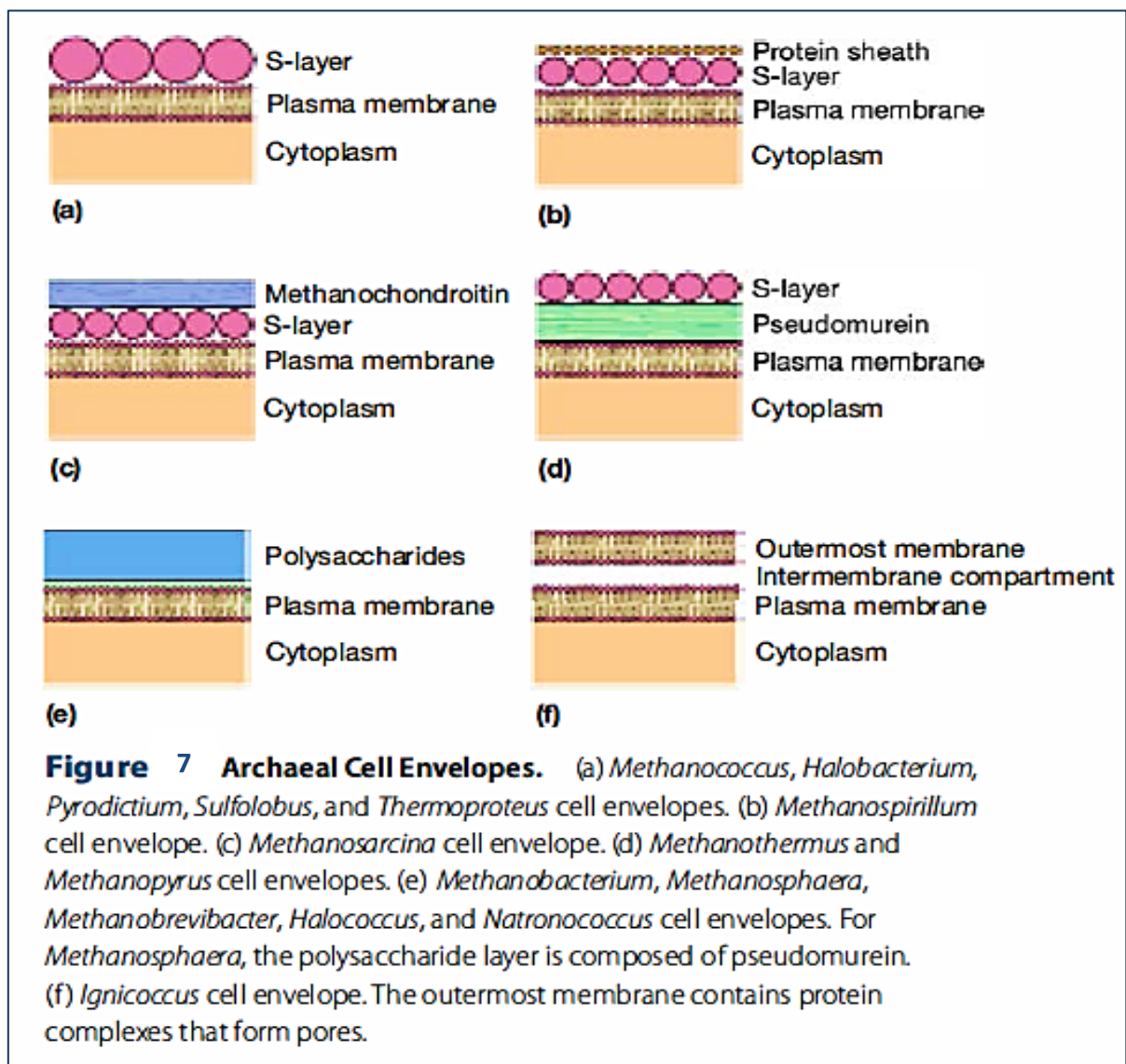
e. The last type of archaeal cell wall **consists of a single, thick homogeneous layer resembling that in Gram-positive bacteria** (figure 7 e). These archaea lack an S-layer and often stain Gram positive. Their wall chemistry varies from species to species but usually **consists of complex polysaccharides** such as pseudomurein.

f. Some archaea **lack any layer resembling a cell wall**.

\* For instance, members of the acidophilic genera *Ferroplasma* and *Thermoplasma* have envelopes consisting only of a plasma membrane covered by a **layer of slime**. The slime, which is referred to as a glycocalyx, may provide some of the protection needed for these archaea to survive in their acidic habitats.



\* The most unique wall-less archaeon is *Ignicoccus hospitalis*. Its envelope consists only of the plasma membrane and an outermost membrane, with an intermembrane compartment between them (figure 7 f). The outermost membrane contains protein complexes that form pores, much like bacterial porin proteins create pores in the outer membrane of typical Gram-negative bacteria.





## 2. The Plasma (Cytoplasmic) Membrane

The **plasma (cytoplasmic) membrane** (or **inner membrane**) is a thin structure lying inside the cell wall and enclosing the cytoplasm of the cell.

### \* **Bacterial plasma membrane**

#### **The chemical composition of plasma membrane**

The structure, first proposed by S. J. Singer and G. L. Nicolson, is called the **fluid mosaic model**.

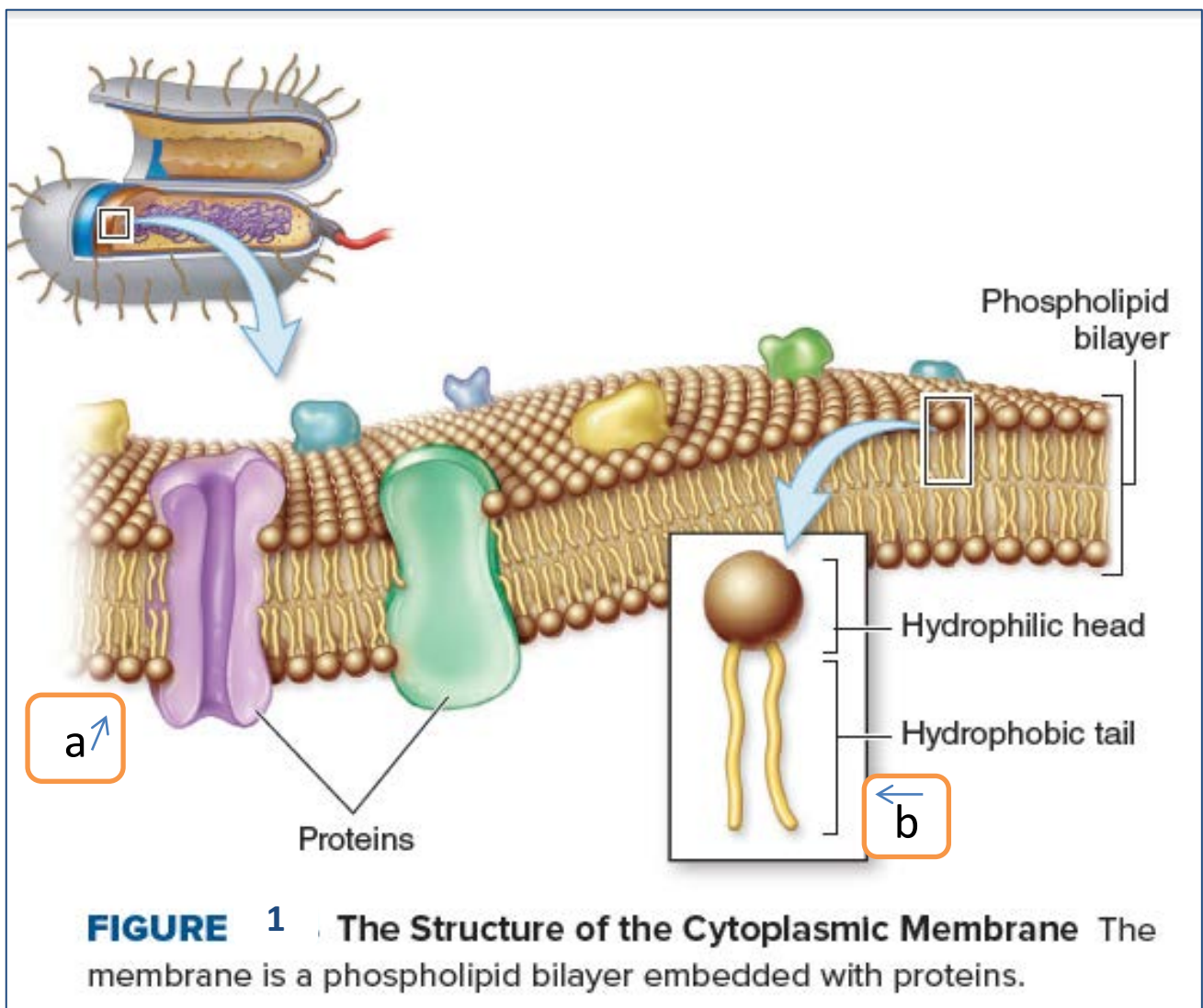
The **fluid mosaic model** describes our current understanding of membrane structure.

The term **mosaic** indicates that the membrane proteins are arranged in a way that resembles the tiles in a mosaic, and **fluid** indicates that the proteins and lipids are free to flow laterally within a membrane.

Cytoplasmic membranes are about 8 nm thick and composed of **phospholipids** and associated **proteins**.

Some bacterial membranes also contain **sterol-like molecules**, called **hopanoids**, that help stabilize the membrane.

The structure of a cytoplasmic membrane is referred to as a **phospholipid bilayer** (Figure 1 a).



### \*Phospholipids

- A phospholipid molecule is **bipolar**; that is, the two ends of the molecule are different (Figure 2 b).

\_The phosphate-containing **heads** of each phospholipid molecule are **hydrophilic**; that is, they are attracted to water at the two surfaces of the membrane.

\_The hydrocarbon **tails** of each phospholipid molecule are **hydrophobic** and huddle together with other tails in the interior of the membrane, away from water.

- Phospholipids placed in a watery environment naturally form a bilayer because of their bipolar nature.

### **\*Proteins**

- The protein molecules in the membrane can be arranged in a variety of ways (Figure 1 a):
  1. Some, called **peripheral proteins**, are easily removed from the membrane by mild treatments and lie at the inner or outer surface of the membrane.
  2. Other proteins, called **integral proteins**, can be removed from the membrane only after disrupting the lipid bilayer (by using detergents).
- Proteins of cell membranes may act as **recognition proteins, enzymes, receptors, carriers, or channels.**

In some locations, the cell membrane forms internal folds in the cytoplasm called **mesosomes**. These are prominent in gram-positive bacteria but are harder to see in gram-negative bacteria because of their relatively small size.

- **Mesosomes** presumably increase the internal surface area available for membrane activities.
- It has been proposed that **mesosomes** participate in cell wall synthesis and guiding the duplicated bacterial chromosomes into the two daughter cells during cell division.

## Functions of the Cell Membrane

1. The most important function of the plasma membrane is to serve as a **selective barrier** through which materials enter and exit the cell. In this function, plasma membranes have **selective permeability** (sometimes called **semipermeability**). This term indicates that certain molecules and ions pass through the membrane, but that others are prevented from passing through it.
2. Plasma membranes are also important to the breakdown of nutrients and the production of energy. The plasma membranes of bacteria contain **enzymes capable of catalyzing the chemical reactions that break down nutrients and produce ATP.**
3. In some bacteria, **pigments and enzymes involved in photosynthesis** are found in infoldings of the plasma membrane that extend into the cytoplasm. These membranous structures are called **chromatophores** or **thylakoids**.

## The Movement of Materials across Membranes

Materials move across plasma membranes of both prokaryotic and eukaryotic cells by **two kinds of processes**: **passive** and **active**.

In **passive processes**, substances cross the membrane **from an area of high concentration to an area of low concentration** (move with the concentration gradient, or difference), **without** any expenditure of **energy** (ATP) by the cell.

In **active processes**, the cell **must use energy (ATP)** to move substances **from areas of low concentration to areas of high concentration** (against the concentration gradient).

- **Passive Processes**

**Passive processes** include **simple diffusion**, **facilitated diffusion** and **osmosis** (Figure 2).

**Simple diffusion** is the net (overall) movement of molecules or ions from an area of high concentration to an area of low concentration (Figure 2a). The movement continues until the molecules or ions are evenly distributed.

Example: such as oxygen and carbon dioxide, across their cell membranes.

In **facilitated diffusion**, **integral membrane proteins** function as **carriers that facilitate the movement of ions or large molecules across the plasma membrane**. Such integral proteins are called **transporters or permeases** ( Figure 2 b&c ).

- Facilitated diffusion is similar to simple diffusion in that the cell **does not** expend energy, because the substance moves from a high to a low concentration.
- Facilitated diffusion differs from simple diffusion in its use of transporters.

In some cases, molecules that bacteria need are too large to be transported into the cells by these methods. Most bacteria, however, **produce enzymes** that can break down large molecules into simpler ones (such as proteins into amino acids, or polysaccharides into simple sugars). Such enzymes, which are released by the bacteria into the surrounding medium, are appropriately called **extracellular enzymes**. Once the enzymes degrade the large molecules, the subunits move into the cell with the help of transporters. For example, specific carriers retrieve DNA bases, such as the purine guanine, from extracellular media (substances outside the cell) and bring them into the cell's cytoplasm.

**Osmosis** ( Figure 2 d ) is the special name given to the diffusion of water across a semipermeable membrane—that is, across a membrane that is permeable to water molecules but not to most solutes that are present, such as proteins, amino acids, salts, or glucose. Because these solutes cannot freely penetrate the membrane, they cannot diffuse across the membrane, no matter how unequal their concentrations on either side may be. Instead, the water diffuses. Water molecules cross from the side of the membrane that contains a higher concentration of water (lower concentration of solute) to the side that contains a lower concentration of water (higher concentration of solute).

**In osmosis, water moves across the membrane until equilibrium is reached, or until the pressure of water is equal to the force of osmosis** ( Figure 3 a & b ).

We commonly compare solutions according to their concentrations of solutes.

- When solutions on either side of a selectively permeable membrane have the same concentration of solutes, the two solutions are said to be **isotonic**. In an isotonic situation, neither side of a selectively permeable membrane will experience a net loss or gain of water( Figure 3 c ).

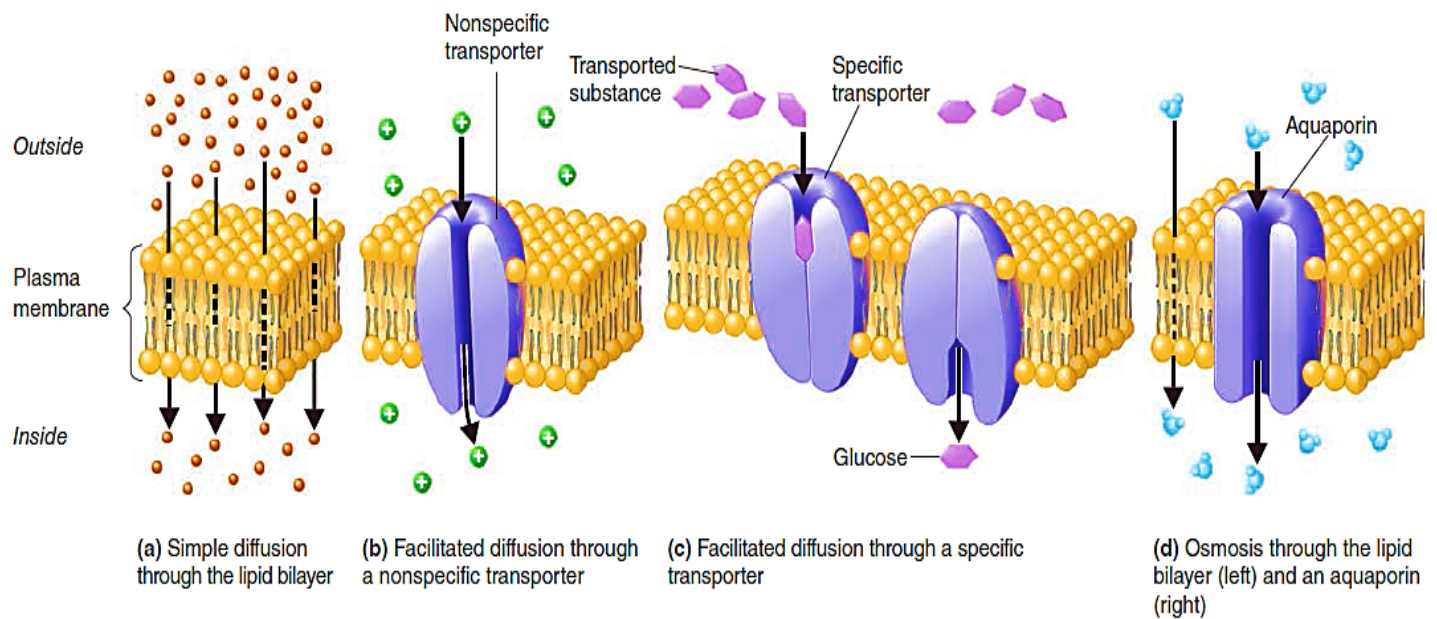
- When the concentrations of solutions are unequal, the solution with the higher concentration of solutes is said to be **hypertonic** to the other. The solution with a lower concentration of solutes is **hypotonic** in comparison.

Obviously, a **hypertonic solution, with its higher concentration of solutes, necessarily means a lower concentration of water; that is, a hypertonic solution has a lower concentration of water than does a hypotonic solution.** Like other chemicals, water moves down its concentration gradient from a hypotonic solution into a hypertonic solution.

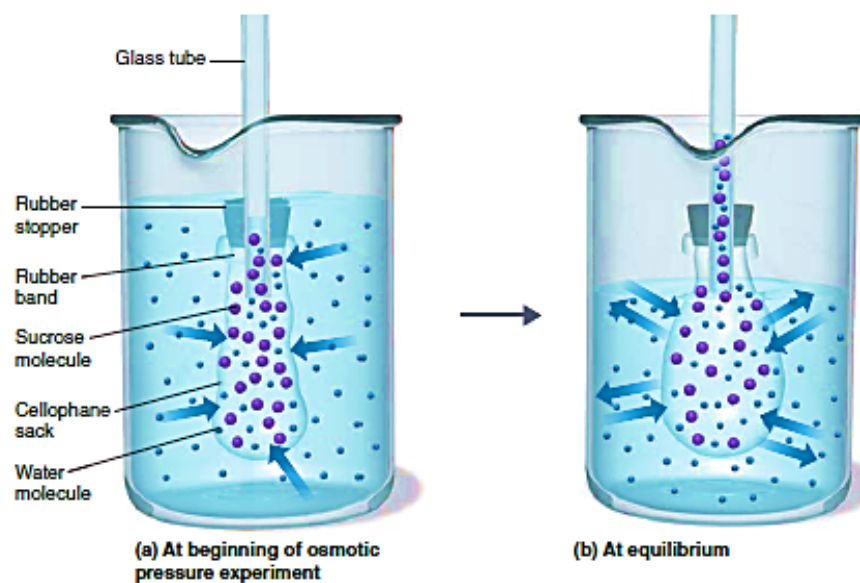
- A cell placed in a **hypertonic** solution will therefore lose water and shrivel ( Figure 3 e ).
- On the other hand, water will diffuse into a cell placed in a **hypotonic** solution because the cell has a higher solutes-to-water concentration. As water moves into the cell, water pressure against its cytoplasmic membrane increases, and the cell expands( Figure 3 d ).

One function of a cell wall, such as the peptidoglycan of bacteria, is to resist further osmosis and prevent cells from bursting.



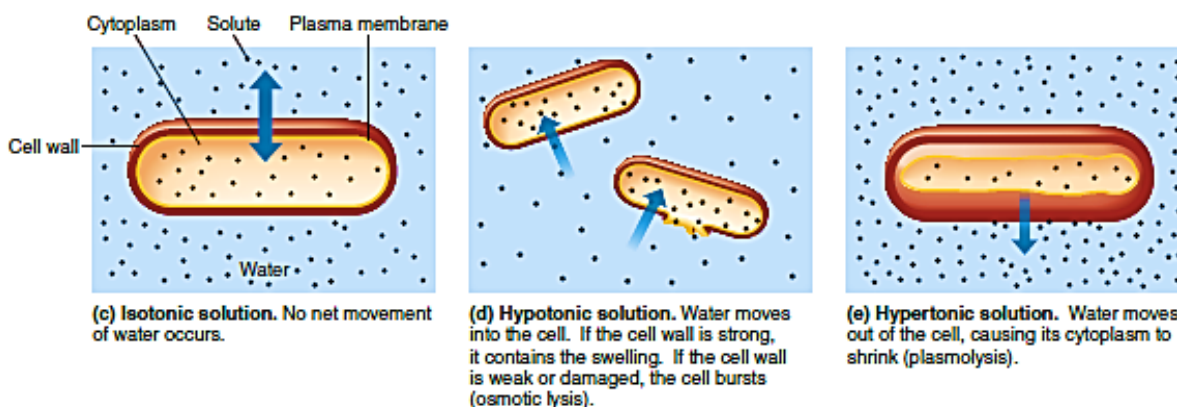


**Figure 2** Passive processes.



**Figure 3** The principle of osmosis. (a) Setup at the beginning of an osmotic pressure experiment. Water molecules start to move from the beaker into the sack along the concentration gradient. (b) Setup at equilibrium. The osmotic pressure exerted by the solution in the sack pushes water molecules from the sack back into the beaker to balance the rate of water entry into the sack. The height of the solution in the glass tube at equilibrium is a measure of the osmotic pressure. (c)–(e) The effects of various solutions on bacterial cells.

**Q** Why is osmosis important?



- **Active transport**

Many nutrients are concentrated more than a thousand-fold as a result of active transport.

\_ Active transport resembles facilitated diffusion in that it **involves carrier proteins**.

\_ Nevertheless, active transport differs from facilitated diffusion in its use of metabolic energy and its ability to concentrate substances.

**Three types of active transport are observed in bacteria:**

1. **Primary active transport.**
2. **Secondary active transport.**
3. **Group translocation.**

**They differ in terms of the energy used to drive transport and on whether or not the transported molecule is modified as it enters.**

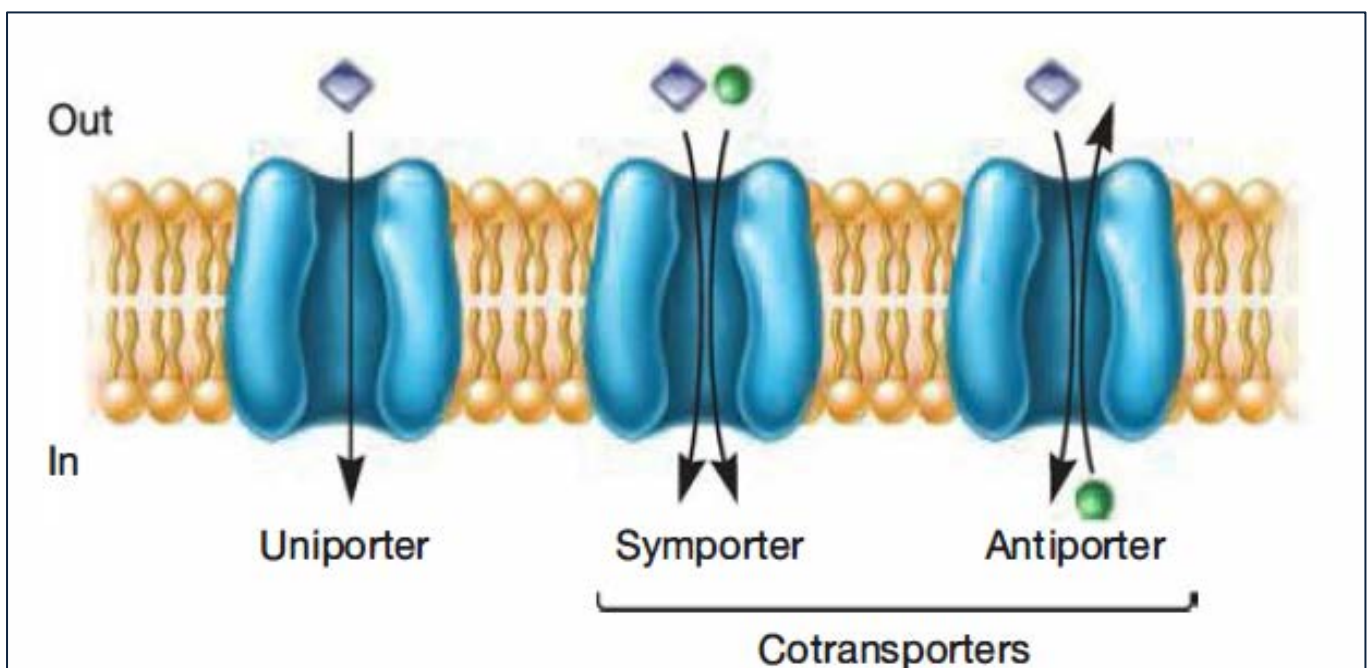
\_ **Primary active transport** is mediated by carriers called primary active transporters. They use the energy provided by ATP hydrolysis to move substances against a concentration gradient without modifying them. Primary active transporters are **uniporters**; that is, **they move a single molecule across the membrane** (figure 5). **ATP-binding cassette transporters (ABC transporters) are important primary active transporters.**

ABC transporters employ substrate-binding proteins. The substrate binding proteins bind the molecule to be transported and then interact with the transporter proteins to move the molecule into the cell.

*E. coli* transports a variety of sugars and amino acids by this mechanism.

**Secondary active transport** couples the potential energy of ion gradients to transport of substances without modifying them. Secondary active transporters are **cotransporters** (figure 5). **They move two substances simultaneously: the ion whose gradient powers transport and the substance being moved across the membrane.**

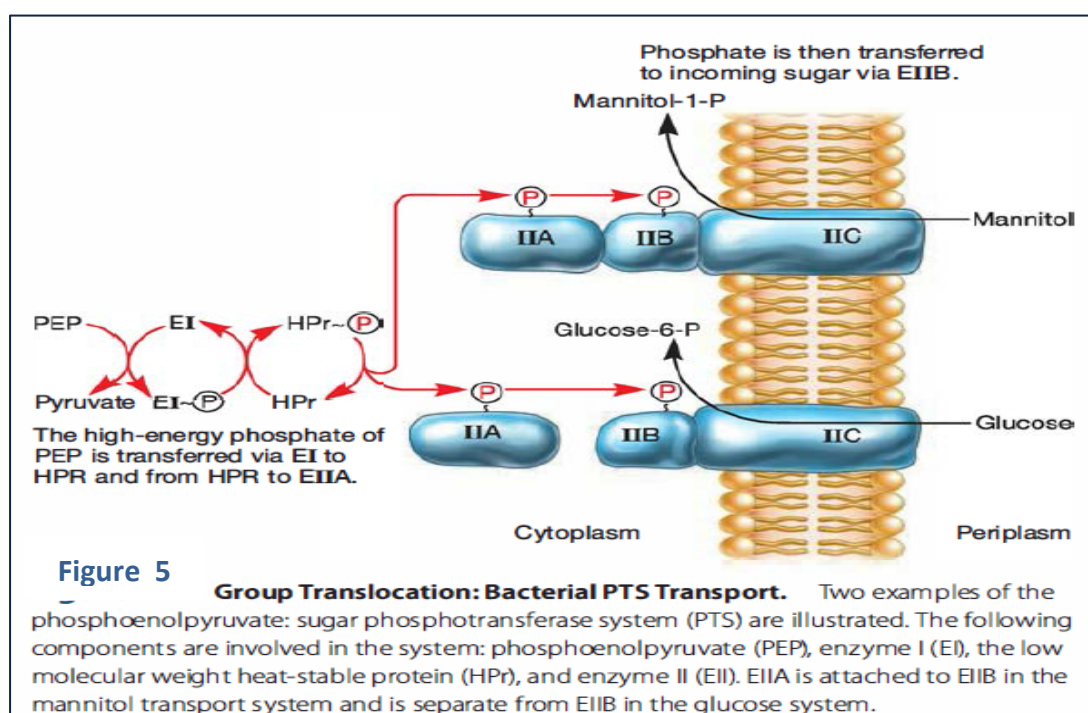
- When the ion and other substance both move in the **same direction**, it is called **symport**.
- When they move in **opposite directions**, it is called **antiport**.



**Figure 4 Carrier Proteins Can Be Uniporters or Cotransporters.**

Uniporters move a single substance into the cell. Cotransporters simultaneously move two substances across the membrane. When both substances move in the same direction, the carrier is a symporter. When the two substances move in opposite directions, the carrier is an antiporter.

**-Group Translocation** : the distinguishing characteristic of group translocation is that a **molecule is chemically modified** as it is brought into the cell (figure 5). The best-known group translocation system is the **phosphoenolpyruvate: sugar phosphotransferase system (PTS)**, which is observed in many bacteria. The PTS transports a variety of sugars while phosphorylating them, using phosphoenolpyruvate (PEP) as the phosphate donor. PEP is an important intermediate of a biochemical pathway used by many bacteria to extract energy from organic energy sources. PEP is a high-energy molecule that can be used to synthesize ATP, the cell's energy currency. However, when it is used in PTS reactions, the energy present in PEP is used to energize uptake rather than ATP synthesis.



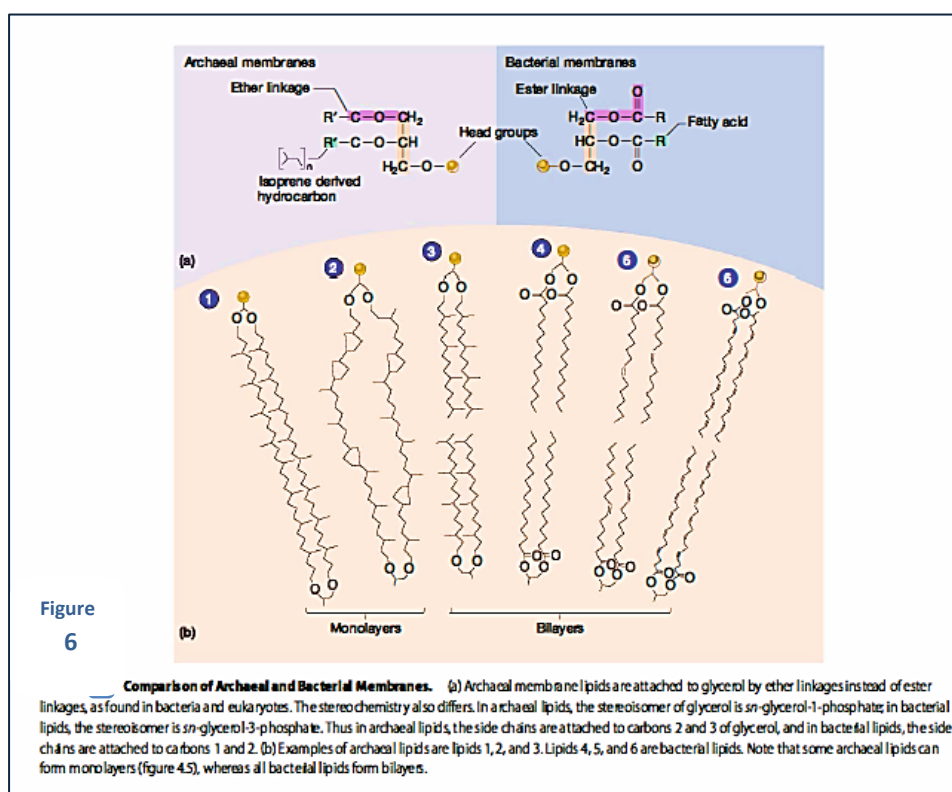


## \*Archaeal Plasma Membranes

Archaeal membranes are composed primarily of lipids that differ from bacterial and eukaryotic lipids in two ways.

First, they contain hydrocarbons derived from isoprene units- five-carbon, branched molecules (figure 6 ). This affects the way the lipids pack together, which in turn affects the fluidity of the membrane and its permeability. This is especially important for extremophilic archaea for which membrane fluidity and permeability could be compromised by extreme conditions.

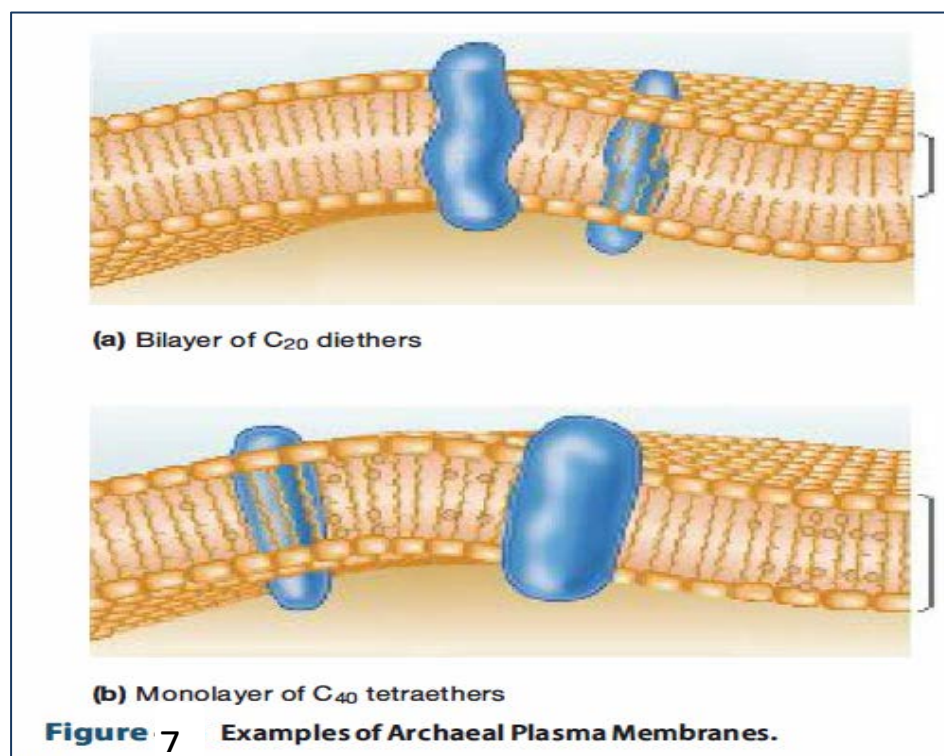
Second, the hydrocarbons are attached to glycerol by ether links rather than ester links . Ether linkages are more resistant to chemical attack and heat than are ester links



Despite the significant differences in membrane lipids, the basic design of archaeal membranes is **similar to that of bacterial and eukaryotic membranes: there are two hydrophilic surfaces and a hydrophobic core.**

- When C20 diethers are used, a typical bilayer membrane is formed (figure 7a).
- When the membrane is constructed of C40 tetraethers, a monolayer membrane with much more rigidity is formed (figure 7 b).

The addition of pentacyclic rings further increases this rigidity. As might be expected from their need for stability, the membranes of extreme thermophiles such as *Thermoplasma* and *Sulfolobus*, which grow best at temperatures over 85°C, are almost completely tetraether monolayers. Archaea that live in moderately hot environments have membranes containing some regions with monolayers and some with bilayers.



## Bacterial Internal Structure

### Cytoplasm

**Cytosol** : is the water-like fluid found in bacterial cells. The cytosol contains all the other internal compounds and components the bacteria needs for survival. The fluid and all its dissolved or suspended particles is called the cytoplasm of the cell. Its major component is water (70%-80%), which serves as a solvent for a complex mixture of nutrients including Proteins, amino acids, sugars, nucleotides, salts, vitamins, enzymes. The major structures in the cytoplasm of prokaryotes are reserve deposits called **inclusions Bodies**, **ribosomes**, **nucleoid** (containing DNA) and the **Cytoskeleton**.

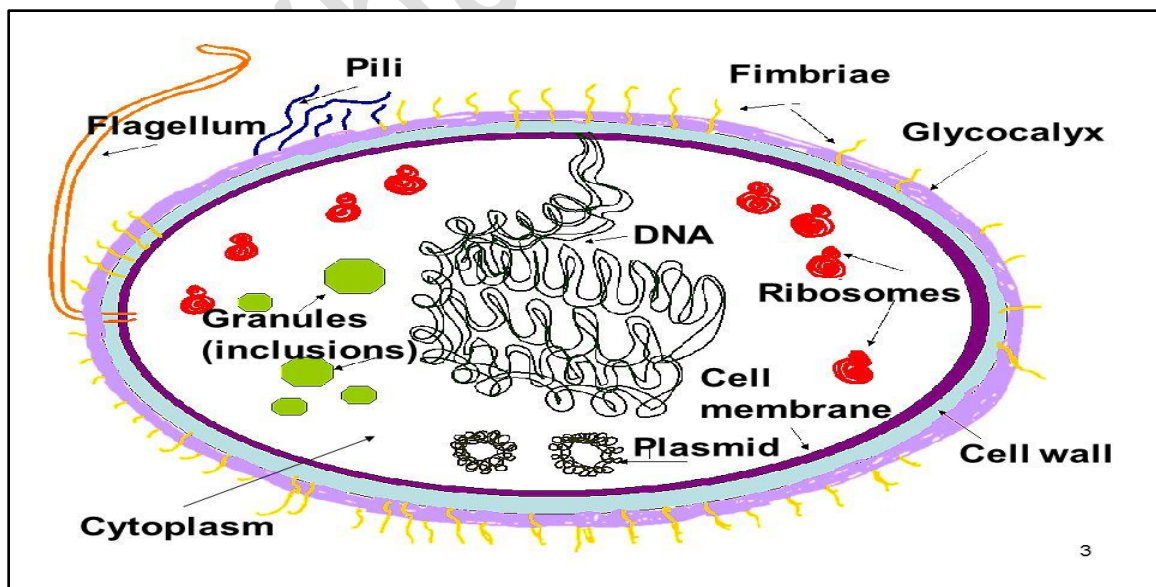


Figure 1: Bacterial internal structure

## 1- Inclusion Bodies (:

### 1-1 Volutin Granules or Metachromatin Granules

Many bacteria and microalgae accumulate inorganic phosphates in the form of granules of polyphosphates.

Because they were first described in *Spirillum volutans* and because they bring about metachromatic effect (appear red with certain blue when stained with methylene blue), they have also been given the name 'volutin granules' and 'metachromatin granules', respectively. Common in *Corynebacterium diphtheria* and in certain lactic acid bacteria. These granules reserve when nucleic acid synthesis does not occur, or else the polyphosphate granules are degraded and used as sources of phosphate for nucleic acids. Polyphosphates are also used as energy source (ATP) in reactions.



Figure 2: Cells of *Corynebacterium diphtheria* showing metachromatin granules as dark granules



### 1-2 Poly- $\beta$ -hydroxybutyrate (PHB) (:

Poly-  $\beta$  -hydroxybutyrate (PHB), one of the most common inclusion bodies in bacteria, is a **lipid** formed from  $\beta$  -hydroxybutyrate monomers (units). Appear in various species of Mycobacterium, Bacillus, Azotobacter, Spirillum and other. PHB is accumulated by aerobic and facultative bacteria when the cells are deprived of oxygen and must carry out fermentative metabolism.

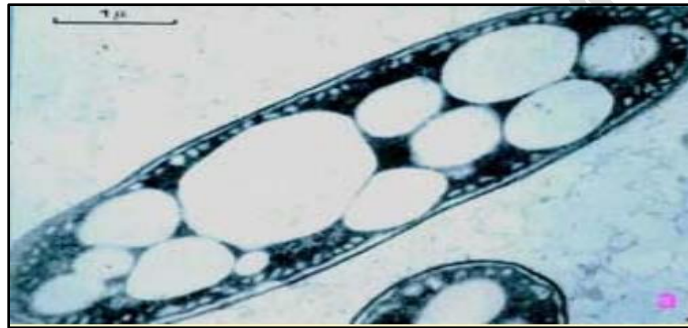


Figure 3: Electron micrograph showing Poly-  $\beta$  -hydroxybutyrate (PHB)

### 1-3 Glycogen:

It is a polymer of glucose units (**polysaccharide**) composed of long chains formed by  $\alpha(1 \rightarrow 4)$  glycosidic bonds. Act as a storage reservoir for carbon and energy. In the presence of iodine, glycogen granules appear reddish brown.

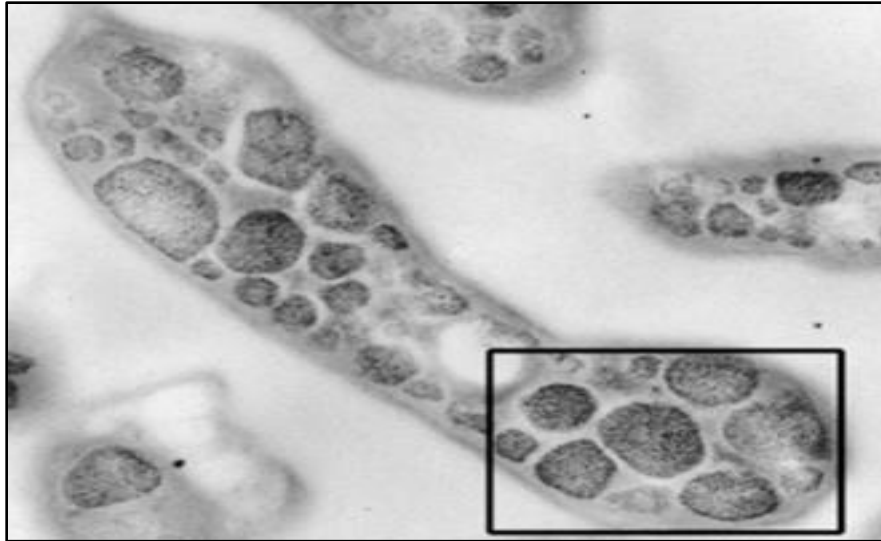
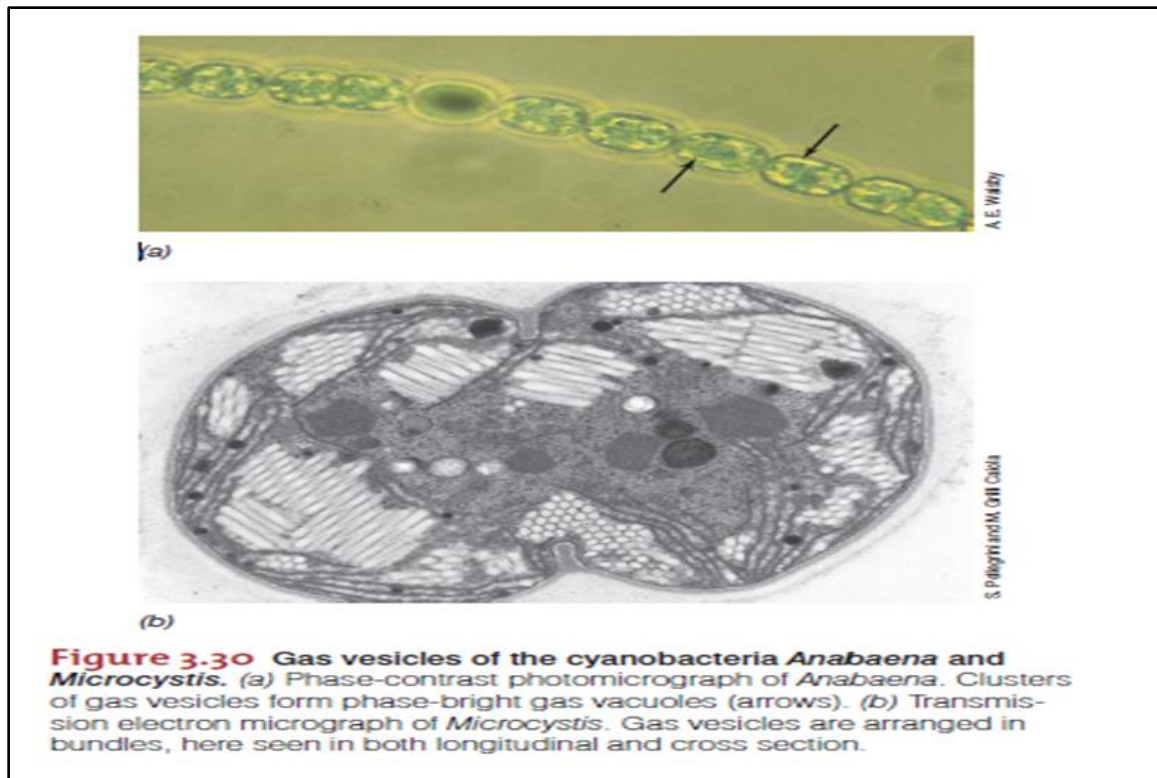


Figure 4: Large particles of Glycogen.

#### 1-4 **Gas vacuoles:**

Hollow cavities found in many aquatic prokaryotes, including cyanobacteria, anoxygenic photosynthetic bacteria and halobacteria are called **Gas vacuoles**. Each vacuole consists of several individual gas vesicles (that store gases in protein sacs), which are hollow cylinders covered by protein. Gas vacuoles maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light, and nutrients.



### 1-5 Magnetosomes:

Magnetosomes are the inorganic inclusion bodies of iron usually in the form of intracellular chains of iron oxide ( $\text{Fe}_3\text{O}_4$ ). Magnetosomes vary in shape from square to rectangular to spike-shaped as their morphology is species-specific. Most of the magnetotactic aquatic bacteria grow best at very low  $\text{O}_2$  concentrations the main function of magnetosomes is probably to guide such bacteria toward the sediment where  $\text{O}_2$  concentration is lower and downward directions and swim down to nutrient-rich sediments or locate the optimum depth in fresh water and marine habitats.

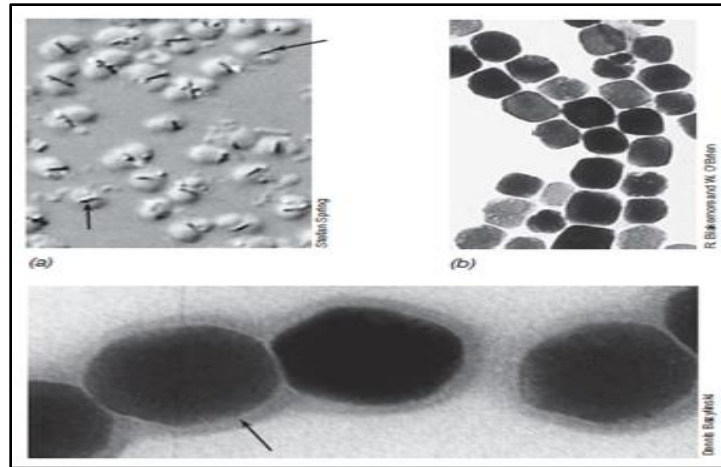


Figure 6: Magnetotactic bacteria and magnetosomes

### 1-6 Sulphur Globules:

Sulphur globules are present in the bacterial cells growing in  $\text{H}_2\text{S}$  rich environment (sulfur bacteria) that belong to the genus *Thiobacillus*. These bacteria oxidize  $\text{H}_2\text{S}$  into elemental sulfur ( $\text{H}_2\text{S} \rightarrow \text{S}^0$ ) which accumulates inside the cell in visible sulfur globules. These sulfur globules of elemental sulfur remain until the  $\text{H}_2\text{S}$  source is reduced. In the latter condition the stored sulfur in these granules is oxidized to sulfate ( $\text{S}^0 \rightarrow \text{SO}_4^{2-}$ ) and the globules slowly disappear.

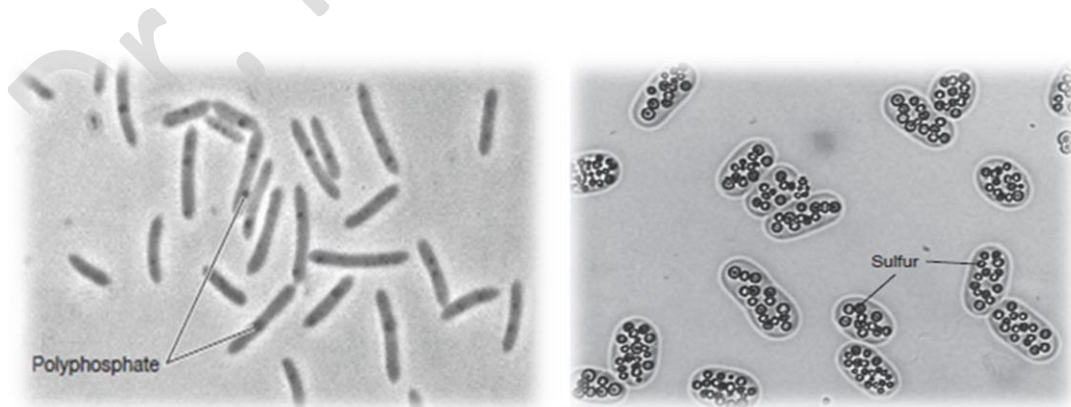


Figure 7: Sulphur Globules

### 1-7 Carboxysomes:

Carboxysomes are inclusions that contain the enzyme: **ribulose-1, 5-bisphosphate carboxylase**. Photoautotrophic (cyanobacteria) and chemolithoautotrophic (sulfur bacteria, nitrifying bacteria) use carbon dioxide as their source of carbon and require this enzyme for carbon dioxide fixation.

### 2- Ribosomes (:

Ribosomes in bacteria (prokaryotes) are small granular bodies of 10-20 nm in diameter freely lying in the cytoplasm and composed of ribosomal ribonucleic acid (**rRNA**) and **proteins**.

#### Ribosomes Subunits:

Each ribosome has sedimentation coefficient of **70S** and is made up of two subunits **50S and 30S**, each subunit consisting of roughly equal amounts of rRNA and protein. The number 30S, 50S, and 70S **refer to Svedberg units, which are units of sedimentation coefficient of ribosome subunits (30S and 50S) or intact ribosomes (70S) when subjected to centrifugal force in an ultracentrifuge**. Ribosomes are functional only when the two subunits are combined together.

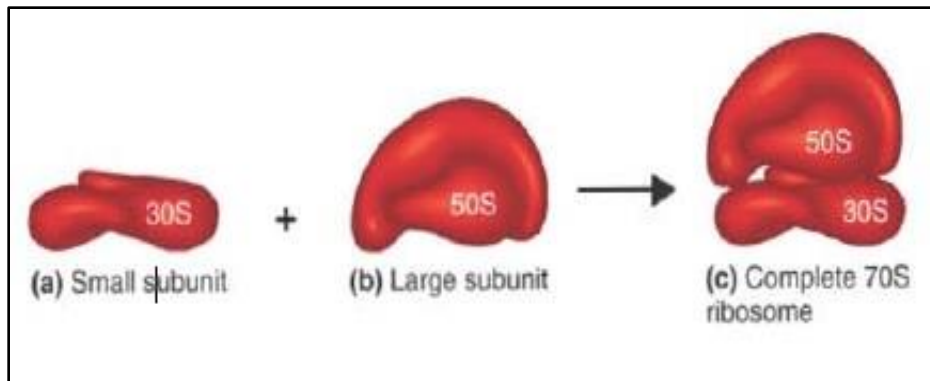


Figure 1: The prokaryotic ribosome

### Ribosomes Functions:

As in eukaryotes, ribosomes are **the sites of protein synthesis in bacteria**. Several antibiotics such as streptomycin, neomycin, tetracyclines, and chloramphenicol specifically inhibit protein synthesis by attacking ribosomes.

### Chromosomes and Plasmids: The source of genetic information (:

Prokaryotes and eukaryotes package their DNA molecules with protein in structures called chromosomes. The vehicle by which hereditary information is transmitted from one generation to the next and which carries all the information required for the cell's structures and functions. Bacterial chromosomes are generally ~1000 times longer than the cells in which they reside.

### The difference between prokaryotes and eukaryotes chromosome:

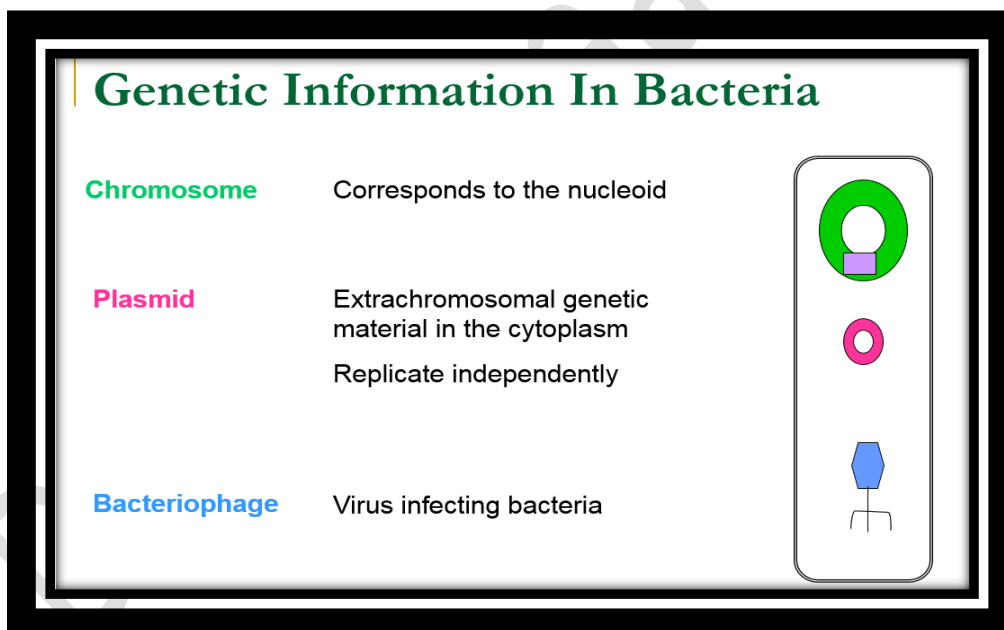
Prokaryote	Eukaryote
1. A circular DNA molecule	Linear DNA
2. No associated proteins	Associated with histone proteins
3. Plasmid often present	No plasmid
4. One chromosome only	Two or more different chromosomes

### 3- Plasmid (:

Is a small **double-stranded DNA molecules** within a cell that is physically separated from a **chromosomal DNA**, and can replicate independently, however plasmids are sometimes present in **archaea and eukaryotic organisms**. Plasmid can be transferred from one bacterium to another. Plasmids are not essential to a cell's survival and many of them carry genes that code for certain phenotypic characteristics of the host cell that enable a bacterium to colonize a host and overcome its defenses.

The following **plasmid types** are medically important:

- Virulence plasmids: Carry determinants of bacterial virulence, e.g., enterotoxin genes or hemolysin genes.
- Resistance plasmids (R plasmid): Carry genetic information causing resistance to anti-infective agents.
- Conjugative plasmid (F plasmid) a plasmid that is transferred from one bacterial cell to another during conjugation.



### Summary of Genetic Information In Bacteria



## 4- Cytoskeleton

The network of protein structures within the cytoplasm, Composed of three elements:

- **microtubules,**
- **microfilaments**
- **intermediate filaments.**

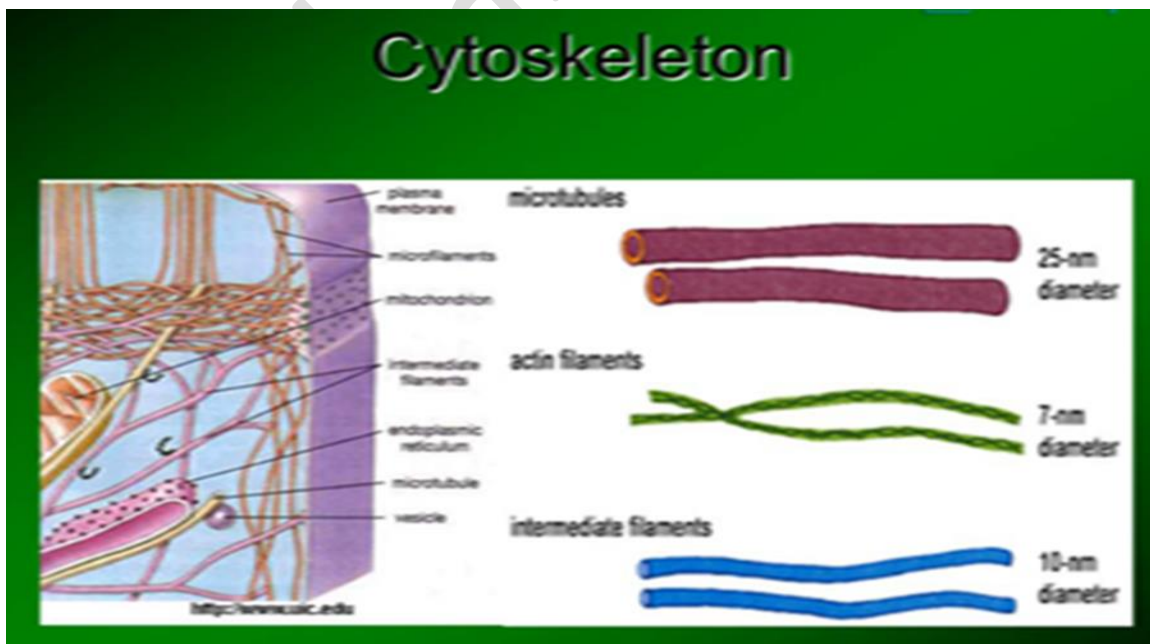
**Microtubules:** the thickest of the cytoskeleton structures 25 nm diameter, are long hollow cylinders composed of protein subunits called tubulin.

### **Function:**

Form the mitotic spindles, the mechanism that partitions chromosomes between two cells in the process of cell division, also function as the framework along which vesicles move within a cell.

**Microfilaments:** 7 nm diameter, enable the cell cytoplasm to move. They are composed of a polymer of actin.

**Intermediate filaments:** function like ropes, 10 nm diameter, strengthening the cell mechanically.



# Molecular Microbiology

**The** nucleic acids, **deoxyribonucleic acid (DNA)\*** and **Ribonucleic acid (RNA)\***, were originally isolated from the cell nucleus. Thereafter, they were also found in other parts of nucleated cells, in cells with no nuclei (bacteria) and in viruses.

## The Levels of Structure and Function of the Genome

The **genome** is the total of genetic material (DNA) carried within a cell. Although most of the genome exists in the form of chromosomes, In general, a **chromosome** is a discrete cellular structure composed of a neatly packaged DNA molecule.

## Compare and contrast the genomes of prokaryotes and eukaryotes



The chromosomes of eukaryotes and bacterial cells differ in several respects. The structure of eukaryotic chromosomes consists of a DNA molecule tightly wound around histone proteins, whereas a bacterial chromosome is condensed and secured into a packet by means of a different type of protein. Eukaryotic chromosomes are located in the nucleus; they vary in number from a few to hundreds; they can occur in pairs (diploid) or singles (haploid); and they are linear in format. In contrast, most bacteria have a single, circular chromosome, although some have multiple chromosomes and a few have linear chromosomes.

All chromosomes contain a series of basic informational “packets” called genes. A **gene** can be defined as a specific segment of DNA that contains the

necessary codes to make a molecule of protein genes that code for proteins, genes that code for RNA, and regulatory genes that control gene expression. The collection of all of these types of genes constitutes an organism's distinctive genetic makeup, or **genotype**. The expression of the genotype creates traits (certain structures or functions) referred to as the **phenotype**.

### **The Structure of RNA & A Double Helix DNA:**

**Describe the structure of DNA, and discuss how it facilitates the ability of DNA to act as genetic material.**



Nucleic acids are polymers of basic building blocks called nucleotides. Each nucleotide is made up of phosphate attached to a *nucleoside*, which is in turn made up of a pentose sugar (ribose in RNA and deoxyribose in DNA) attached to one of five nitrogenous bases: guanine (G), cytosine (C), thymine (T), adenine (A), or uracil (U).

The bases of nucleotides hydrogen-bond to one another in specific ways called complementary base pairs (bp): in DNA, the complementary bases thymine and adenine bond to one another with two hydrogen bonds, whereas in RNA, uracil, not thymine, forms two hydrogen bonds with adenine. In both DNA and RNA, the complementary bases guanine and cytosine bond to one another with three hydrogen bonds. Deoxyribonucleotides are linked through their sugars and phosphates to form the two backbones of a helical, double-stranded DNA (dsDNA) molecule. The carbon atoms of deoxyribose are numbered 1' (pronounced "one prime") through 5' ("five prime"). One end of a DNA strand is called the 5' end because it terminates in a phosphate group attached to a 5' carbon; the opposite (3') end terminates with a hydroxyl group bound to a 3' carbon of deoxyribose. The two strands are constructed similarly but are

oriented in opposite directions to each other; one strand runs in a 5' to 3' direction, while the other runs 3' to 5'. Scientists say the two strands are *antiparallel*. The base pairs extend into the middle of the molecule in a way reminiscent of the steps of a spiral staircase.

The lengths of DNA molecules are not usually given in metric units; instead, the length of a DNA molecule is expressed in base pairs. For example, the genome of the bacterium *Carsonella ruddii* is 159,662 bp long, making it the smallest known cellular genome.

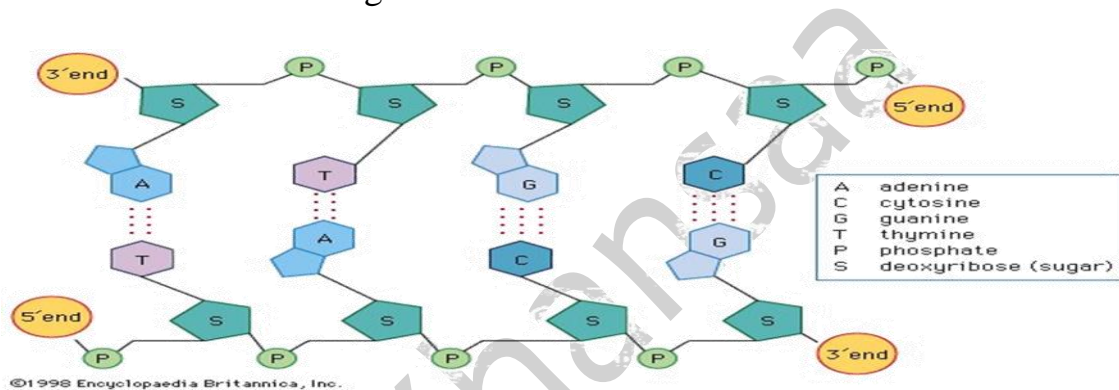


Figure 1: DNA molecules.

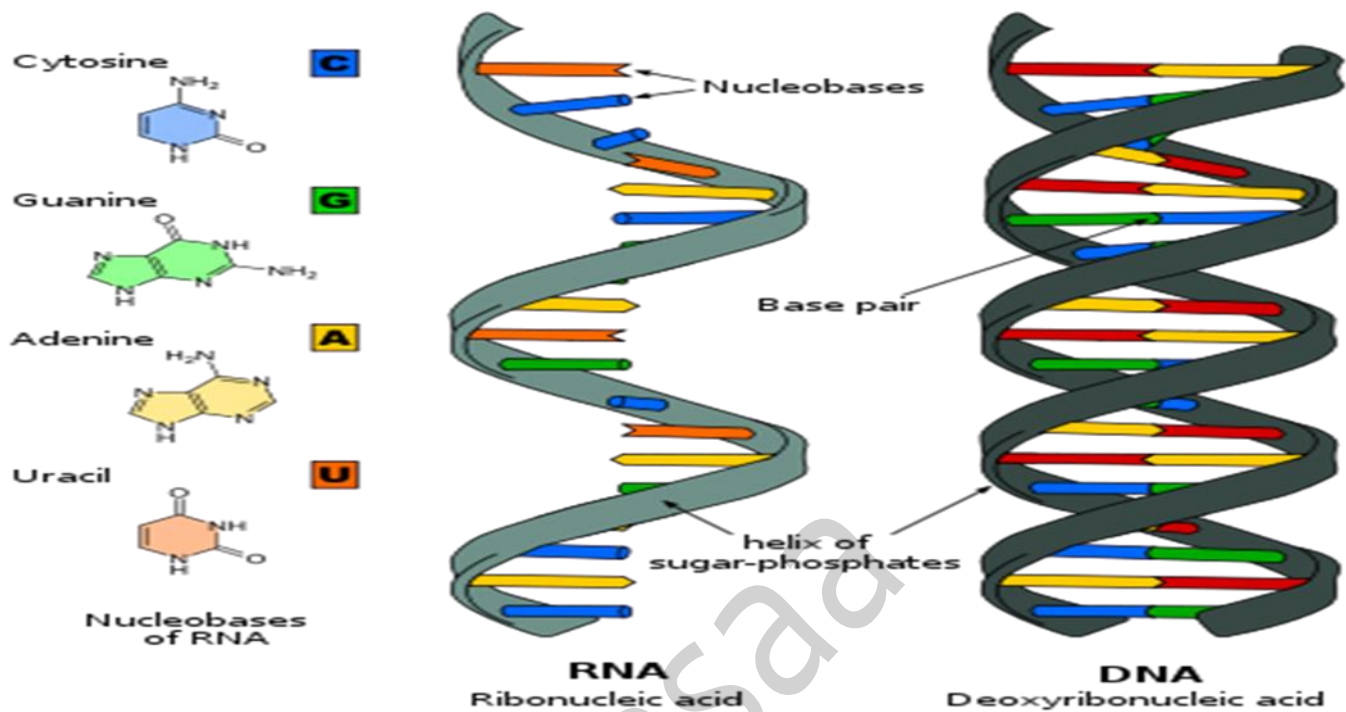


Figure 2: DNA and RNA.

**TABLE 7.1** Characteristics of Microbial Genomes

	Bacteria	Archaea	Eukarya
Number of chromosomes	Single (haploid) copies of one or rarely two	One (haploid)	With one exception, two or more, typically diploid
Plasmids present?	In some cells; frequently more than one per cell	In some cells	In some fungi, algae, and protozoa
Type of nucleic acid	Circular or linear dsDNA	Circular dsDNA	Linear dsDNA in nucleus; circular dsDNA in mitochondria, chloroplasts, and plasmids
Location of DNA	In nucleoid of cytoplasm and in plasmids	In nucleoid of cytoplasm and in plasmids	In nucleus and in mitochondria, chloroplasts, and plasmids in cytosol
Histones present?	No, though chromosome is associated with a small amount of nonhistone protein	Yes	Yes

Table 1: Characteristics of microbial genomes.

**TABLE 2.5** Comparison of Nucleic Acids

Characteristic	DNA	RNA
Sugar	Deoxyribose	Ribose
Purine nucleotides	A and G	A and G
Pyrimidine nucleotides	T and C	U and C
Number of strands	Double stranded in cells and in most DNA viruses; single stranded in parvoviruses	Single stranded in cells and in most RNA viruses; double stranded in reoviruses
Function	Genetic material of all cells and DNA viruses	Protein synthesis in all cells; genetic material of RNA viruses

Table 2: Comparison of nucleic acids.

**Important definitions in DNA replication:**

**DNA polymerase III :** Adding bases to the new DNA chain; proofreading the chain for mistakes.

**DNA polymerase I :** Removing RNA primers, replacing gaps between Okazaki fragments with correct nucleotides, repairing mismatched bases.

**Primase:** Synthesizing an RNA primer.

**Ligase:** Final binding of nicks in DNA during synthesis and repair

**Gyrase:** Supercoiling.

**Replication fork:** The Y-shaped point on a replicating DNA molecule where the DNA polymerase is synthesizing new strands of DNA.

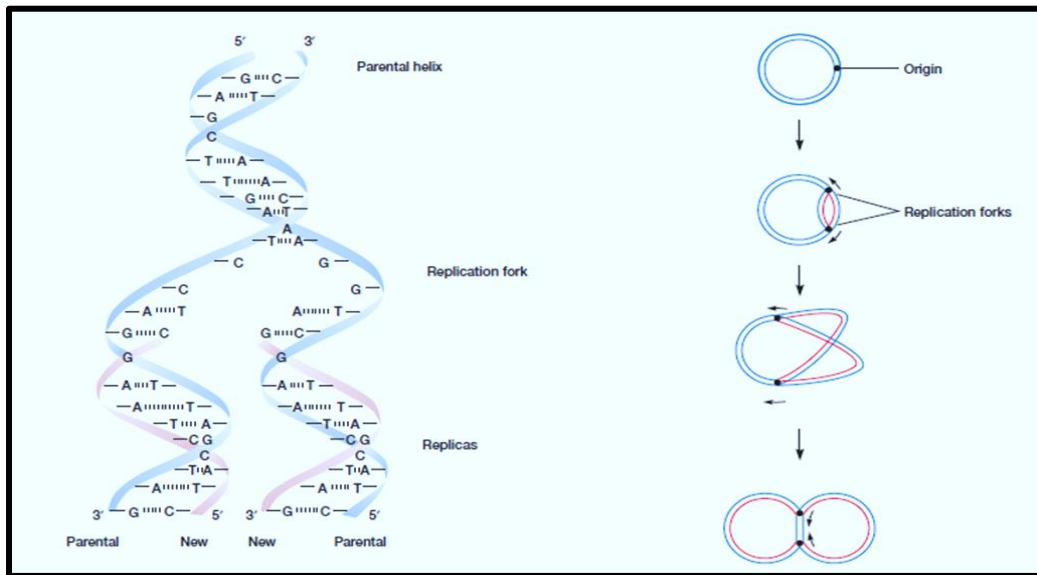


Figure 3: Semiconservative Replication

Bidirectional Replication

## DNA Replication (:

Compare and contrast the synthesis of leading and lagging strands in

DNA



A simplified version of replication is shown in (**figure 4**) and includes the following:

### A :) Synthesis of the Leading Strand

A cell synthesizes a leading strand toward the replication fork. Because DNA polymerase III can only synthesize new DNA in the 5' to 3' direction, just one strand, called the **leading strand**, will be synthesized continuously. After addition of an RNA primer, a DNA polymerase complex enters and moves



along the template strand in the 3' to 5' order toward the replication fork, adding nucleotides in the 5' to 3' order on the new strand.

- 1- An enzyme called primase synthesizes a short RNA molecule that is complementary to the template DNA strand. This RNA primer provides the 3' hydroxyl group required by DNA polymerase III.
- 2- Triphosphate deoxyribonucleotides form hydrogen bonds with their complements in the parental strand. Adenine nucleotides bind to thymine nucleotides, and guanine nucleotides bind to cytosine nucleotides.
- 3- Using the energy in the high-energy bonds of the triphosphate deoxyribonucleotides, DNA polymerase III covalently joins the nucleotides one at a time to the leading strand. DNA polymerase III can add about 500 to 1000 nucleotides per second to a new strand.
- 4- DNA polymerase III also performs a proofreading function. About one out of every 100,000 nucleotides is mismatched with its template; for instance, a guanine might become incorrectly paired with a thymine. DNA polymerase III recognizes most of these errors and removes the incorrect nucleotides before proceeding with synthesis. This role, known as the proofreading exonuclease function, acts like the backspace key on a keyboard, removing the most recent error.
- 5- Another DNA polymerase—DNA polymerase I replaces the RNA primer with DNA.

### **B :) Synthesis of the Lagging Strand**

Because DNA polymerase III adds nucleotides only to the 3' end of the new strand, the enzyme moves away from the replication fork as it synthesizes a



lagging strand. As a result, the lagging strand is synthesized discontinuously and always lags behind the process occurring in the leading strand. The steps in the synthesis of a lagging strand are as follows.

- 1- Primase synthesizes RNA primers, but in contrast to its action on the leading strand, primase synthesizes **multiple primers**—one every 1000 to 2000 .
- 2- Nucleotides pair up with their complements in the template—adenine with thymine and cytosine with guanine.
- 3- DNA polymerase III joins neighboring nucleotides and proofreads. In contrast to synthesis of the leading strand, however, the lagging strand is synthesized in discontinuous segments called **Okazaki fragments**, named for the Japanese scientist Reiji Okazaki (1930–1975), who first identified them. Each Okazaki fragment uses one of the new RNA primers, so each fragment consists of 1000 to 2000 nucleotides.
- 4- DNA polymerase I replaces the RNA primers of Okazaki fragments with DNA and proofreads the short DNA segment it has synthesized.
- 5- DNA ligase seals the gaps between adjacent Okazaki fragments to form a continuous DNA strand.

In summary, synthesis of the leading strand proceeds continuously toward the replication fork from a single RNA primer at the origin, following helicase and the replication fork down the DNA. The lagging strand is synthesized away from the replication fork discontinuously as a series of Okazaki fragments, each of which begins with its own RNA primer. All the primers are eventually replaced with DNA nucleotides, and ligase joins the Okazaki fragments.

As noted earlier, DNA replication is semiconservative; each daughter molecule is composed of one parental strand and one daughter strand. The replication process produces doublestranded daughter molecules with a nucleotide sequence.

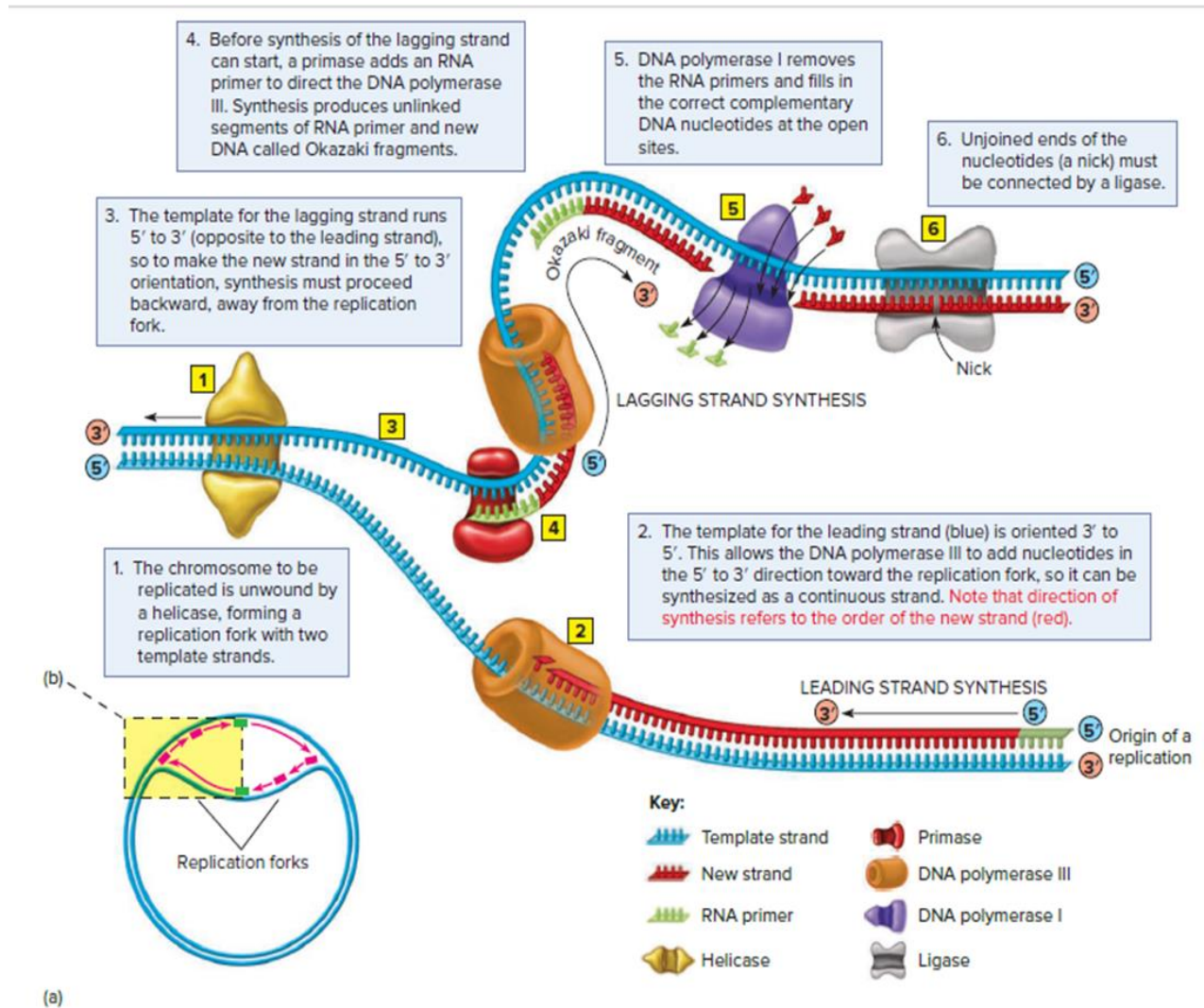


Figure 4: **DNA replication** in a circular bacterial chromosome (a): bacterial chromosome showing the overall pattern of replication, (b): an enlarged view of left replication fork to see the details of replication.

# Transcription and Translation (Gene Expression)

## 1- Transcription:

Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA (especially mRNA) by the enzyme RNA polymerase.

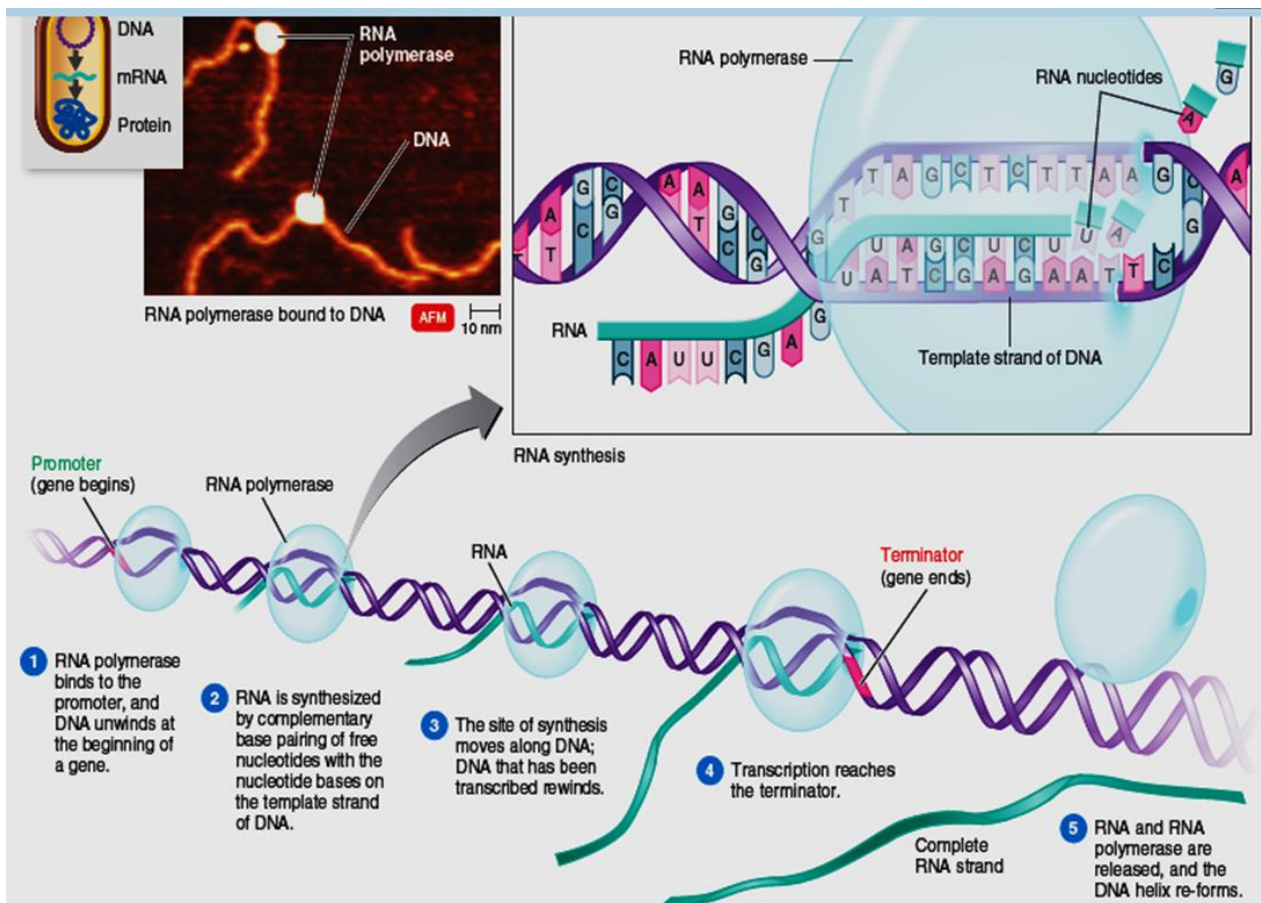


Figure 5: The major events in transcription, using mRNA.

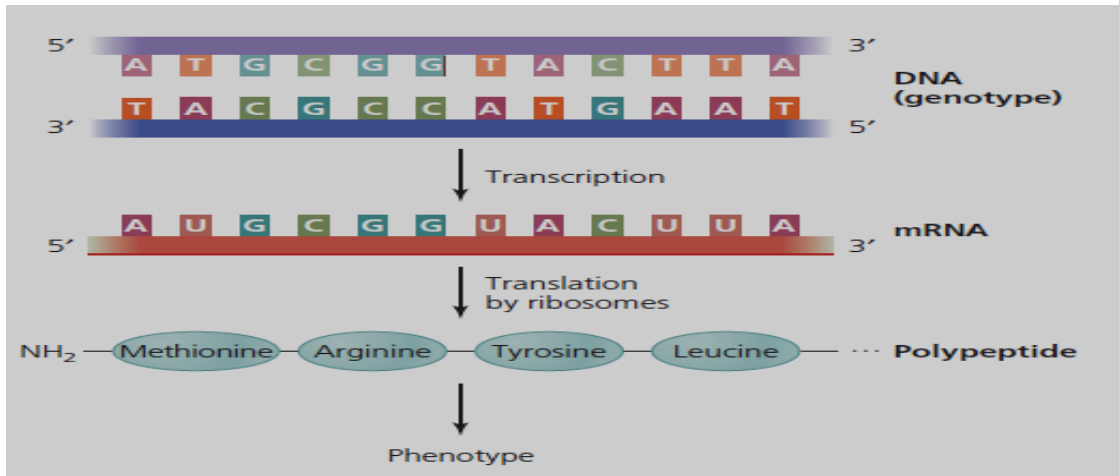


TABLE 9.2 Major Types of Ribonucleic Acid Involved In Protein Synthesis			
RNA Type	Contains Codes For	Function In Cell	Translated
Messenger (mRNA)	Sequence of amino acids in protein	Carries the DNA master code to the ribosome	Yes
Transfer (tRNA)	Specifying a given amino acid	Carries amino acids to ribosome during translation	No
Ribosomal (rRNA)	Several large structural rRNA molecules	Forms the major part of a ribosome and participates in protein synthesis	No
Primer	An RNA that can begin DNA replication	Primes DNA	No

Table 3: RNA types involved in protein synthesis

## 2- The Second Stage of Gene Expression: Translation (Protein synthesis :)

In translation, all of the elements needed to synthesize a protein, from the mRNA to the tRNAs with amino acids, are brought together on the ribosomes. The process occurs in five stages: **initiation**, **elongation**, **termination**, and **protein folding and processing**.

- **The Beginning (initiation) of Protein Synthesis**

With mRNA serving as the guide, the stage is finally set for actual protein assembly. The correct tRNA (labeled **1** on **figure 6**) enters the P site and binds to the **start codon (AUG)** presented by the mRNA. Rules of pairing dictate that the **anticodon** of this tRNA will complement the mRNA codon AUG; thus, the tRNA with anticodon UAC will first occupy site P. It happens that the amino acid carried by the initiator tRNA in bacteria is *formyl methionine* (fMet), though in many cases, it may not remain a permanent part of the finished protein.

- **Elongation begins** with the filling of the A site by a second tRNA (**step1**). The identity of this tRNA and its amino acid is dictated by the second mRNA codon.
- From this point on, **peptide elongation proceeds** repetitively by this same series of actions out to the end of the mRNA (**steps 2,... and 8**).



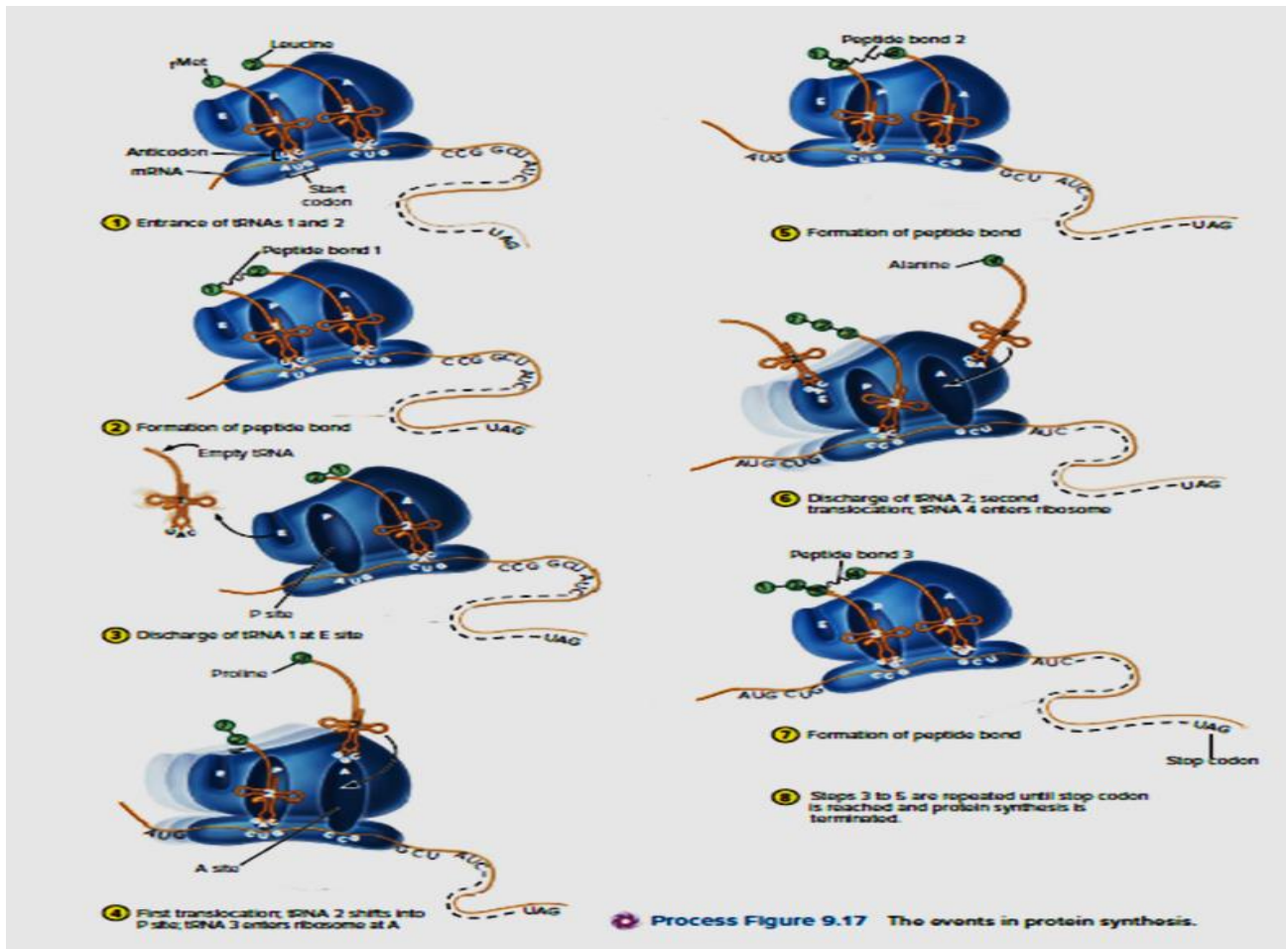


Figure 6: Protein synthesis – Translation

## DNA Recombination:

Bacterial recombination is a type of genetic recombination in bacteria characterized by DNA transfer from one organism called donor to another organism as recipient. This process occurs in three main ways:

1-Transformation, 2-Transduction, and 3-Conjugation. In general, any organism that has acquired genes that originated in another organism is called a **recombinant**.

## 1. Transformation

It is the transfer of “naked” DNA after cell lysis and this transformation process has been observed mainly in the genera *Streptococcus*, *Neisseria*, *Helicobacter* and *Haemophilus*. **Example** of genes transferred: Polysaccharide capsule; metabolic enzymes.

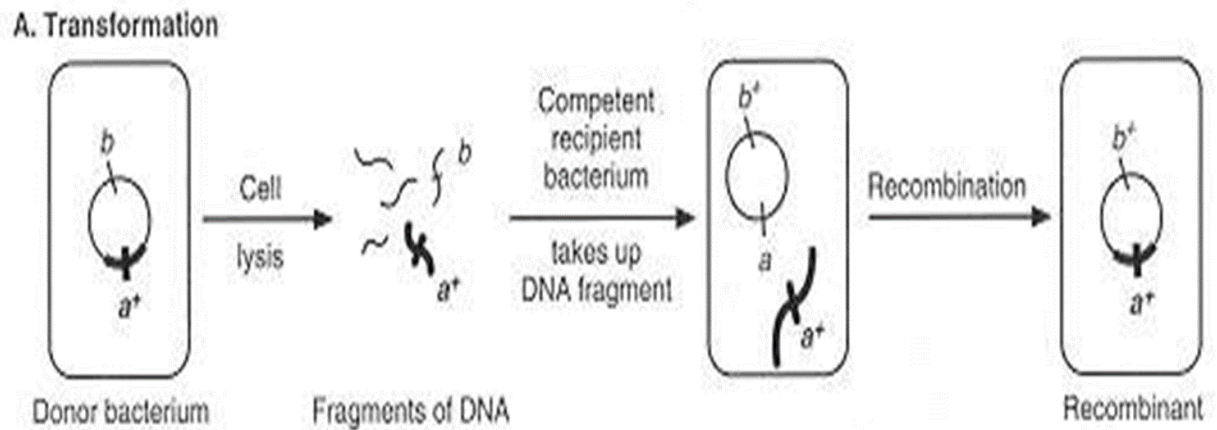
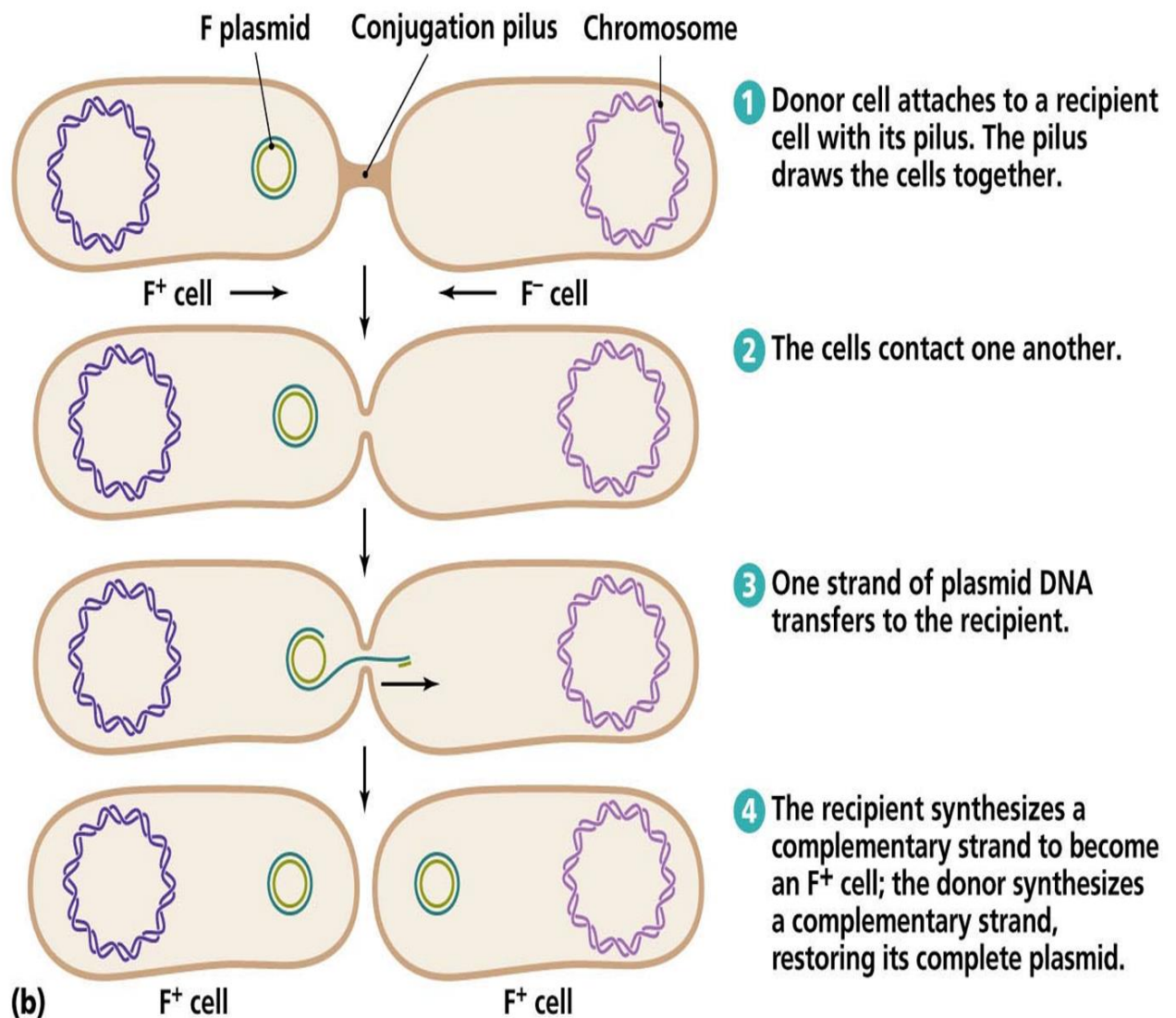


Figure 7: Transformation

## 2- Conjugation

It is the transfer of DNA from a donor to a receptor in a conjugation process involving cell-to-cell contact. Conjugation is made possible by two genetic elements: the conjugative plasmids and the conjugative pilli. Conjugation is seen frequently in Gram-negative rods (*Enterobacteriaceae*), in which the phenomenon has been most thoroughly researched, and enterococci. **Example** of genes transferred: Drug resistance; resistance to metals, toxin production and enzymes; adherence molecules.



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Figure 8: Conjugation



### 3- Transduction

It is the transfer of DNA from a donor to a receptor with the help of transport bacteriophages. **Example** of genes transferred: Exotoxins; enzymes for sugar fermentation; drug resistance.

Bacteriophages



Infection of another bacterium



Transfer of host bacterial DNA to the new bacterium



Acquisition of new characteristics coded by the donor DNA.

Following injection of the phage genome, it is integrated into the chromosome by means of region-specific recombination employing an integrase and this process is called **Lysogeny** .

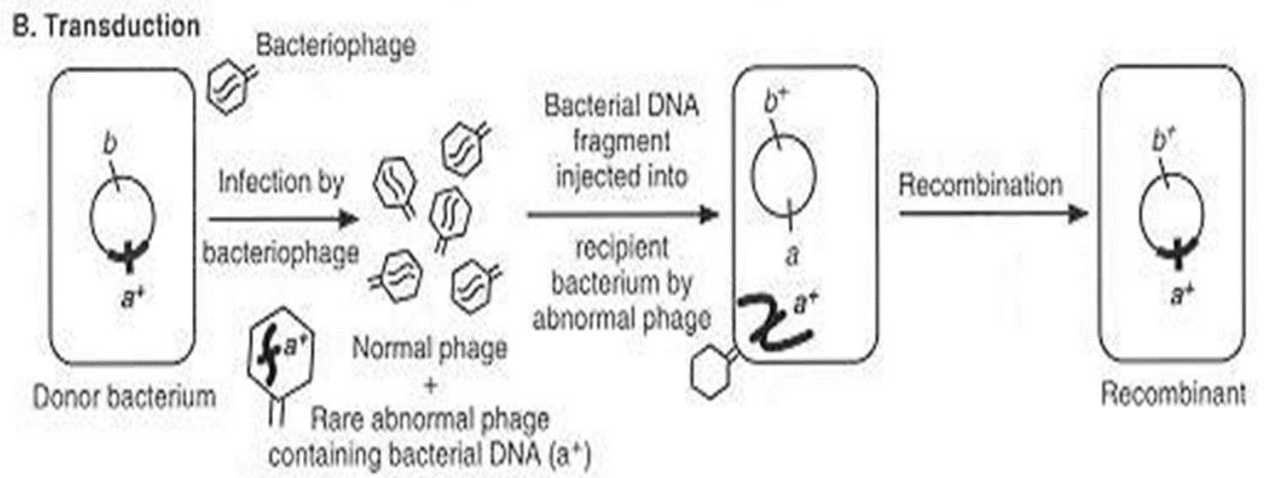


Figure 9: Transduction

## Endospores

Some bacteria, notably **certain gram-positive bacteria: *Bacillus* and *Clostridium* (rod), and *Sporosarcina* (cocci)** are characterized by the ability to produce unique structures called **endospores**, which are important for several reasons, including **their durability and potential pathogenicity**.

Although true endospores are found in gram-positive bacteria, **one gram-negative species, *Coxiella burnetii*** , the cause of Q fever, **forms endosporelike structures** that resist heat and chemicals and can be stained with endospore stains.

Though some people refer to endospores simply as “spores,” endospores should not be confused with the reproductive spores of actinobacteria, algae, and fungi. **A single bacterial cell, called a *vegetative cell* to distinguish it from an endospore, transforms into only one endospore, which then germinates to grow into only one vegetative cell; therefore, endospores are not reproductive structures.** Instead, **endospores constitute a defensive strategy against hostile or unfavorable conditions.** So, **endospores are specialized resistant, “resting” cells .**

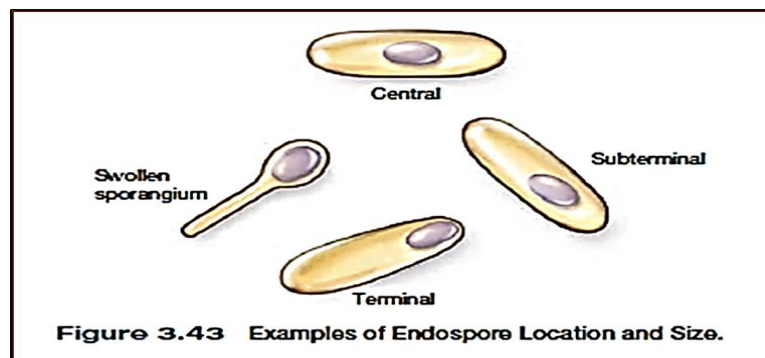
A vegetative cell normally transforms itself into an endospore only when one or more nutrients (such as carbon or nitrogen) are in limited supply.

The **diameter** of the endospore may be:

the **same** as, **smaller** than, or **larger** than the diameter of the vegetative cell.

Depending on the species, the endospore might be **located**:

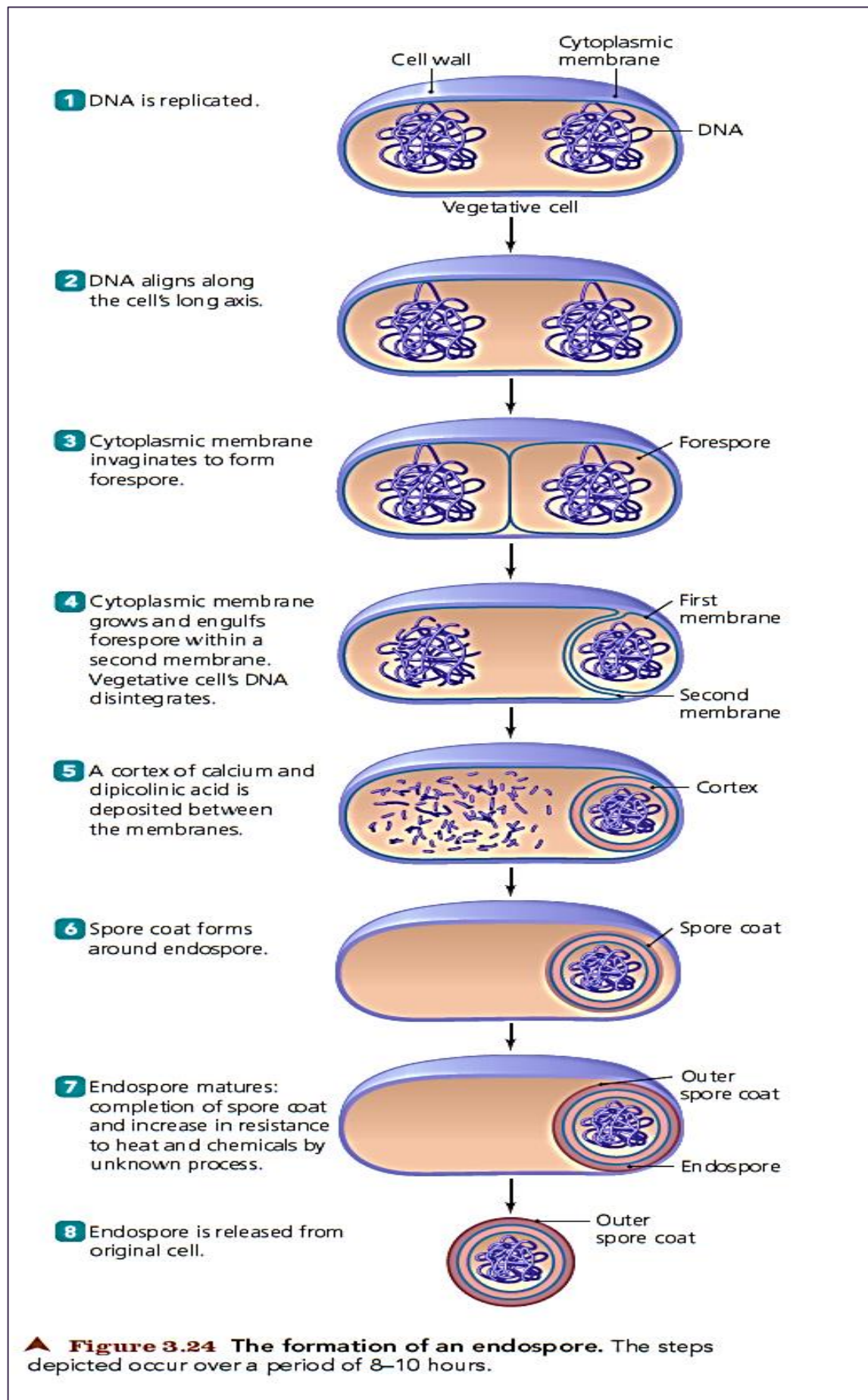
**terminally** (at one end), **subterminally** (near one end), or **centrally** inside the vegetative cell (Figure 1).



### **Sporulation or Sporogenesis:**

The process of endospore formation, called **sporulation**, requires 8 to 10 hours and proceeds in many steps (Figure 2).

During the process, newly replicated bacterial chromosome and a small portion of cytoplasm are isolated by an ingrowth of the plasma membrane called a *spore septum*. Then, two membranes, a **thick layer of peptidoglycan**, and a **spore coat** form around a **copy of the cell's DNA and a small portion of cytoplasm**. The cell deposits large quantities of **dipicolinic acid, calcium, and DNA-binding proteins within the endospore while removing most of the water**. When the endospore matures, the vegetative cell wall ruptures (lyses), killing the cell, and the endospore is freed.



## Structure of the endospore:

The structure of the endospore as seen with the electron microscope differs distinctly from that of the vegetative cell (**Figure 3**). In particular, the endospore is structurally more complex in that it has many layers that are absent from the vegetative cell.

1. The outermost layer is the **exosporium**, a thin protein covering.
2. Within this are the **spore coats**, composed of layers of spore-specific proteins (Figure 3-b).
3. Below the spore coat is the **cortex**, which consists of loosely cross-linked peptidoglycan.
4. Inside the cortex is the **core**, which contains the core wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes, and other cellular essentials.

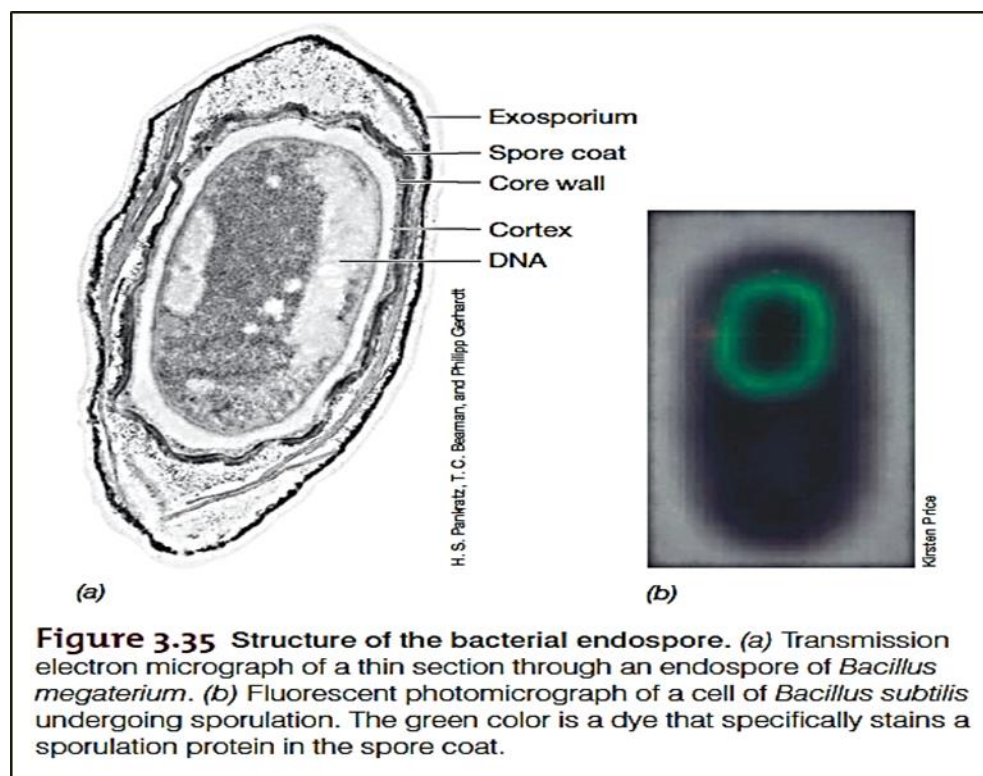
Thus, the endospore differs structurally from the vegetative cell primarily in the kinds of structures found outside the core wall.

### Chemical composition of endospores:

One substance that is **characteristic of endospores but absent from vegetative cells** is **dipicolinic acid**, which **accumulates in the core**. Endospores are also enriched in **calcium** ( $\text{Ca}^{+2}$ ), most of which is complexed with dipicolinic acid .

### The calcium–dipicolinic acid complex functions:

1. To bind free water within the endospore, thus helping to dehydrate it.
2. In addition, the complex intercalates (inserts between bases) in DNA, which stabilizes DNA against heat denaturation.





**Properties of endospores:**

1. Endospores are **extremely resistant** to drying, heat, radiation, and lethal chemicals. For example, they remain alive in boiling water for several hours; are unharmed by alcohol, peroxide, bleach, and other toxic chemicals; and can tolerate over 400 rad of radiation, which is more than five times the dose that is lethal to most humans.
2. Endospores are stable resting stages that barely metabolize, and they germinate only when conditions improve.

Scientists suggest how endospores are able to resist harsh conditions: it appears that the double membrane, spore coats, dipicolinic acid, calcium, and DNA-binding proteins serve to stabilize DNA and enzymes, protecting them from adverse conditions.

**Note : No Archaea have been shown to form endospores.**

**Germination:**

Endospores can remain dormant for thousands of years. An endospore returns to its vegetative state by a process called **germination** (Figure 4).

Germination is triggered by physical or chemical damage to the endospore's coat. The endospore's enzymes then break down the extra layers surrounding the endospore, water enters, and metabolism resumes. *Because one vegetative cell forms a single endospore, which, after germination, remains one cell, sporulation in bacteria is not a means of reproduction. This process does not increase the number of cells.*

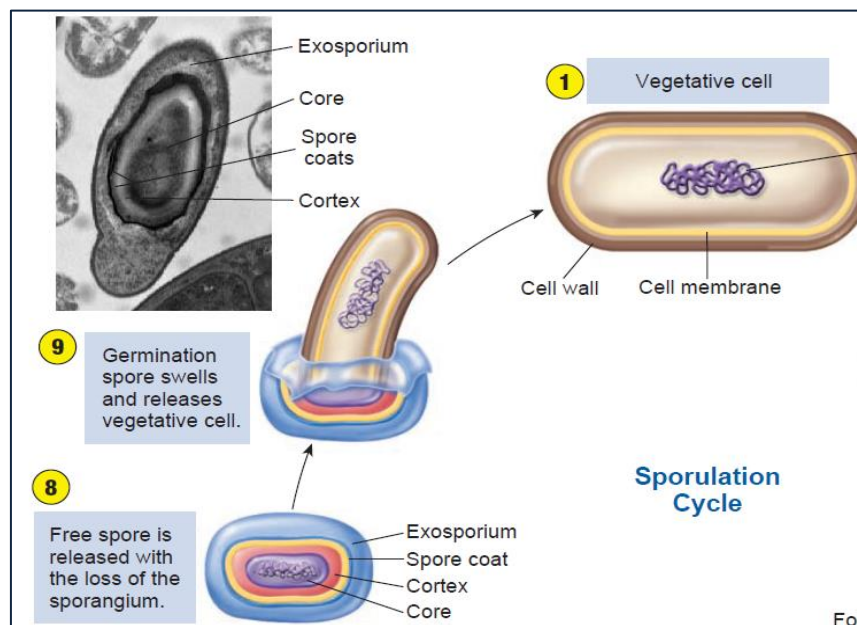


Figure 4: Germination of endospores.

**Importance of endospores in clinical and industrial views:**

Endospore formation is a serious concern to food processors, and health care professionals, because:

1. endospores are resistant to treatments that inhibit other microbes.
2. endospore-forming bacteria produce deadly toxins that cause such fatal diseases as anthrax, tetanus, and gangrene.

*Some members of the genus Clostridium cause diseases such as gangrene, tetanus, botulism, and food poisoning.*

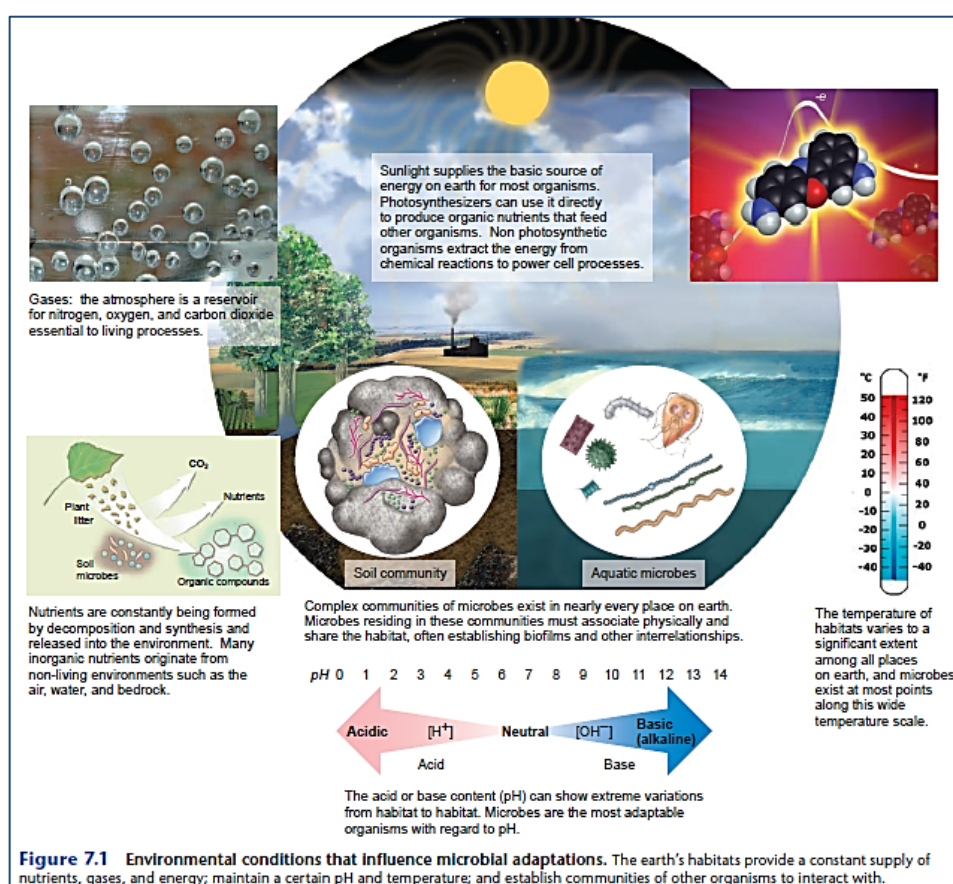
*Some members of the genus Bacillus cause anthrax and food poisoning.*

# Microbial Nutrition, Ecology, and Growth

## Introduction

There are millions of habitats on earth, of both natural and human origin. In these settings, microorganisms are exposed to a tremendous variety of conditions that affect their survival. Environmental factors with the greatest impact on microorganisms are **nutrient** and **energy sources**, **temperature**, **gas content**, **water**, **salt**, **pH**, **radiation**, and **other organisms** ( figure 1 ).

Microbes survive in their habitats through the process of gradual adjustment of anatomy and physiology, a process called **adaptation** (**Changes in structure and function that improve an organism's survival in a given environment**). It is this adaptability that allows microbes to inhabit all parts of the biosphere. The process that selects for favorable adaptations is also a major force behind the evolution of species.



**Nutrition** is a process by which chemical substances called **nutrients** are acquired from the environment and used in cellular activities such as metabolism and growth. With respect to nutrition, microbes are not really so different from humans.

Before a cell can replicate, it **must coordinate many different chemical reactions and organize many different molecules into specific structures**. Collectively, these reactions are called **metabolism**.

**Metabolic reactions** are either **catabolic**, which means **energy releasing**, or **anabolic**, which means **energy requiring**. **Catabolism breaks molecular structures down, releasing energy in the process**, and **anabolism uses energy to build larger molecules from smaller ones**.

In general, all living things have an absolute need for the bioelements, traditionally listed as carbon, hydrogen, oxygen, phosphorus, potassium, nitrogen, sulfur, calcium, iron, sodium, chlorine, magnesium, and certain other elements. Beyond these basic requirements, microbes have significant differences in the source, chemical form, and amount of the elements they use.

# Microbial Nutrition

## Elements of life

Chemical analysis of cells shows that over 95% of cell dry weight is made up of a few major elements: **carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron**. These are called **macroelements** or **macronutrients** because **they are required in relatively large amounts**.

The first six (C, O, H, N, S, and P) are components of carbohydrates, lipids, proteins, and nucleic acids.

The remaining four macroelements exist in the cell as cations and play a variety of roles. For example:

- **Potassium ( $K^+$ )** is required for activity by a number of enzymes, including some involved in protein synthesis.
- **Calcium ( $Ca^{2+}$ )** contributes to the heat resistance of bacterial endospores.
- **Magnesium ( $Mg^{2+}$ )** serves as a cofactor for many enzymes, and stabilizes ribosomes and cell membranes.
- **Iron ( $Fe^{2+}$  and  $Fe^{3+}$ )** is a part of some molecules involved in the synthesis of ATP by electron transport-related processes.

In addition to macroelements, all microorganisms require several **nutrients in small amounts, in nature, they are ubiquitous and usually present in adequate amounts to support the growth of microbes**. These nutrients are called **micronutrients** or **trace elements**. The micronutrients—**manganese, zinc, cobalt, molybdenum, nickel, and copper**—are needed by most cells.

\* Micronutrients are normally a **part of enzymes and cofactors**, and they **aid in the catalysis of reactions** and **maintenance of protein structure**.

For example:

\* **Zinc ( $\text{Zn}^{2+}$ )** is present at the active site of some enzymes.

\* **Manganese ( $\text{Mn}^{2+}$ )** aids many enzymes that catalyze the transfer of phosphate.

\* **Molybdenum ( $\text{Mo}^{2+}$ )** is required for nitrogen fixation.

\* **Cobalt ( $\text{Co}^{2+}$ )** is a component of vitamin B<sub>12</sub>.

**Growth factors** :organic compounds that are essential cell components or precursors of such components but cannot be synthesized by the organism.

There are **three major classes** of growth factors:

1. **Amino acids.**
2. **Purines and pyrimidines.**
3. **Vitamins.**



## Nutritional Types of Microorganisms

Sources of **Carbon**, **Energy**, and **Electrons** (figure 2) & (table 1):

- **Carbon** is needed to synthesize the organic molecules from which organisms are built.

- **Source of carbon**

Organisms can be categorized into two broad groups based on their **source of carbon**.

1. Organisms that utilize an **inorganic** source of carbon (that is, **carbon dioxide**) as their sole source of carbon are called **autotrophs**, so named because they “feed themselves.” More precisely, autotrophs make organic compounds from CO<sub>2</sub> and thus need not acquire carbon from organic compounds from other organisms.
2. In contrast, organisms called **heterotrophs** catabolize reduced **organic molecules** (such as **proteins, carbohydrates, amino acids, and fatty acids**) they acquire from other organisms.

- **Source of energy**

Organisms can also be categorized according to whether they use chemicals or light as a **source of energy** for such cellular processes as anabolism, intracellular transport, and motility.

1. Organisms that acquire energy from redox reactions involving **inorganic** and **organic chemicals** are called **chemotrophs** ( these reactions are either aerobic respiration, anaerobic respiration, or fermentation, depending on the final electron acceptor).
2. Organisms that use **light** as their energy source are called **phototrophs**.

Thus we see that organisms can be categorized based on their carbon and energy sources into one of four basic groups: **photoautotrophs**, **chemoautotrophs**, **photoheterotrophs**, and **chemoheterotrophs**.

- **Source of electrons**
- Electrons are needed for **two reasons**:
  1. The movement of electrons through electron transport chains and during other oxidation-reduction reactions can provide **energy** for use in cellular work.
  2. Electrons also are needed to reduce molecules during biosynthesis (e.g., the reduction of CO<sub>2</sub> to form organic molecules).

Additionally, the cells of all organisms require electrons or hydrogen atoms for redox reactions.

1. **Heterotrophs** acquire electrons (typically as part of hydrogen atoms) from the same organic molecules that provide them carbon and are called **organotrophs**.
2. **Autotrophic** organisms acquire electrons or hydrogen atoms from inorganic molecules (such as H<sub>2</sub>, NO<sub>2</sub><sup>-</sup>, H<sub>2</sub>S, and Fe<sup>2+</sup>) and are called **lithotrophs**.

		Energy source	
		Light ( <i>photo-</i> )	Chemical compounds ( <i>chemo-</i> )
Carbon source	Carbon dioxide ( <i>auto-</i> )	<b>Photoautotrophs</b> <ul style="list-style-type: none"> <li>Plants, algae, and cyanobacteria use H<sub>2</sub>O to reduce CO<sub>2</sub>, producing O<sub>2</sub> as a by-product</li> <li>Green sulfur bacteria and purple sulfur bacteria do not use H<sub>2</sub>O nor produce O<sub>2</sub></li> </ul>	<b>Chemoautotrophs</b> <ul style="list-style-type: none"> <li>Hydrogen, sulfur, and nitrifying bacteria, some archaea</li> </ul>
	Organic compounds ( <i>hetero-</i> )	<b>Photoheterotrophs</b> <ul style="list-style-type: none"> <li>Green nonsulfur bacteria and purple nonsulfur bacteria, some archaea</li> </ul>	<b>Chemoheterotrophs</b> <ul style="list-style-type: none"> <li>Aerobic respiration: most animals, fungi, and protozoa, and many bacteria</li> <li>Anaerobic respiration: some animals, protozoa, bacteria, and archaea</li> <li>Fermentation: some bacteria, yeasts, and archaea</li> </ul>

**Figure 2 :** Four basic groups of organisms based on their carbon and energy sources

<b>Table 1 Major Nutritional Types of Microorganisms</b>				
Nutritional Type	Carbon Source	Energy Source	Electron Source	Representative Microorganisms
Photolithoautotroph	CO <sub>2</sub>	Light	Inorganic e <sup>-</sup> donor	Purple and green sulfur bacteria, cyanobacteria
Photoorganoheterotroph	Organic carbon	Light	Organic e <sup>-</sup> donor	Purple nonsulfur bacteria, green nonsulfur bacteria
Chemolithoautotroph	CO <sub>2</sub>	Inorganic chemicals	Inorganic e <sup>-</sup> donor	Sulfur-oxidizing bacteria, hydrogen-oxidizing bacteria, methanogens, nitrifying bacteria, iron-oxidizing bacteria
Chemolithoheterotroph	Organic carbon	Inorganic chemicals	Inorganic e <sup>-</sup> donor	Some sulfur-oxidizing bacteria (e.g., <i>Beggiatoa</i> )
Chemoorganoheterotroph	Organic carbon	Organic chemicals often same as C source	Organic e <sup>-</sup> donor, often same as C source	Most nonphotosynthetic microbes, including most pathogens, fungi, and many protists and archaea

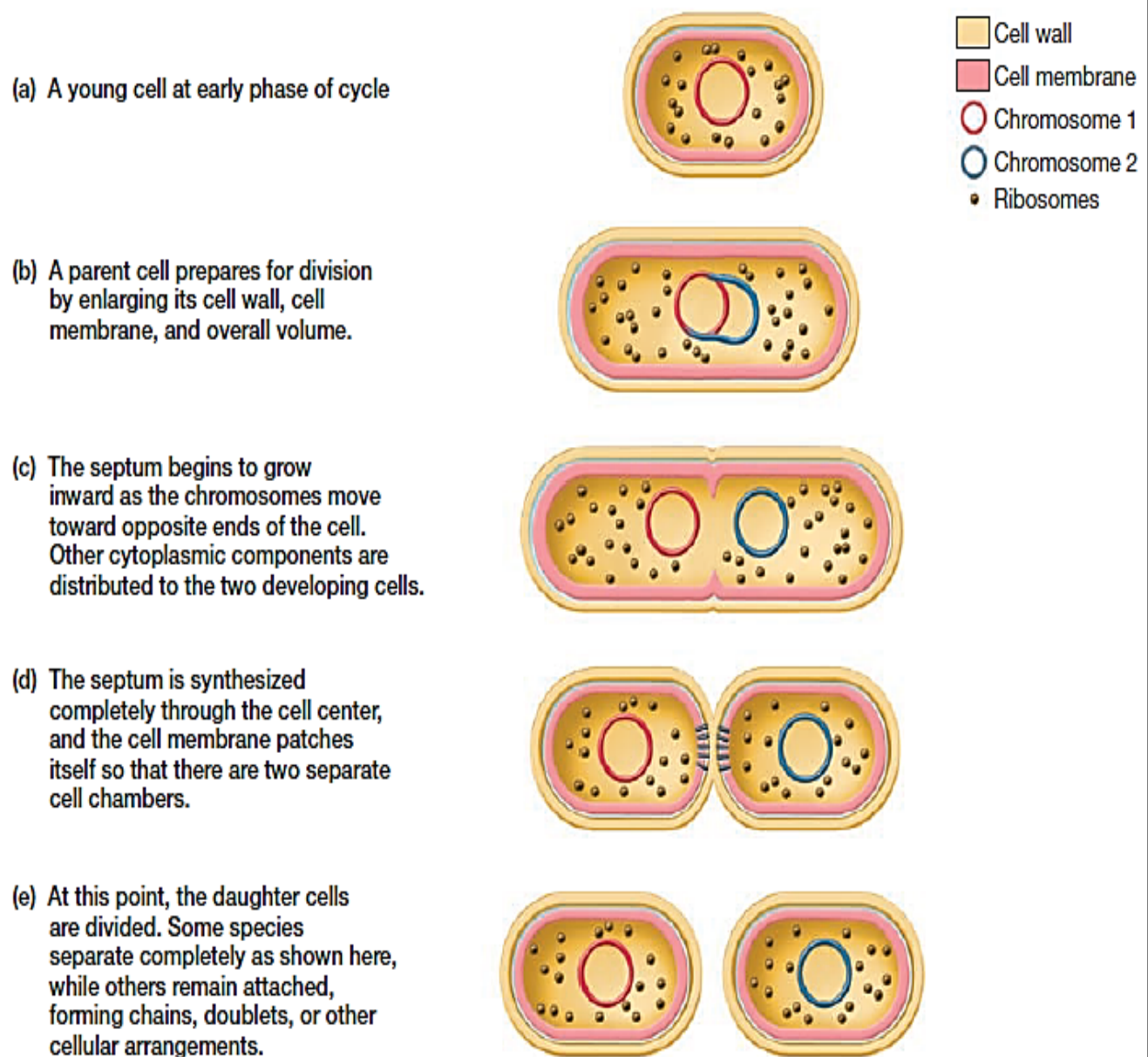
## BACTERIAL CELL CYCLE

The **cell cycle** is the complete sequence of events extending from the formation of a new cell through the next division.

Although some prokaryotes reproduce by budding, fragmentation, and other means, most prokaryotes reproduce by **binary fission** (figure 1).

**Binary fission is a relatively simple type of cell division. The cell:**

1. **Elongates.**
2. **Replicates its chromosome.**
3. **Separates the newly formed DNA molecules** so there is one chromosome in each half of the cell.
4. **Finally, a septum (cross wall) is formed at midcell**, dividing the parent cell into two progeny cells, each having its own chromosome and a complement of other cellular constituents.



**Figure 1** Binary Fission.

## **GROWTH CURVE**

Binary fission and other cell division processes bring about an increase in the number of cells in a population.

Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in **liquid medium**, they usually are grown in a **batch culture** —that is, they are incubated in a **closed culture vessel** with a single batch of medium.

### **-Because:**

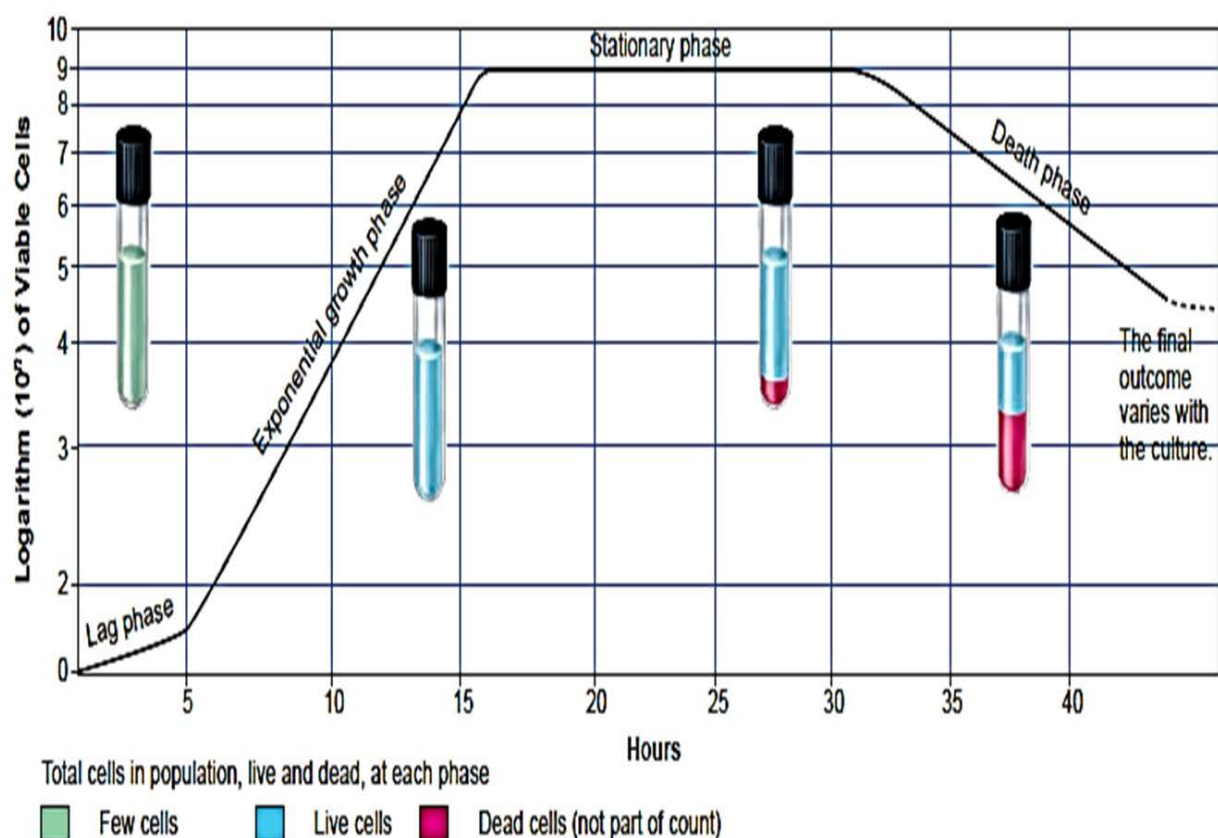
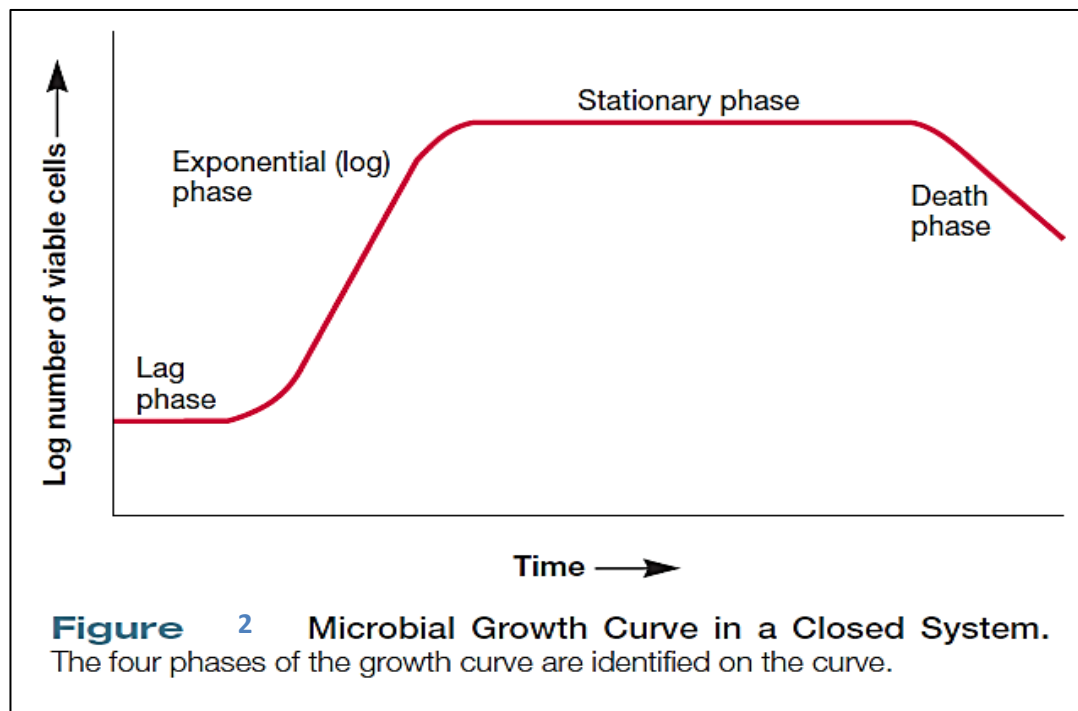
- **no fresh medium is provided during incubation.**
- **nutrient concentrations decline.**
- **and concentrations of wastes increase.**

**-The growth of microorganisms** reproducing by binary fission **can be plotted as the logarithm of the number of viable cells versus the incubation time.**

**-The resulting curve ( figures 2 & 3 ) has four distinct phases :**

- 1. Lag Phase.**
- 2. Exponential Phase.**
- 3. Stationary Phase.**
- 4. Death Phase.**





## **Lag Phase**

The **lag phase** ( figures 2 & 3 ) is an early “flat” period on the graph when the population appears **not to be growing** or **is growing at less than the exponential rate**.

### **Growth lags primarily because :**

1. The newly inoculated cells require a period of adjustment, enlargement, and synthesis of DNA, enzymes, and ribosomes. The medium may be different from the one the microorganism was growing in previously. Here new enzymes would be needed to use different nutrients.
2. Possibly the microorganisms have been injured and require time to recover.
3. The cells are not yet multiplying at their maximum rate.
4. The population of cells is so sparse or dilute that the sampling misses them.

The length of the lag period varies from one population to another, depending on **the condition of the microbes** and **medium**.

It is important to note that even though the population of cells is not increasing (growing), individual cells are metabolically active as they increase their contents and prepare to divide.

## **Exponential growth (logarithmic or log) phase**

During the **exponential (log) phase** (figures 2 & 3), microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the environmental conditions.

The growth rate of microorganisms is constant during the exponential phase; that is, they are **completing the cell cycle** and **doubling in number at regular intervals**.

This phase will continue as long as cells have adequate nutrients and the environment is favorable.

The population is most uniform in terms of chemical and physiological properties during this phase; **therefore exponential phase cultures are usually used in biochemical and physiological studies**.

### **Stationary growth phase:**

In a closed system such as a batch culture, population growth eventually ceases and the growth curve becomes **horizontal** (figures 2 & 3).

At the **stationary growth phase**, the population enters a survival mode in which **cells stop growing** or **grow slowly**.

In the stationary phase, the total number of viable microorganisms **remains constant**. This may result from a **balance between cell division and cell death**, or **the population may simply cease to divide but remain metabolically active**.

#### **Microbial populations enter the stationary phase for several reasons:**

1. Nutrient limitation; if an essential nutrient is severely depleted, population growth will slow.
2. Aerobic organisms often are limited by O<sub>2</sub> availability.
3. Population growth also may cease due to the accumulation of toxic waste products.
4. Finally, some evidence exists that growth may cease when a critical population level is reached.

**Thus entrance into the stationary phase may result from several factors operating in concert.**

## **Death phase**

As the limiting factors intensify, cells begin to die at an exponential rate (literally perishing in their own wastes), and most are unable to multiply.

The curve now dips downward as the **death phase** (figures 2 & 3 ) begins. The speed with which death occurs depends on **the relative resistance of the species** and **how toxic the conditions are**, but it is usually slower than the exponential growth phase.

In the laboratory, refrigeration is used to slow the progression of the death phase so that cultures will remain viable as long as possible.

## Mathematics of Growth

During the exponential phase, each microorganism is dividing at constant intervals. Thus the population doubles in number during a specific length of time called the **generation (doubling) time** .

**The time required for a bacterial cell to grow and divide is its generation time.**

Viewed another way, generation time is also the time required for a population of cells to double in number.

This can be illustrated with a simple example. Suppose that a culture tube is inoculated with one cell that divides every 20 minutes (table 4). The population will be 2 cells after 20 minutes, 4 cells after 40 minutes, and so forth. Because the population is doubling every generation, the increase in population is always  $2^n$  where  $n$  is the number of generations. The resulting population increase is exponential-that is, logarithmic (figure 4 ).

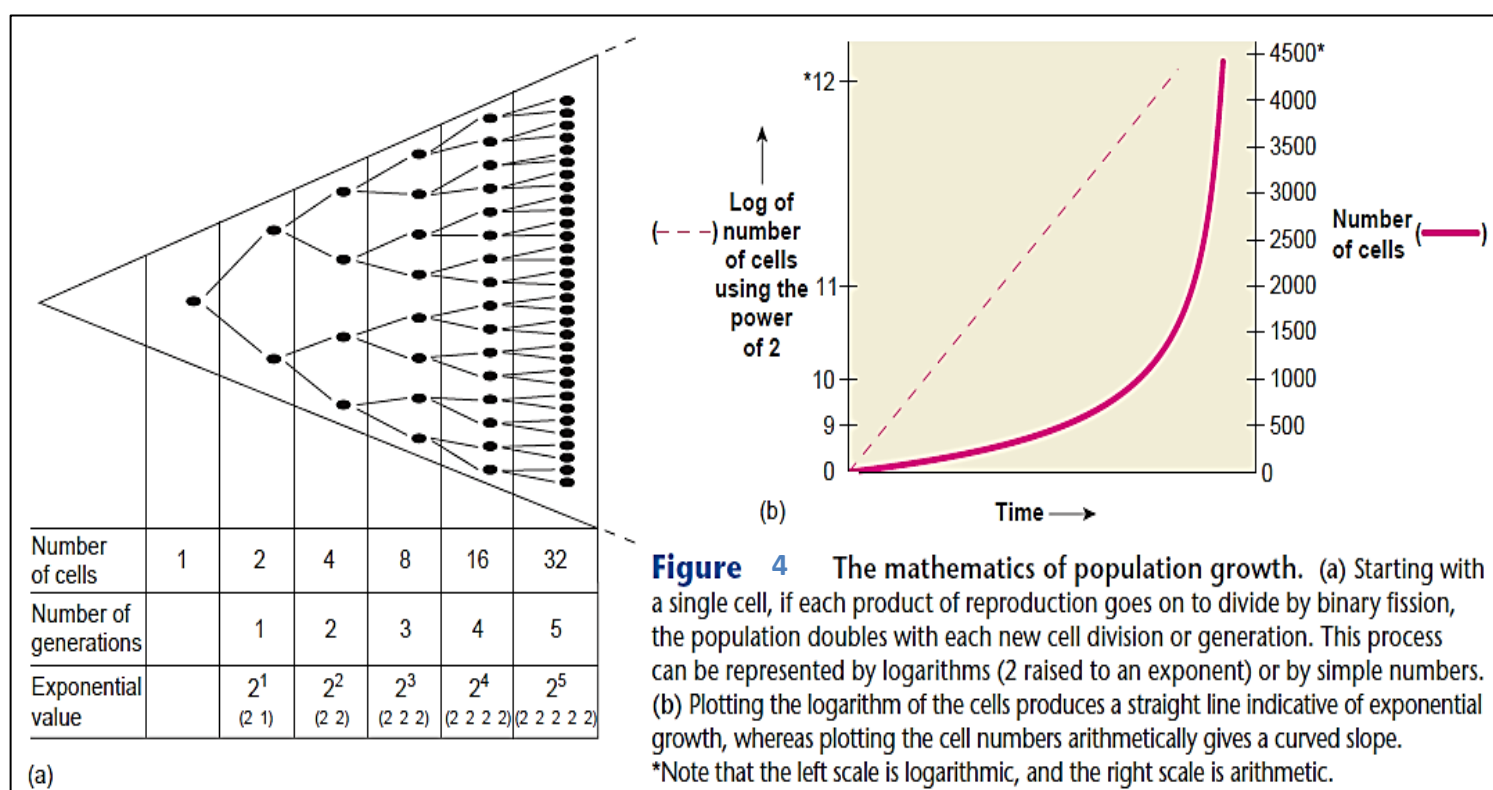
<div>Table 1</div> <div>An Example of Exponential Growth</div>				
Time <sup>a</sup>	Division Number	$2^n$	Population <sup>b</sup> ( $N_0 \times 2^n$ )	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204

<sup>a</sup>The hypothetical culture begins with one cell having a 20-minute generation time.  
<sup>b</sup>Number of cells in the culture.

$N_0$  \_ the initial population number

$N_t$  \_ the population at time  $t$

$n$  \_ the number of generations in time  $t$



**Figure 4** The mathematics of population growth. (a) Starting with a single cell, if each product of reproduction goes on to divide by binary fission, the population doubles with each new cell division or generation. This process can be represented by logarithms (2 raised to an exponent) or by simple numbers. (b) Plotting the logarithm of the cells produces a straight line indicative of exponential growth, whereas plotting the cell numbers arithmetically gives a curved slope.



**Generation times vary markedly with:**

1. The species of microorganism.
2. Environmental conditions.

They range from less than 10 minutes (0.17 hours) to several days ( table 2 ).

Generation times in nature are usually much longer than in culture.

<div>Table 2</div> <div>Examples of Generation Times<sup>a</sup></div>		
Microorganism	Incubation Temperature (°C)	Generation Time (Hours)
<b>Bacteria</b>		
<i>Escherichia coli</i>	40	0.35
<i>Bacillus subtilis</i>	40	0.43
<i>Staphylococcus aureus</i>	37	0.47
<i>Pseudomonas aeruginosa</i>	37	0.58
<i>Clostridium botulinum</i>	37	0.58
<i>Mycobacterium tuberculosis</i>	37	≈ 12
<i>Treponema pallidum</i>	37	33

The mathematics of growth during the exponential phase are illustrated in (figure 5), which shows the calculation of two important values. The growth rate constant ( $k$ ) is the number of generations per unit time and is often expressed as generations per hour. It can be used to calculate the generation time. As can be seen in (figure 5), the generation time is simply the reciprocal of the growth rate constant.

#### Calculation of the growth rate constant

Let  $N_0$  = the initial population number

$N_t$  = the population at time  $t$

$n$  = the number of generations in time  $t$

For populations reproducing by binary fission

$$N_t = N_0 \times 2^n$$

Solving for  $n$ , the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The growth rate constant ( $k$ ) is the number of generations per unit time ( $\frac{n}{t}$ ). Thus

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

#### Calculation of generation (doubling) time

If a population doubles, then

$$N_t = 2N_0$$

Substitute  $2N_0$  into the growth rate constant equation and solve for

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The generation time is the reciprocal of the growth rate constant.

$$g = \frac{1}{k}$$

**Figure 5 Calculation of the Growth Rate Constant and Generation Time.** The calculations are only valid for the exponential phase of growth, when the growth rate is constant.

## INFLUENCES OF ENVIRONMENTAL FACTORS ON GROWTH

Microorganisms are greatly affected by the chemical and physical nature of their surroundings.

An understanding of environmental influences aids in:

1. The control of microbial growth.
2. The study of the ecological distribution of microorganisms.

### 1. Water Activity and Osmosis

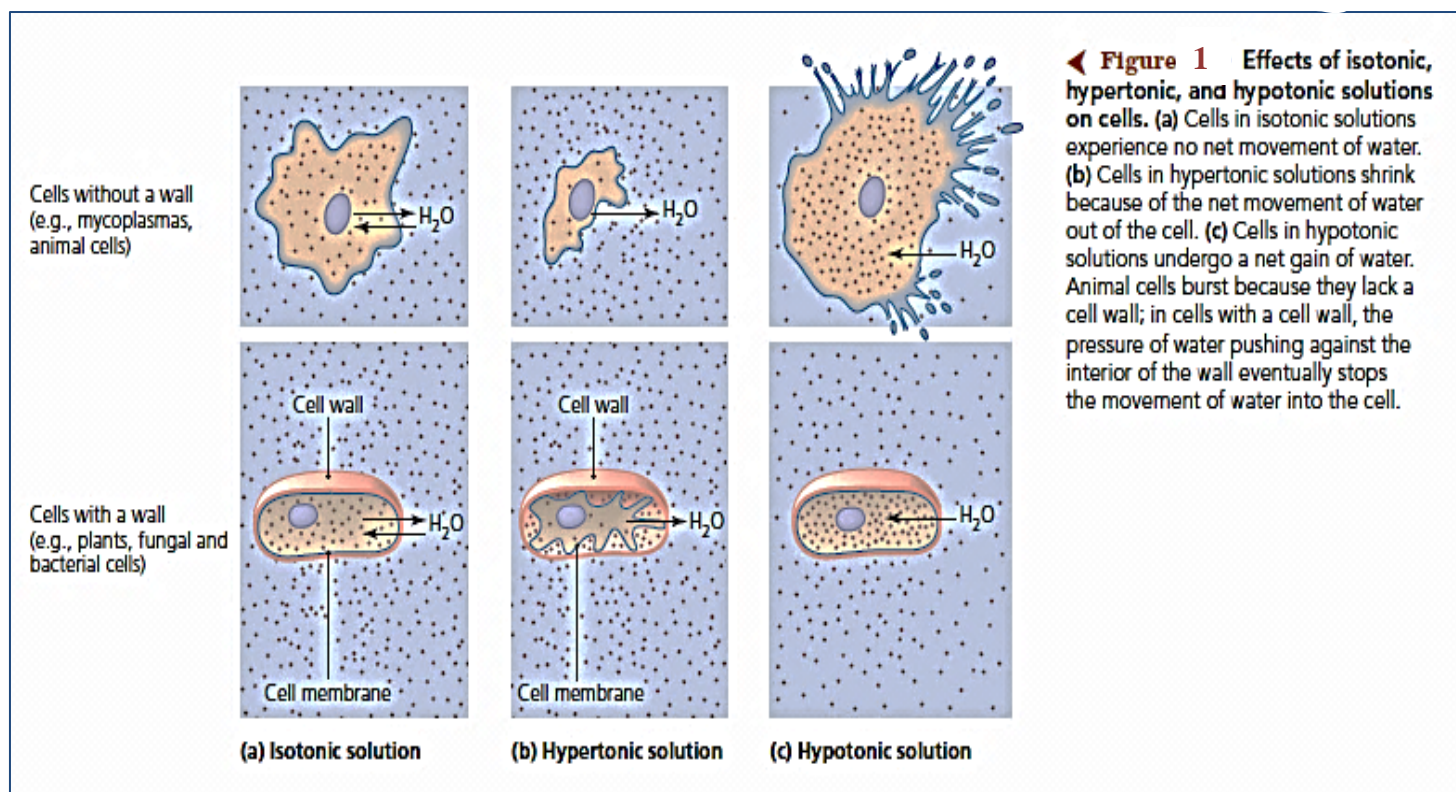
Microorganisms require water; they must be in a moist environment if they are to be metabolically active.

**Water is needed to:**

1. Dissolve enzymes and nutrients.
  2. Also, it is an important reactant in many metabolic reactions.
- Even though most cells die in the absence of water. Some microorganisms—for example, the bacterium *Mycobacterium tuberculosis*—have cell walls that retain water, allowing them to survive for months under dry conditions.
  - Additionally, the spores and cysts of some other single-celled microbes cease most metabolic activity in a dry environment for years; these cells are in essence in a state of suspended animation because they neither grow nor reproduce in their dry condition.

Because a selectively permeable plasma membrane separates microorganisms from their environment, they can be affected by changes in the osmotic concentration of their surroundings.

- If it is placed in a **hypertonic solution** (one with a **higher osmotic concentration**), **water will flow out of the cell**. In microbes that have cell walls, the membrane shrinks away from the cell wall—a process called **plasmolysis**. **Dehydration** of the cell in hypertonic environments may damage the cell membrane and cause the **cell to become metabolically inactive**.(figure 1-b).
- Conversely if a microorganism is placed in a **hypotonic solution** (one with a **lower osmotic concentration**), **water will enter the cell** and cause it to **burst** unless something is done to prevent the influx or inhibit plasma membrane expansion. (figure 1-c).
- Clearly it is important that microbes be able to respond to changes in the osmotic concentrations of their environment.



**Because** the osmotic concentration of a habitat has such profound effects on microorganisms, it is useful to **express quantitatively the degree of water availability**. Microbiologists generally use water activity ( $a_w$ ) for this purpose (**water availability also may be expressed as water potential, which is related to  $a_w$** ). The water activity of a solution is 1/100 the relative humidity of the solution (when expressed as a percent). It is also equivalent to the ratio of the solution's vapor pressure ( $P_{\text{soln}}$ ) to that of pure water ( $P_{\text{water}}$ ).

Thus, values of  $a_w$  vary between 0 and 1.

$$a_w = \frac{P_{\text{soln}}}{P_{\text{water}}}$$

- **Halophile:** A microorganism that requires high levels of sodium chloride NaCl for growth, *Halobacterium*.
- **Halotolerant:** A microorganism that capable of growing in the presence of sodium chloride NaCl, **but not requiring it**.
- **Extreme halophiles:** Organisms capable of growth in very salty environments. These organisms require 15–30% NaCl, *depending on the species*, for optimum growth.
- **Osmotolerant:** Organisms that grow over a wide range of water activity or solute concentration, *Staphylococcus aureus*.
- **Osmophiles :**Organisms able to live in environments high in sugar as a solute.
- **Xerophiles:** Those able to grow in very dry environments (made dry by lack of water rather than from dissolved solutes).

## 2.pH

pH is a measure of the relative acidity of a solution and is defined as the negative logarithm of the hydrogen ion concentration (expressed in terms of molarity).

Each species has a definite pH growth range and pH growth optimum.

- **Acidophiles** have their growth optimum between **pH 0 and 5.5**.
- **Neutrophiles** have their growth optimum between **pH 5.5 and 8.0**.
- **Alkalophiles (alkaliphiles)** have their growth optimum, between pH **8.0 and 11.5**.
- **Extreme alkalophiles** have growth optima at **pH 10 or higher**.

In general, different microbial groups have characteristic pH preferences.

- Most bacteria and protists are neutrophiles.
- Most fungi prefer more acidic surroundings, about pH 4 to 6;
- Many archaea are acidophiles. For example, the archaeon *Sulfolobus acidocaldarius* is a common inhabitant of acidic hot springs; it grows well from pH 1 to 3 and at high temperatures. The archaea *Ferroplasma acidarmanus* can actually grow very close to pH 0.
- Alkalophiles are distributed among all three domains of life.



### 3. Temperature

Microorganisms are particularly susceptible to external temperatures because **they cannot regulate their internal temperature.**

1. An important factor influencing the effect of temperature on growth is **the temperature sensitivity of enzyme-catalyzed reactions.**

- Each enzyme has a temperature at which it **functions optimally.**
- At some **temperature below the optimum**, it ceases to be catalytic.
- As the **temperature rises from this low point**, the rate of catalysis **increases to that observed for the optimal temperature.**
- High temperatures **denature enzymes, transport carriers, and other proteins.**

2. Temperature also has a **significant effect on microbial membranes.**

- At **very low temperatures**, membranes solidify.
- At **high temperatures**, the **lipid bilayer** simply **melts and disintegrates.**

\*\* Thus when organisms are **above their optimum temperature**, both **function** and cell **structure** are affected.

\*\* If **temperatures** are **very low**, **function** is affected but **not necessarily** cell chemical composition and structure.

Because of these opposing temperature influences, microbial growth has a characteristic temperature dependence with distinct **cardinal temperatures** — **minimum**, **optimum**, and **maximum** growth temperatures ( figure 2 )(table 1).

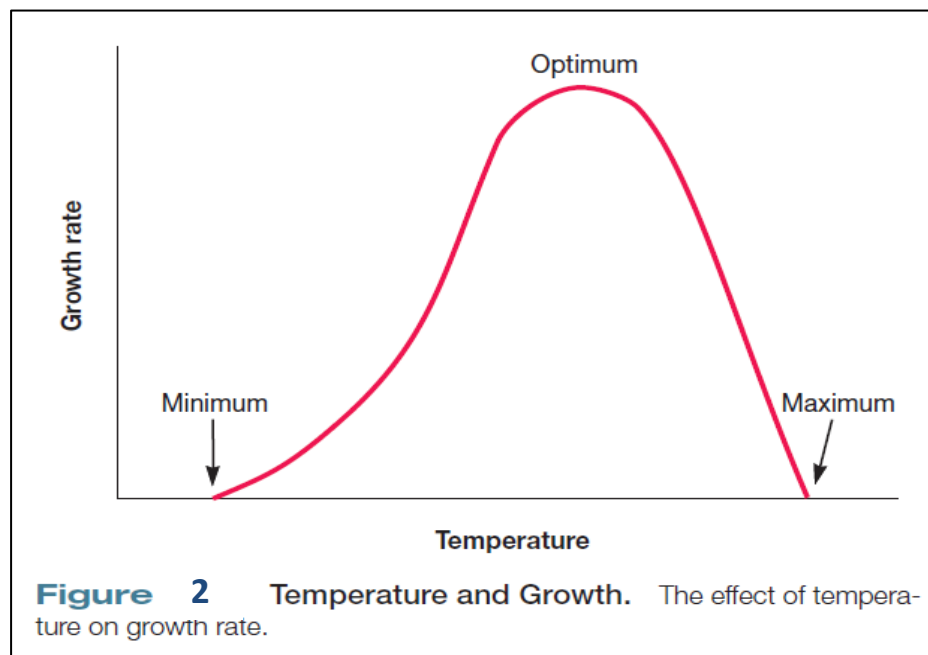
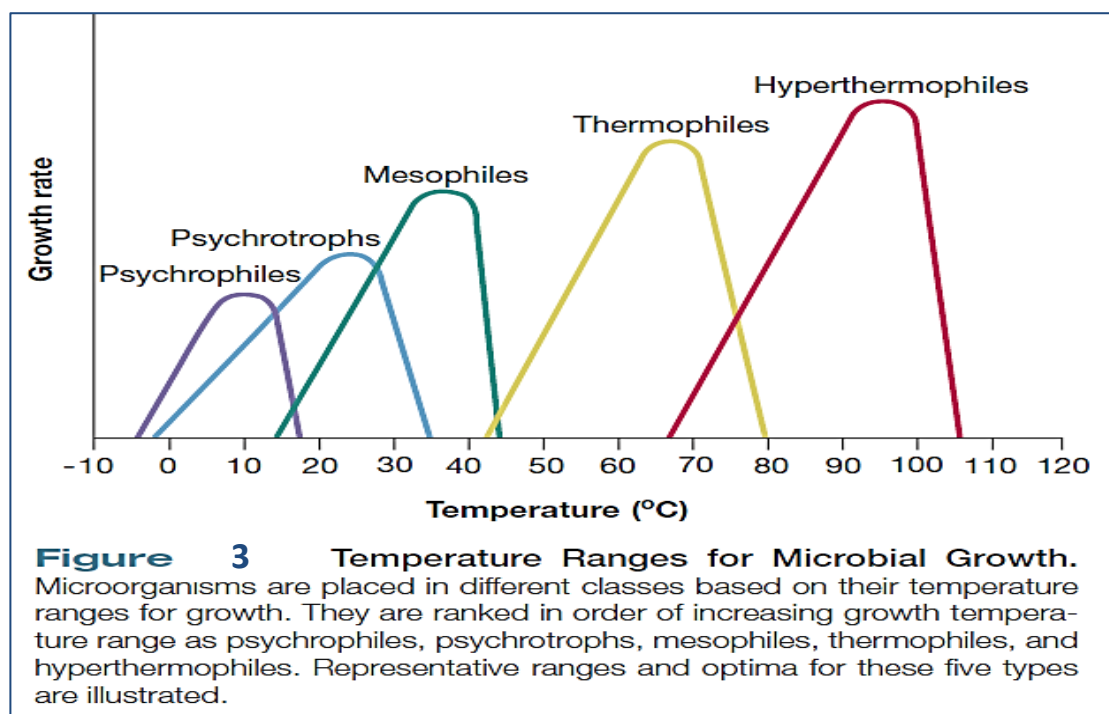


Table 1 Temperature Ranges for Microbial Growth			
Cardinal Temperatures (°C)			
Microorganism	Minimum	Optimum	Maximum
<b>Nonphotosynthetic Procaryotes</b>			
<i>Bacillus psychrophilus</i>	-10	23-24	28-30
<i>Pseudomonas fluorescens</i>	4	25-30	40
<i>Enterococcus faecalis</i>	0	37	44
<i>Escherichia coli</i>	10	37	45
<i>Neisseria gonorrhoeae</i>	30	35-36	38
<i>Thermoplasma acidophilum</i>	45	59	62
<i>Thermus aquaticus</i>	40	70-72	79
<i>Pyrococcus abyssi</i>	67	96	102
<i>Pyrodictium occultum</i>	82	105	110
<i>Pyrolobus fumarii</i>	90	106	113

**Microorganisms can be placed in one of five classes based on their temperature ranges for growth (figure 3):**

1. **Psychrophiles:** grow at 0°C and have an optimum growth temperature of 10°C or lower; the maximum is around 15°C. They are readily isolated from Arctic and Antarctic habitats.
2. **Psychrotrophs (facultative psychrophiles)** :grow at 0 to 7°C even though they have optima between 20 and 30°C, and maxima at about 35°C. Psychrotrophic bacteria and fungi are major causes of refrigerated food spoilage.
3. **Mesophiles:** have a temperature minimum of 15 to 20°C, and their maximum is about 45°C or lower. Most microorganisms probably fall within this category. Almost all human pathogens are mesophiles, as might be expected because the human body is a fairly constant 37°C.
4. **Thermophiles:** their growth minimum is usually around 45°C, and they often have optima between 55 and 65°C, maximum 85°C. These organisms flourish in many habitats including composts, hot waterlines, and hot springs.
5. **Hyperthermophiles** have growth optima between 85°C and about 113°C. They usually do not grow well below 55°C.



*(Psychrophilic microorganisms have adapted to their environment in several ways. Their enzymes, transport systems, and protein synthetic machinery function well at low temperatures. The cell membranes of psychrophilic microorganisms have high levels of unsaturated fatty acids and remain semifluid when cold. Indeed, many psychrophiles begin to leak cellular constituents at temperatures higher than 20°C because of cell membrane disruption.)*

*(Thermophiles and hyperthermophiles differ from mesophiles in many ways. They have heat-stable enzymes and protein synthesis systems that function properly at high temperatures. These proteins are stable for a variety of reasons. Heat-stable proteins have highly organized hydrophobic interiors and more hydrogen and other noncovalent bonds. Larger quantities of amino acids such as proline also make polypeptide chains less flexible and more heat stable. In addition, the proteins are stabilized and aided in folding by proteins called chaperone proteins. Evidence exists that histonelike proteins stabilize the DNA of thermophilic bacteria. The membrane lipids of thermophiles and hyperthermophiles are also quite temperature stable. They tend to be more saturated, more branched, and of higher molecular weight. This increases the melting points of membrane lipids. Archaeal thermophiles have membrane lipids with ether linkages, which protect the lipids from hydrolysis at high temperatures. Sometimes archaeal lipids actually span the membrane to form a rigid, stable monolayer).*

## 4. Pressure

Organisms that spend their lives on land or the surface of water are always subjected to a pressure of 1 atmosphere (atm) and are never affected significantly by pressure. *It is thought that high hydrostatic pressure affects membrane fluidity and membrane associated function.*

Yet many procaryotes live in the deep sea (ocean depths of 1,000 m or more) where the hydrostatic pressure can reach 600 to 1,100 atm and the temperature is about 2 to 3°C. Pressure in marine environments increases 1atm/10 meters and can reach 1,000 atm at its greatest depths.

**Barotolerant:** best growth is at 1 atmospheric pressures, and can grow at up to 400 atm. Many procaryotes are **barotolerant**.

**Barophiles:** grow best at 400-600 atm. Some procaryotes are truly **barophilic** —they grow more rapidly at high pressures.

**Extreme barophiles:** grow only at higher pressures 700 atm or more.

Barophiles may play an important role in nutrient recycling in the deep sea.

## 5. Oxygen Concentration

Oxygen is **essential** for **obligate aerobes** because it **serves as the final electron acceptor of electron transport chains**, which produce most of the ATP in these organisms.

By contrast, oxygen is a deadly poison for **obligate anaerobes**.

**Q:** How can oxygen be essential for one group of organisms and yet be a fatal toxin for others?

The key to understanding this apparent incongruity is understanding that neither atmospheric oxygen ( $O_2$ ) nor covalently bound oxygen in compounds such as carbohydrates and water is poisonous.

Rather, **the toxic forms of oxygen are those that are highly reactive.**

**They are toxic for the same reason that oxygen is the final electron acceptor for aerobes: They are excellent oxidizing agents, so they steal electrons from other compounds, which in turn steal electrons from still other compounds. The resulting chain of vigorous oxidations causes irreparable damage to cells by oxidizing important compounds, including proteins and lipids.**

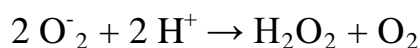
**There are four toxic forms of oxygen:**

- **Singlet oxygen ( $^1\text{O}_2$ ).** Singlet oxygen is a very reactive oxidizing agent.

*Phagocytic cells, such as certain human white blood cells, use it to oxidize pathogens.*

- **Superoxide radical ( $\text{O}_2^-$ ).** A few superoxide radicals form during the incomplete reduction of  $\text{O}_2$  during electron transport in aerobes and during metabolism by anaerobes in the presence of oxygen. Superoxide radicals are so reactive and toxic.

**\*\* Aerobic organisms** must produce enzymes called **superoxide dismutases** to detoxify them. These enzymes, *depending on the organism*, combine two superoxide radicals and two protons to form hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and molecular oxygen ( $\text{O}_2$ ):

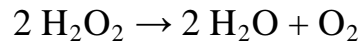


**\*\* One reason that anaerobes are susceptible to oxygen** is that they **lack superoxide dismutase**; they die as a result of the oxidizing reactions of superoxide radicals formed in the presence of oxygen.

- **Peroxide anion ( $\text{O}_2^{2-}$ ).** Hydrogen peroxide formed during reactions catalyzed by superoxide dismutase (and during other metabolic reactions) contains peroxide anion, another highly reactive oxidant. It is peroxide anion that makes hydrogen peroxide an antimicrobial agent.

**\*\* Aerobes** contain either **catalase** or **peroxidase**, enzymes that detoxify peroxide anion.

**Catalase** converts hydrogen peroxide to water and molecular oxygen:



*A simple test for catalase involves adding a sample from a bacterial colony to a drop of hydrogen peroxide. The production of bubbles of oxygen indicates the presence of catalase.*

**Peroxidase** breaks down hydrogen peroxide without forming oxygen, using a reducing agent such as the coenzyme NADH:



**\*\* Obligate anaerobes** either **lack both catalase and peroxidase** or **have only a small amount of them**, so they are susceptible to the toxic action of hydrogen peroxide.

• **Hydroxyl radical (OH•)**. Hydroxyl radicals result from ionizing radiation and from the incomplete reduction of hydrogen peroxide. Hydroxyl radicals are the most reactive of the four toxic forms of oxygen.

**\*\* Because hydrogen peroxide does not accumulate in aerobic cells** (because of the action of catalase and peroxidase), the threat of hydroxyl radical is virtually eliminated in aerobic cells.

Not all organisms are either obligate or strict aerobes or anaerobes; many organisms can live in various oxygen concentrations between these two extremes.



## Oxygen Classes of Microorganisms

1. **Obligate aerobes:** organisms are completely dependent on atmospheric O<sub>2</sub> for growth.

Oxygen serves as the terminal electron acceptor for the **electron transport chain ETC** in the metabolic process called **aerobic respiration**.

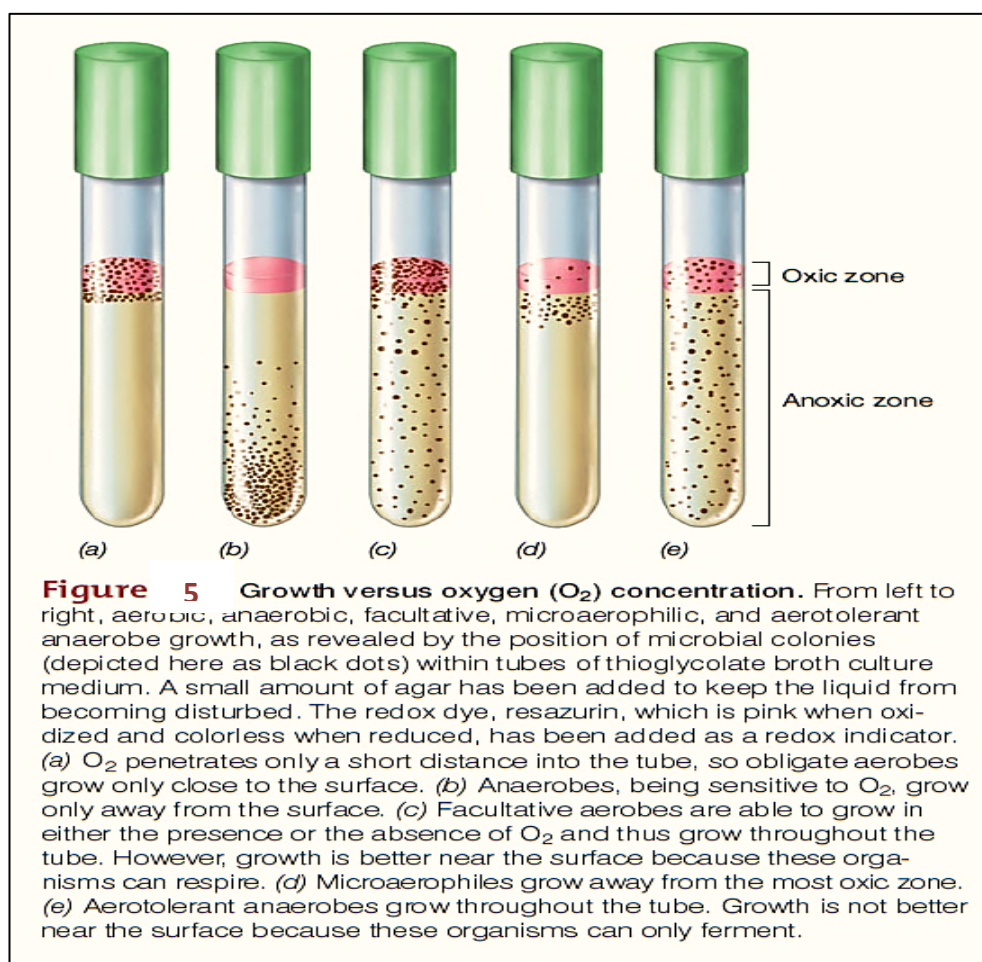
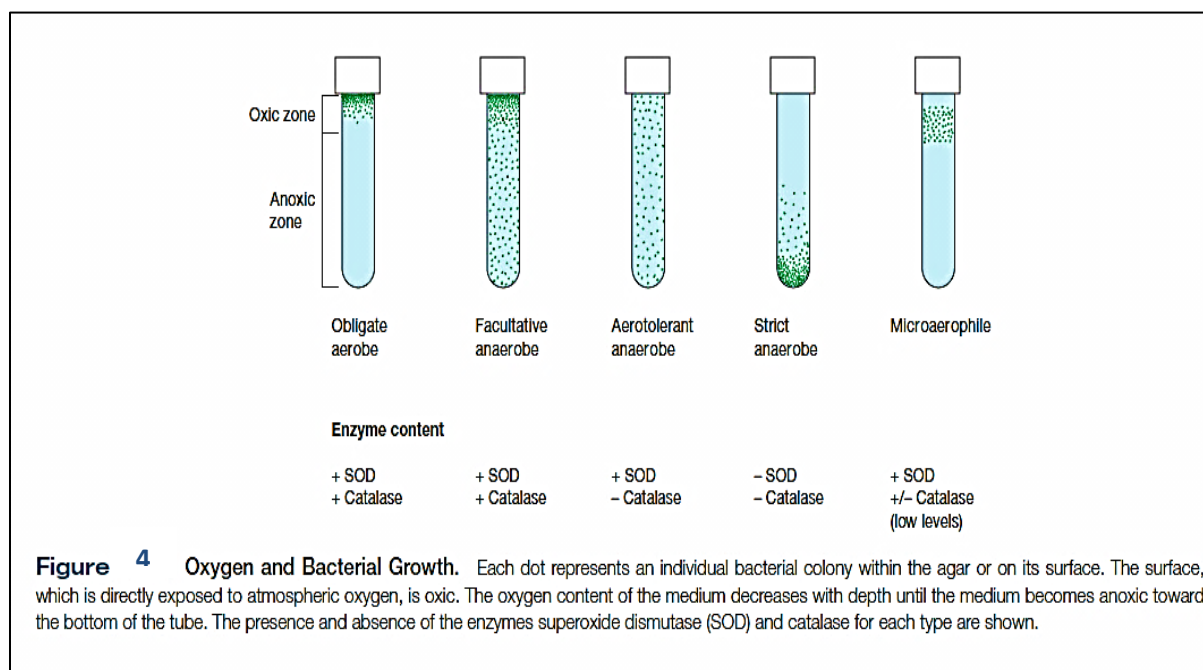
2. **Microaerophiles** such as *Campylobacter* are damaged by the normal atmospheric level of O<sub>2</sub> (20%) and require O<sub>2</sub> levels in the range of 2 to 10% for growth.

3. **Facultative anaerobes** do not require O<sub>2</sub> for growth but grow better in its presence. In the presence of oxygen, they use O<sub>2</sub> as the terminal electron acceptor during aerobic respiration.

4. **Aerotolerant anaerobes** such as *Enterococcus faecalis* simply ignore O<sub>2</sub> and grow equally well whether it is present or not; chemotrophic aerotolerant anaerobes are often described as having strictly fermentative metabolism.

5. **Strict or obligate anaerobes** (e.g., *Bacteroides*, *Clostridium pasteurianum*, *Methanococcus* ) are usually killed in the presence of O<sub>2</sub>. Strict anaerobes cannot generate energy through aerobic respiration and employ other metabolic strategies such as fermentation or anaerobic respiration, neither of which requires O<sub>2</sub>.

The nature of bacterial O<sub>2</sub> responses can be readily determined by growing the bacteria in culture tubes filled with a solid culture medium (figures 4 & 5).



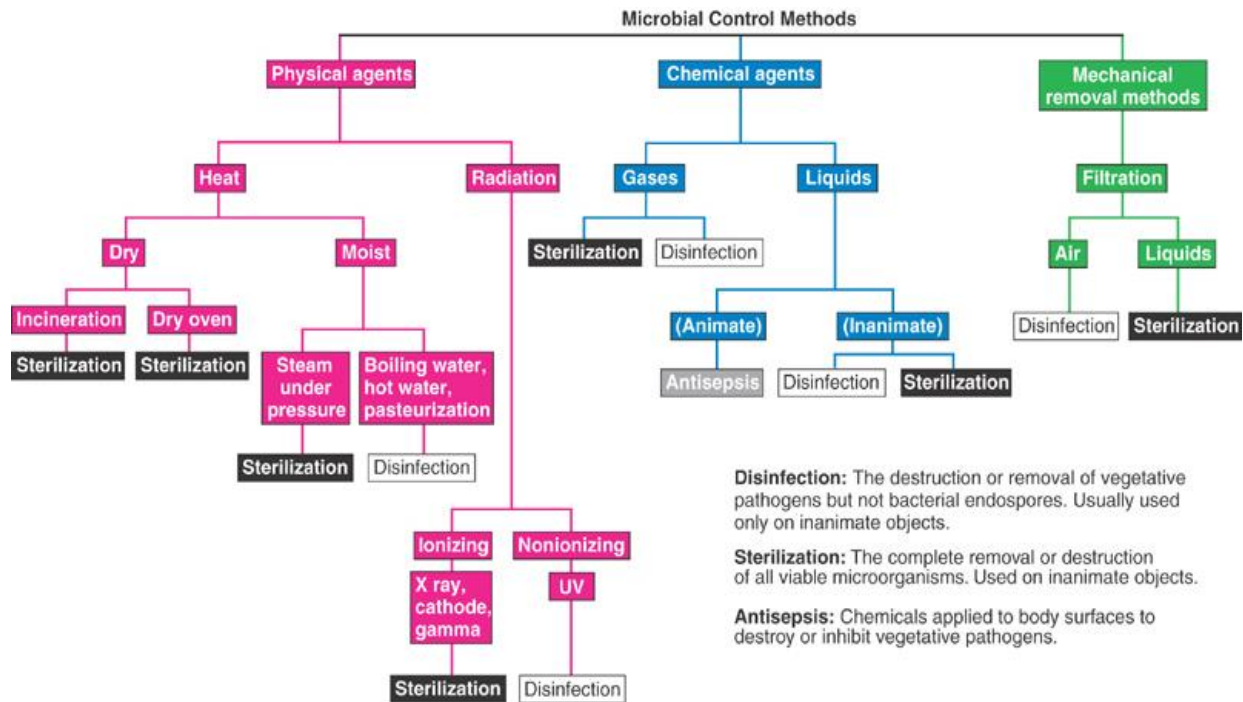
## 6. Radiation

Sunlight is the major source of radiation on Earth. It includes visible light, ultraviolet (UV) radiation, infrared rays, and radio waves. Visible light is a most conspicuous and important aspect of our environment: most life depends on the ability of photosynthetic organisms to trap the light energy of the sun.

Many forms of electromagnetic radiation are very harmful to microorganisms. This is particularly true of **ionizing radiation**, radiation of very short wavelength and high energy, which can cause atoms to lose electrons (ionize). Two major forms of ionizing radiation are (1) **X rays**, and (2) **gamma rays**, which are emitted during radioisotope decay. **Low levels of ionizing radiation may produce mutations and may indirectly result in death, whereas higher levels are directly lethal.**

**Ultraviolet (UV) radiation** can kill microorganisms due to its short wavelength (approximately from 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. The primary mechanism of UV damage is the formation of thymine dimers in DNA.

## Physical and Chemical Agents for Microbial Control



### Terminology and Methods of Control

**Sterilization** – a process that destroys all viable microbes, including viruses and endospores and is carried out in health-care facilities by physical or chemical methods.

**Disinfection** – a process to destroy vegetative pathogens, not endospores; inanimate objects.

**Antiseptics:** Those chemicals that can be safely applied over skin and mucus membranes.

**Sanitization** – any cleansing technique that mechanically removes microbes.

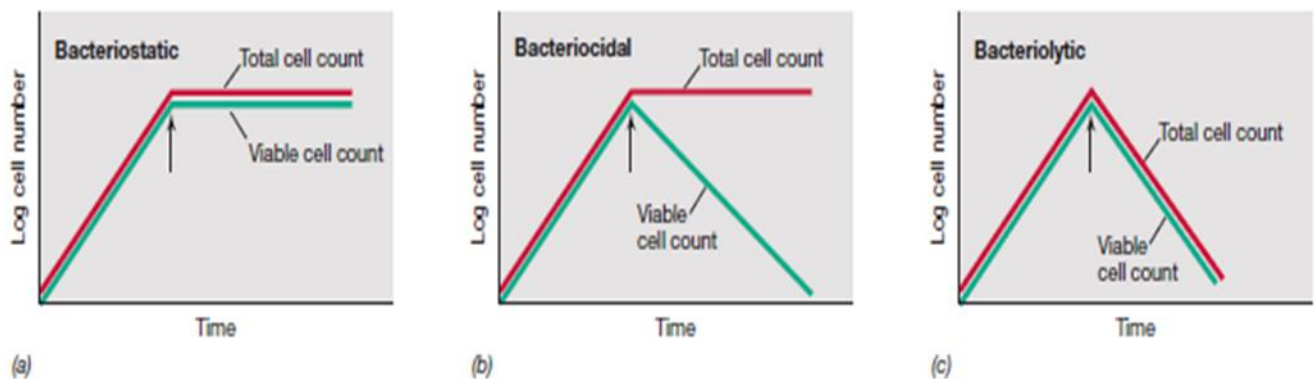
**Degermation** – reduces the number of microbes.

### **Bacteriocidal and Bacteriostatic (prefix cidal vs. static)**

Agents that kill organisms are called –cidal agents, they are called bacteriocidal, fungicidal, and viricidal agents because they kill bacteria, fungi, and viruses, respectively.

Agents that do not kill but only inhibit growth are called –static agents. These include **bacteriostatic**, **fungistatic**, and **viristatic** compounds.

**lytic agents:** Some -cidal agents are also -lytic agents, killing by cell lysis and release of cytoplasmic contents. Lysis decreases the viable cell number and also the total cell number, shown by a decrease in culture turbidity.



## **Antimicrobial Agents' Modes of Action**

Cellular targets of physical and chemical agents:

1. The cell wall – cell wall becomes fragile and cell lyses; some antimicrobial drugs, detergents, and alcohol

2. The cell membrane - loses integrity; detergent surfactants
3. Cellular synthetic processes (DNA, RNA) – prevention of replication, transcription; some antimicrobial drugs, radiation, formaldehyde, ethylene oxide
4. Proteins – interfere at ribosomes to prevent translation, disrupt or denature proteins; alcohols, phenols, acids, heat.

### **(I) Methods of Physical Control:**

1. Heat – moist and dry
2. Cold temperatures
3. Radiation
4. Filtration

#### **1- Heat:**

**Moist heat** – lower temperatures and shorter exposure time; coagulation and denaturation of proteins

#### **Moist Heat Methods:**

- Steam under pressure
- Autoclave 15 psi/121°C/10-40 min killing of heatresistant endospores.
- Steam must reach surface of item being sterilized
- Item must not be heat or moisture sensitive
- Mode of action – denaturation of proteins, destruction of membranes and DNA

- **Boiling Water:** Boiling at 100°C for 30 minutes to destroy non-spore-forming pathogens.

**Dry Heat** - using higher temperatures than moist heat – flame or electric heating, reduces microbes and other substances.

Dry ovens – 150-180°C- coagulate proteins.

**2- Cold:** Microbiostatic – slows the growth of microbes

- Refrigeration 0-15°C and freezing <0°C
- Used to preserve food, media and cultures

**3- Radiation:**

Ionizing radiation – deep penetrating power that has sufficient energy to break DNA, gamma rays, X-rays, used to sterilize medical supplies and food products.

Nonionizing radiation – little penetrating power – must be directly exposed, UV light creates thymine dimers, which interfere with replication. For example, laboratory laminar flow hoods, designed to maintain clean work areas, are equipped with a “germicidal” UV light to decontaminate the work surface after use.

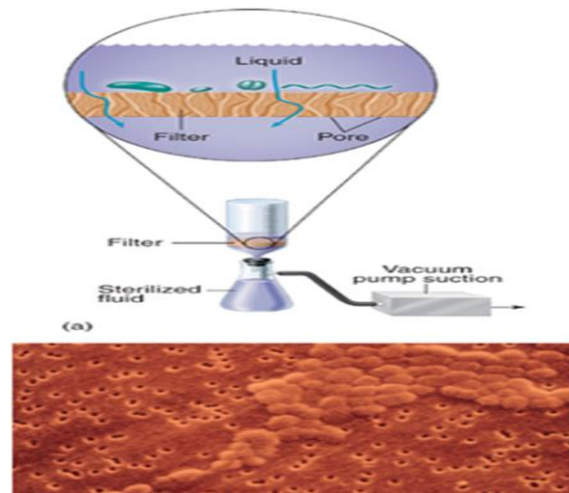




#### 4- Filter Sterilization:

Physical removal of microbes by passing a gas or liquid through filter

Used to sterilize heat sensitive liquids and air in hospital isolation units and industrial clean rooms.



## (II) Chemical Agents in Microbial Control Classification of disinfectants:

1. Based on consistency

(a) Liquid (E.g., Alcohols, Phenols)

(b) Gaseous (Formaldehyde vapour)

2. Based on spectrum of activity

(a) High level

(b) Intermediate level

(c) Low level

3. Based on mechanism of action

(a) Action on membrane (E.g., Alcohol, detergent)

(b) Denaturation of cellular proteins (E.g., Alcohol, Phenol)



(c) Oxidation of essential sulphhydryl groups of enzymes (E.g., H<sub>2</sub>O<sub>2</sub>, Halogens)

(d) Damage to nucleic acids (Formaldehyde)

**Alcohols:**

**Mode of action:** Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein. **Examples:** Ethyl alcohol.

**Aldehydes:**

**Mode of action:** Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores. **Example:** Formaldehyde

**Phenol:**

**Mode of action:** Act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

### (III) Antimicrobial Agents Used *In Vivo*

Up to this point, we have considered the effects of physical and chemical agents used to inhibit microbial growth outside the human body.

- **Antibiotics** are common metabolic products of aerobic spore-forming bacteria and fungi.
  - bacteria in genera *Streptomyces* and *Bacillus*
  - molds in genera *Penicillium* and *Cephalosporium*
- By inhibiting the other microbes in the same habitat, antibiotic producers have less competition for nutrients and space.

Antibiotic production has been particularly associated with soil microorganisms. Although the majority of antibacterial agents in clinical use today are derived from natural products of fermentation, most are then chemically modified (semi-synthetic) to improve their antibacterial or pharmacologic properties. However, some agents are totally synthetic (e.g. sulphonamides, quinolones). Therefore, the term ‘antibacterial or ‘antimicrobial’ agent is often used in preference to ‘antibiotic’. Agents used against fungi, parasites, and viruses can also be included under antimicrobials, but the terms antifungals, antiprotozoans, anthelmintics, and antivirals are more often used.

### SELECTIVE TOXICITY

Selective toxicity is achieved by exploiting differences in the structure and metabolism of microorganisms and host cells; ideally, the antimicrobial agent should kill or inhibit microbial cells without damaging host tissues. This is more likely to be achievable in microorganisms that are prokaryotes than in

those that are eukaryotes, as the former are structurally more distinct from the host cells.

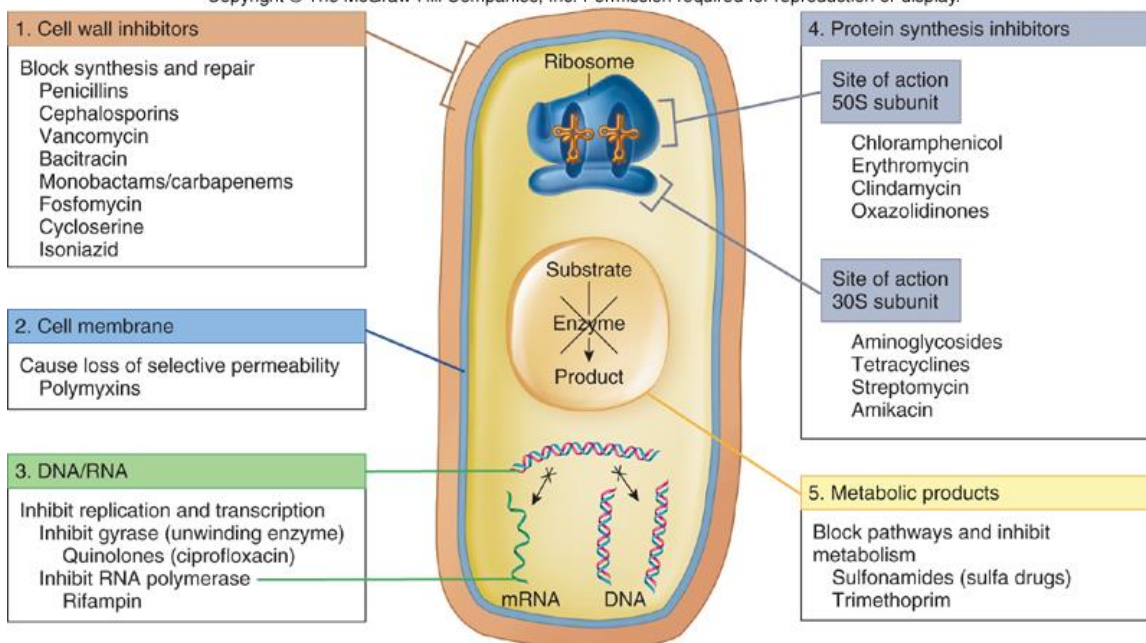
## **CLASSIFICATION OF ANTIBACTERIAL AGENTS**

There are three ways of classifying antibacterial agents:

1. According to whether they are bactericidal or bacteriostatic
2. by target site
3. by chemical structure

**There are five main target sites for antibacterial action**

- cell wall synthesis
- protein synthesis
- nucleic acid synthesis
- metabolic pathways
- cell membrane function.



## MODE OF ACTION OF ANTIBACTERIAL AGENTS

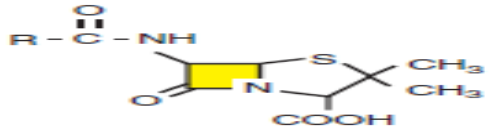
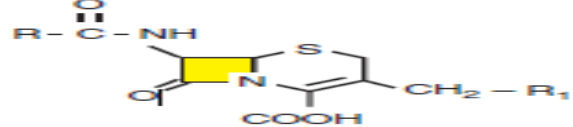
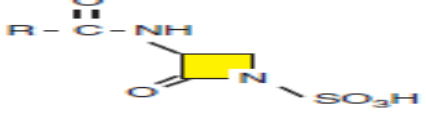

### 1- INHIBITORS OF CELL WALL SYNTHESIS

Peptidoglycan, a vital component of the bacterial cell wall is a compound unique to bacteria and therefore provides an optimum target for selective toxicity.

**$\beta$ -Lactam Antibiotics: Penicillins and Cephalosporins** it was isolated from the fungus *Penicillium chrysogenu*, *Cephalosporium* sp. respectively. One of the most important groups of antibiotics.  **$\beta$ -lactam antibiotics** include the medically important penicillins, cephalosporins, and cephamycins. These antibiotics share a characteristic structural component, the  $\beta$ -lactam ring. The popularity of these agents results from their bactericidal action and lack of toxicity to humans; also, their molecular structures can be manipulated to achieve greater activity for wider therapeutic applications. Penicillin G is active primarily against gram-positive Bacteria because gram-negative Bacteria are impermeable to the antibiotic. Semisynthetic penicillins, are effective against some gram-negative Bacteria. The structural differences in the N-acyl groups of these semisynthetic penicillins allow them to be transported inside the gram-negative outer membrane.

## Mechanism of Action

The  $\beta$ -lactam antibiotics are inhibitors of cell wall synthesis. An important feature of bacterial cell wall synthesis is transpeptidation, the reaction that results in the cross-linking of two glycan-linked peptide chains. The transpeptidase enzymes bind to penicillin or other  $\beta$ -lactam antibiotics. Thus, these transpeptidases are called penicillin-binding proteins (PBPs). When PBPs bind penicillin, they cannot catalyze the transpeptidase reaction. As a result, the newly synthesized bacterial cell wall is no longer cross-linked and cannot maintain its strength. In addition, the antibiotic–PBP complex stimulates the release of autolysins, enzymes that digest the existing cell wall.

Beta-lactam class	Examples	Base molecular structure
Penicillins	Penicillin Ampicillin Piperacillin Mezlocillin	
Cephalosporins	Cefazolin Cefuroxime Cefotetan Cefotaxime Ceftriaxone Ceftazidime Cefepime	
Monobactams	Aztreonam	
Carbapenems	Imipenem Meropenem	

**Figure 11-2** Basic structures and examples of commonly used beta-lactam antibiotics. The core beta-lactam ring is highlighted in yellow in each structure. (Modified from Salyers AA, Whitt DD, editors: *Bacterial pathogenesis: a molecular approach*, Washington, DC, 1994, ASM Press.)

## 2- Inhibitors of Protein Synthesis

Several classes of antibiotics target bacterial protein synthesis. Antibiotic classes that act by inhibiting protein synthesis include aminoglycosides, macrolide, ketolides chloramphenicol, tetracyclines, glycylglycine, and oxazolidinones.

**Aminoglycosides.** Aminoglycosides inhibit bacterial protein synthesis by binding to protein receptors on the organism's 30S ribosomal subunit. This process (protein synthesis) interrupts several steps, including initial formation of the protein synthesis complex, accurate reading of the mRNA code, and formation of the ribosomal-mRNA complex. Aminoglycosides include gentamycin, tobramycin, amikacin, netilmicin, streptomycin, and kanamycin. The spectrum of activity of aminoglycosides includes a wide variety of gram-negative and gram-positive bacteria (broad spectrum of activity).

### **3- Inhibitors of DNA and RNA Synthesis**

The antimicrobial agents that target DNA metabolism are the fluoroquinolones and metronidazole.

**Fluoroquinolones.** Fluoroquinolones, also often simply referred to as *quinolones*, are derivatives of nalidixic acid, an older antibacterial agent. These agents bind to and interfere with DNA gyrase enzymes involved in the regulation of bacterial DNA supercoiling, a process that is essential for DNA replication and transcription.

### **4- Inhibitors of Other Metabolic Processes**

Include sulfonamides and trimethoprim, The folic acid pathway is used by bacteria to produce precursors important for DNA synthesis. Sulfonamides target and bind , dihydropteroate synthase and trimethoprim target dihydrofolate reductase leading to disrupt the folic acid pathway.

## **Mechanisms of antibiotic resistance**

### **A- Intrinsic ( inherent) resistance**

The bacterial resistance of antibiotics can be identified into the following three types :

**(i)** Antibiotic inactivation by the bacteria before they reach their target within the bacteria.

The B-lactamases that cleave the B-lactam ring of the penicillin and cephalosporin antibiotics.

Another hydrolytic enzyme include aminoglycoside-modifying enzymes and chloramphenicol acetyl transferases.

(ii) Access to the target site may be altered (altered uptake or increased exit). This mechanism involves decreasing the amount of drug by either:

- Altering entry, for example by decreasing the permeability of the cell wall
- Pumping the drug out of the cell (known as an efflux mechanism).

(iii) Outer membrane (OM) permeability changes, prevention of antibiotic access to the target site may be occurred either by a permeability barrier or the presence of an efflux pump mechanism. Such mechanism responsible for resistance to macrolides, chloramphenicol, tetracyclines and aminoglycosides.

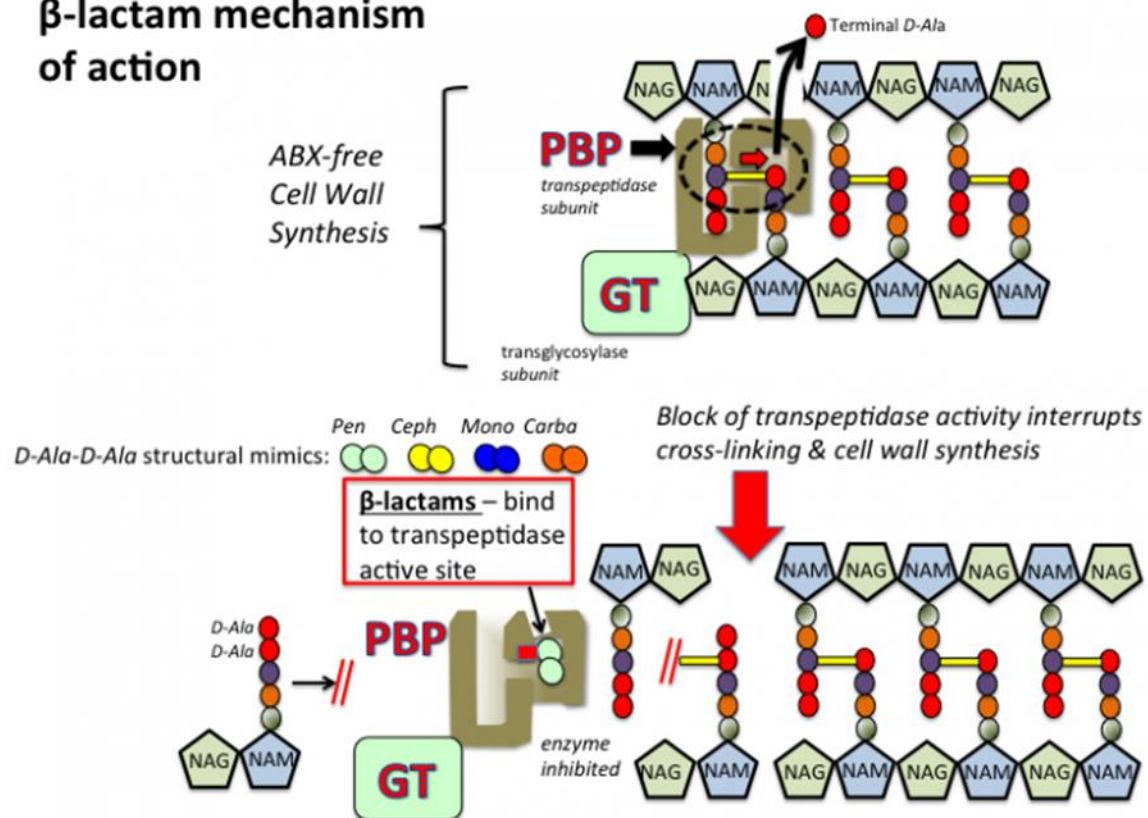
## **B- Acquired Resistance**

Antibiotic resistance that results from altered cellular physiology and structure caused by changes in a microorganism's **genetic structure** is known as acquired resistance. Unlike intrinsic resistance, acquired resistance may be a trait associated with only some strains of a particular organism group or species, but not others. Therefore, the presence of this type of resistance in any clinical isolate is unpredictable, and this unpredictability is the primary reason why laboratory methods to detect resistance are necessary.

Because acquired resistance mechanisms are all genetically encoded, the methods for acquisition basically are those that allow for gene change or exchange. Therefore, resistance may be acquired by:

- Successful genetic mutation
- Gaining of genes from other organisms via gene transfer mechanisms
- A combination of mutational and gene transfer Events

## β-lactam mechanism of action





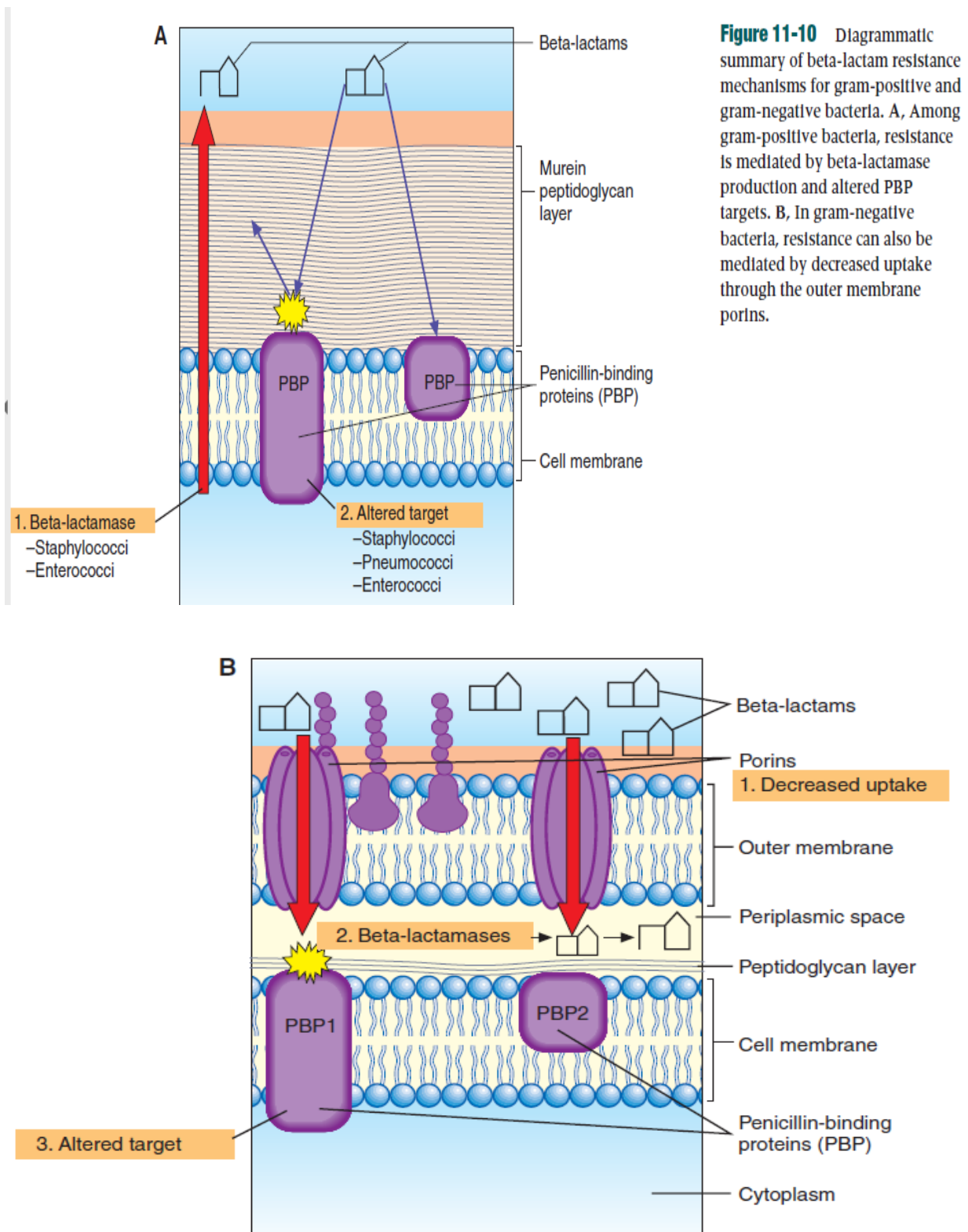


Figure: Beta-Lactam resistance mechanisms

### Cell Wall (peptidoglycan synthesis)

$\beta$ -lactams { Penicillins    Bacitracin  
                  Cephalosporins    Glycopeptides  
                  Carbapenems  
                  Monobactams

### Protein synthesis

#### 30S inhibitors

Aminoglycosides  
Tetracyclines  
Tigecycline

#### 50S inhibitors

Chloramphenicol  
Clindamycin  
Linezolid  
Macrolides  
Streptogramins

### Membrane integrity

Polymyxin B  
Daptomycin

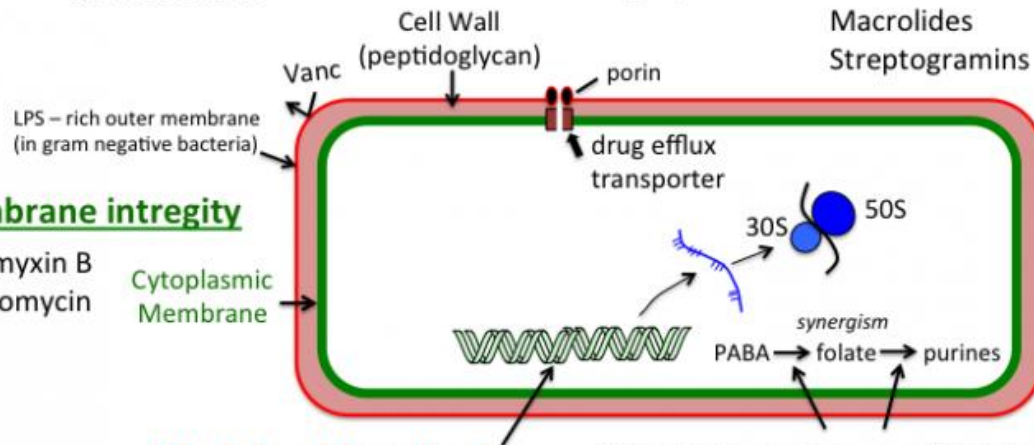
Cytoplasmic  
Membrane

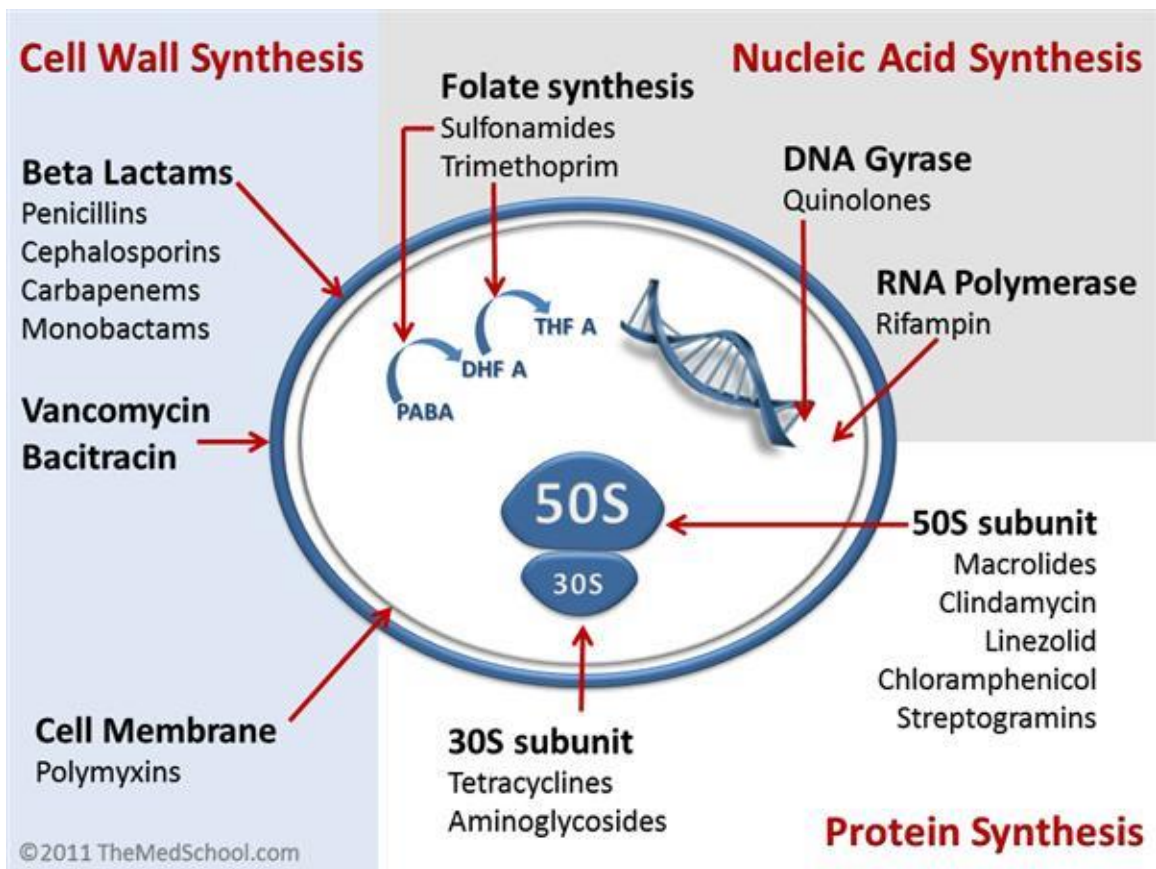
### Nucleic acid synthesis

Fluoroquinolones  
Metronidazole  
Rifamycins

### Metabolic pathways (folate)

Sulfonamides  
Trimethoprim







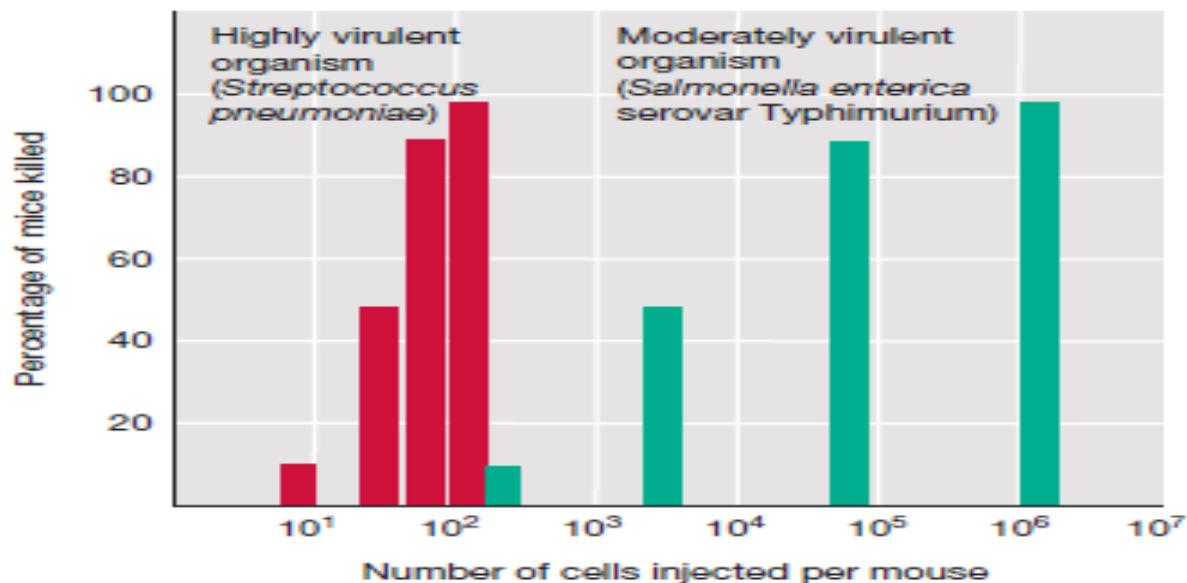
# **Microbial Virulence and Pathogenesis**

**Second lecture**  
**Dr.Khansaa Mohammed**



## Factors impacting outcome of host-pathogen relationships

1. Number of organisms present
2. Virulence factors
3. Host's defenses or degree of resistance
4. Transport of the bacterial pathogen to the host.



**Figure 27.14 Microbial virulence.** Differences in microbial virulence demonstrated by the number of cells of *Streptococcus pneumoniae* and *Salmonella enterica* serovar Typhimurium required to kill mice.



# Measuring Pathogenicity

- ❑ LD<sub>50</sub> - Lethal Dose of a microbes toxin that will kill 50% of experimentally inoculated test animal.
- ❑ ID<sub>50</sub> - infectious dose required to cause disease in 50% of inoculated test animals.
  - Example: ID<sub>50</sub> for *Vibrio cholerea* 10<sup>8</sup> cells.
  - ID<sub>50</sub> for Inhalation Anthrax - 5,000 to 10,000 spores.





# Portals of entry

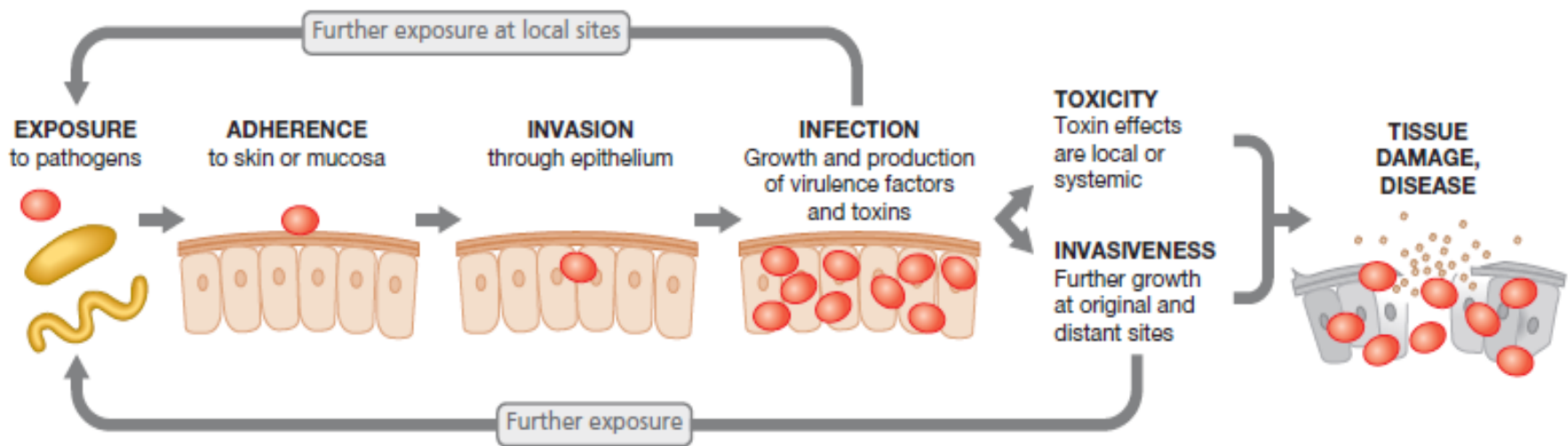
Pathogens can gain entrance to the human body and other hosts through several portals, which are called **portals of entry**.

## Route of entry of Microbial pathogen:

PORTAL OF ENTRY	BACTERIA	VIRUS	FUNGUS
<i>Skin and mucous membrane</i>	<i>Clostridium tetani</i> <i>Leptospira</i>	<i>Hepatitis B virus,</i> <i>HIV</i>	<i>Dermatophytes</i>
<i>Respiratory tract</i>	<i>Streptococcus pneumoniae,</i> <i>Neisseria meningitidis,</i> <i>Mycobacterium tuberculosis.</i>	<i>Rhinovirus,</i> <i>Respiratory syncytical virus,</i> <i>Influenza virus</i>	<i>Cryptococcus neoformans,</i> <i>Histoplasma capsulatum</i>
<i>Gastro-intestinal tract</i>	<i>Shigella sp,</i> <i>salmonella sp, Vibrio sp.</i>	<i>Hepatitis A or E virus, Polio virus</i>	<i>Candida albicans</i>
<i>Genital tract</i>	<i>Neisseria gonorrhoea,</i> <i>Treponema pallidum .</i>	<i>HIV, Human papilloma virus .</i>	<i>Candida albicans</i>

# BACT PATHOGENESIS -STAGES

What are the four stages of pathogenesis?



**Figure 23.9 Microbial pathogenesis.** Following exposure to a pathogenic microorganism, pathogen-directed events can result in disease.

# 1. Adherence

Is the term used to describe the interaction of the bacterium's molecules on its surface with that of the molecules of the host.

The host component that the adhesion binds is sometimes termed the **receptor**. The bacterial adhesion would be **specificity** with the host receptor and can be as diverse as the bacterium and host themselves; proteins, lipids, sugars, from both sides. The contact is so close that specialized injection systems can penetrate the membrane and pump toxins or effectors into the host cytoplasm.

# Adherence Factors

Pathogens adhere to the mucous membrane surfaces with selectivity using:

- Pilus adhesion and Fimbriae
- Slime layer
- Glyco-calyx
- Membrane proteins
- Bacterial Biofilm.

# Bacterial Pilus adherence

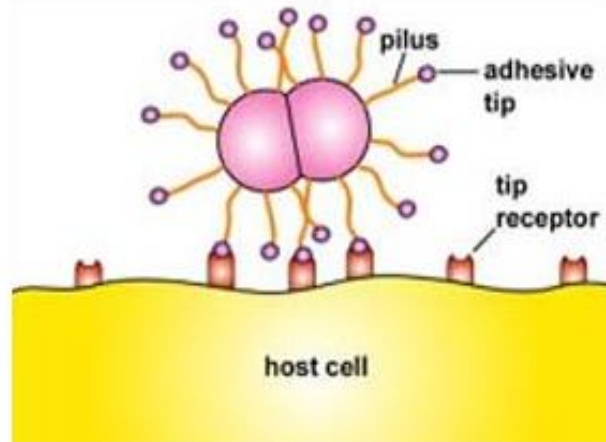


Figure 2.5C. 1: Adhesive Tip of Bacterial Pili Binding to Host Cell Receptors

## 2. Colonisation

The second stage of microbial infection is colonization: the establishment of the pathogen at the appropriate portal of entry. Organisms that infect these regions have usually some ability to overcome or withstand the constant pressure of the host defenses at the surface.

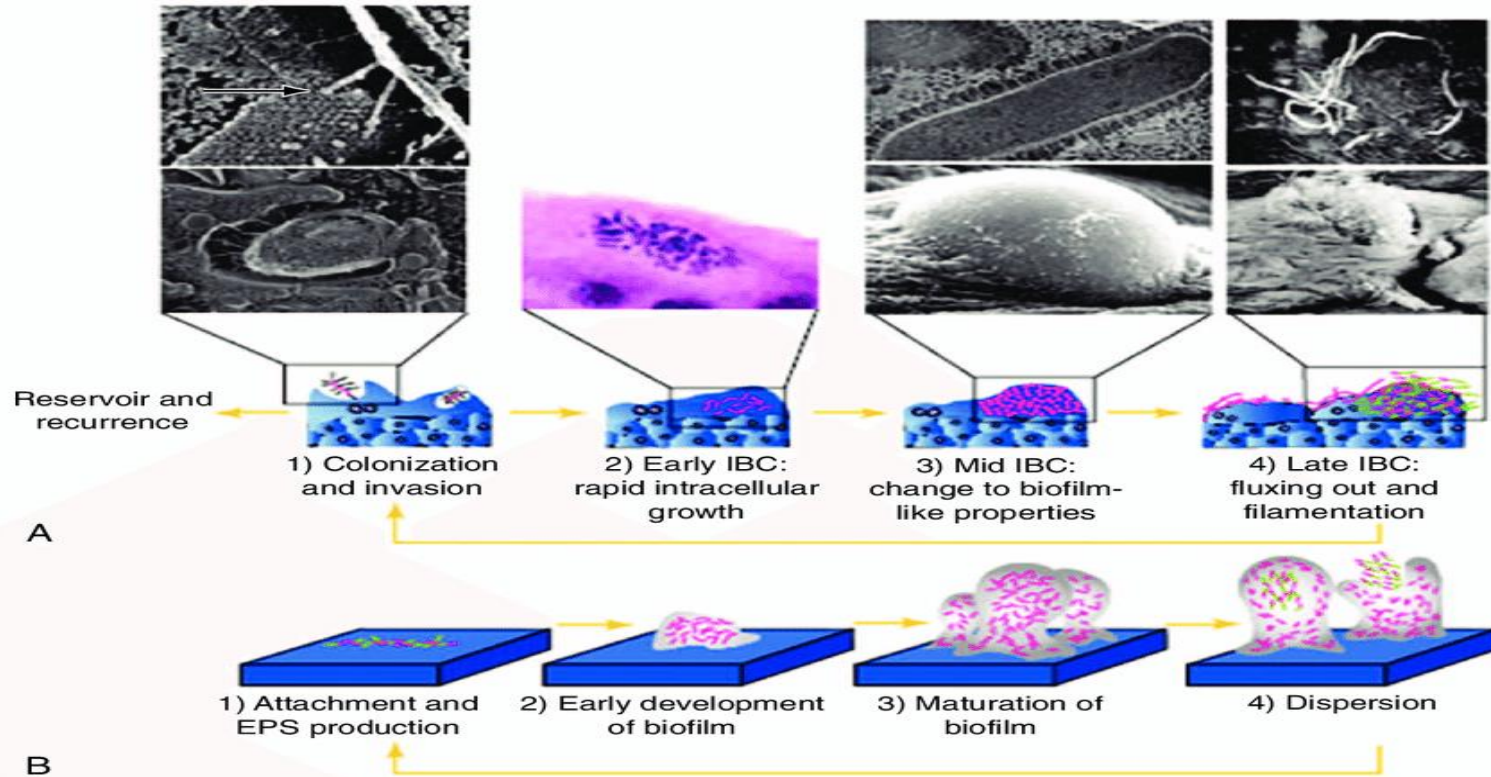


# Biofilm

The grouping of hundreds to thousands of bacterial cells together is sometimes termed a microcolony. When these microcolonies grow in size and by a thick sticky carbohydrates (and other proteins adhesins) with distinct biological and chemical differences the unit can be referred to as a **biofilm**.

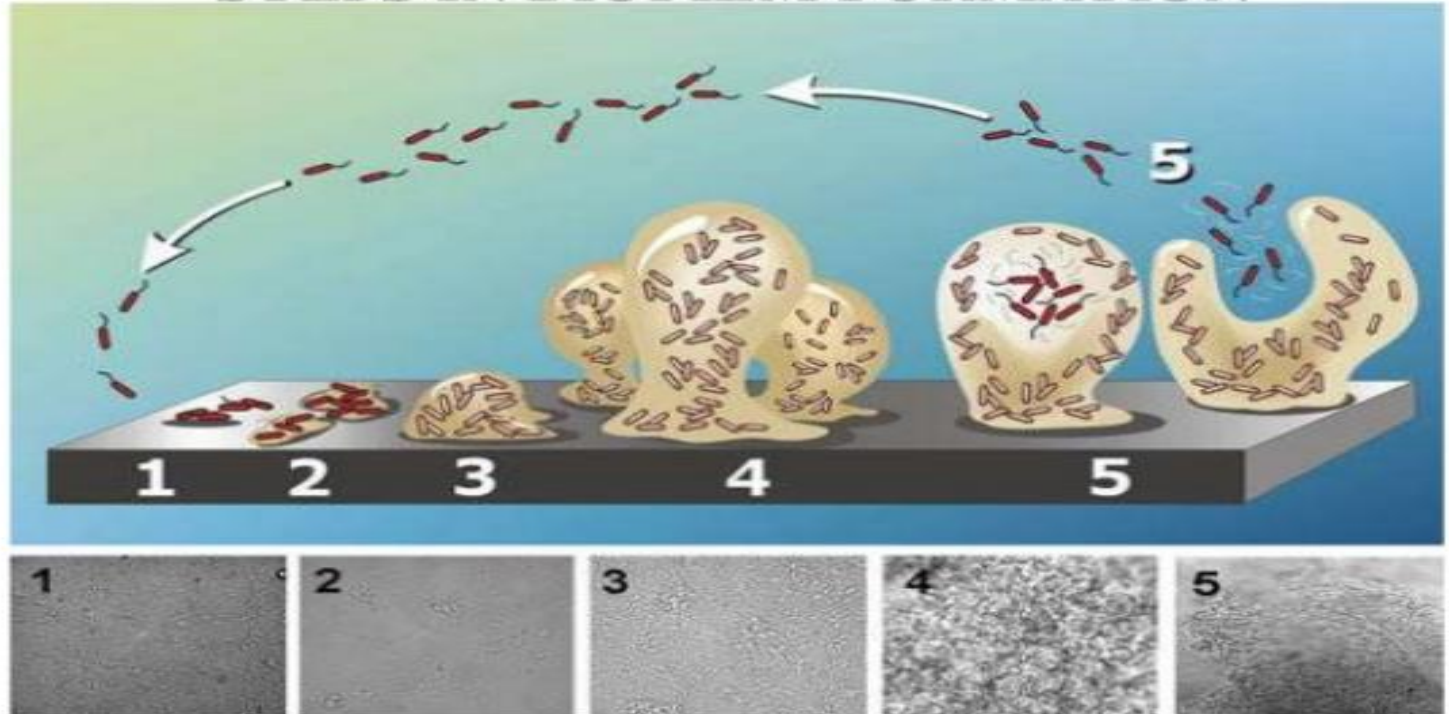


# Biofilm



# Biofilm

## STEPS IN BIOFILM FORMATION



### 3. Invasion

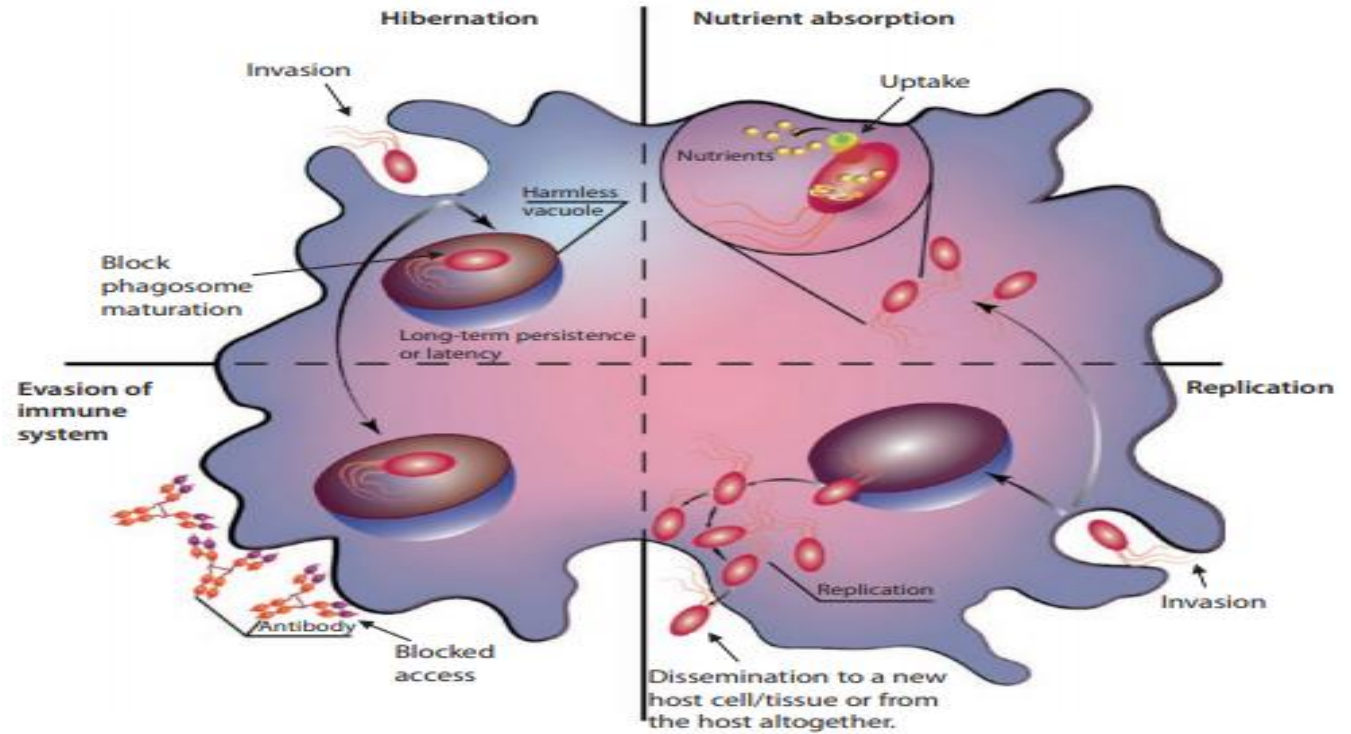
**Invasion.** Once bacteria have successfully colonised their host, they need a way to invade these tissues in order to replicate and cause disease. Invasion can be mediated by: Toxins and Enzymes which penetrate and damage cells.

# The benefits of the invasion of host cells

- Some of the reasons entering host cells can benefit bacteria pathogens. **They include using the host cell as a safe ecological place for long-term persistence, avoiding the immune system including detection by antibodies or phagocytes, greater access to key metabolites and building blocks (nutrient absorption), and use of these nutrients to grow, divide, and finally extend to other areas of the host or exit the host .**



# Invasion



# Invasion

**On the skin**, this may involve a normal inhabitant such as *Staphylococcus aureus*, penetrating to the dermis of the skin, causing an abscess, then becoming systemic.

**In the lungs**, it may involve *Streptococcus pneumoniae*, which can live in the upper respiratory tract but invade the deep alveoli, which leads to pneumonia and inflammation.

**In the gastrointestinal** and urinary tract, it may involve *Escherichia coli*, which invade the epithelium of these tracts and sometimes enter the blood

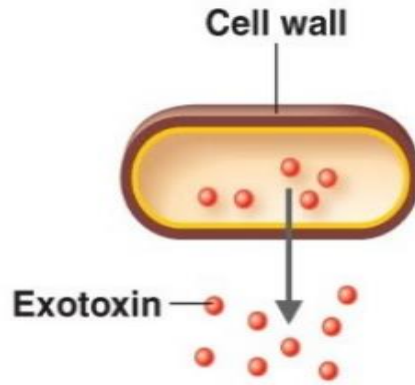
# Invasion: Toxins

Components or products of microorganisms which, when extracted and introduced into host animals, reproduces disease symptoms normally associated with infection

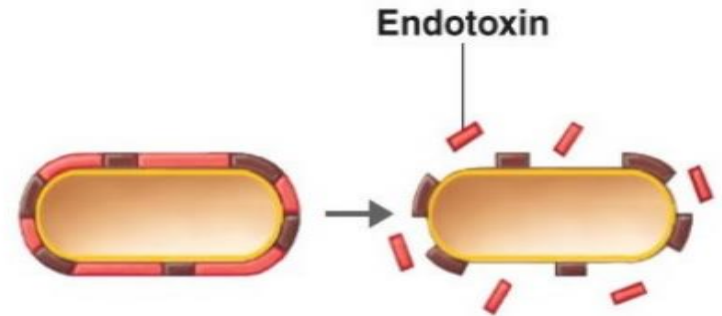
**Toxins are two general types:**

- Endotoxin
- Exotoxin

# Invasion: Toxins



**(a) Exotoxins** are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.



**(b) Endotoxins** are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria.

The endotoxins are liberated when the bacteria die and the cell wall breaks apart.



# Invasion: Toxins

**Table 19.5 Basic properties of exotoxins and endotoxins**

Property	Exotoxin	Endotoxin
Chemical properties	Proteins, excreted by certain gram-positive or gram-negative Bacteria; generally heat-labile	Lipopolysaccharide-lipoprotein complexes released on cell lysis as part of the outer membrane of gram-negative Bacteria; extremely heat-stable
Mode of action; symptoms	Specific; either cytotoxin, enterotoxin, or neurotoxin with defined specific action on cells or tissues	General; fever, diarrhea, vomiting
Toxicity	Highly toxic, often fatal	Weakly toxic, rarely fatal
Immunogenicity	Highly immunogenic; stimulate the production of neutralizing antibody (antitoxin)	Relatively poor immunogen; immune response not sufficient to neutralize toxin
Toxoid potential	Treatment of toxin with formaldehyde will destroy toxicity, but treated toxin (toxoid) remains immunogenic	None
Fever potential	Do not produce fever in host	Pyrogenic, often produce fever in host

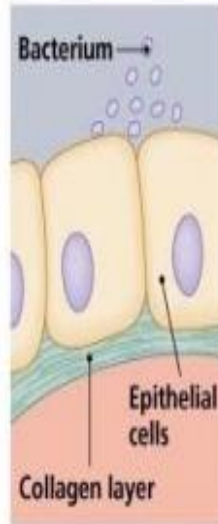
# Invasion: Enzymes

**Enzymes:** Play an important role in inflammatory process.

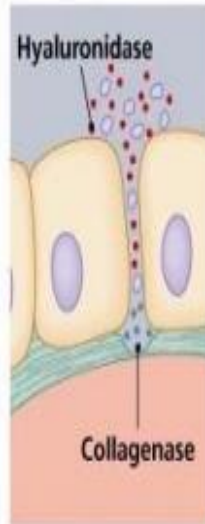
ENZYMES	ORGANISMS INVOLVED	MECHANISM OF ACTION
1. Hyaluronidase	<i>Staphylococci; Group A, B, G streptococci, Clostridium perfringenes</i>	Hydrolyse hyaluronic acid thereby spreading bacteria to spread through subcutaneous tissue
2. Collaginase	<i>Clostridium perfringenes</i>	Hydrolyse collagenase thereby spreading bacteria to spread through subcutaneous tissue
3. Coagulase	<i>Staphylococcus aureus</i>	It convert fibrinogen to fibrin clot, thereby protect bacteria from phagocytosis.
4. Streptokinase	<i>Group A, C, G streptococci.</i>	Bind to plasminogen and activate the production of plasmin.
5. Staphylokinase	<i>Staphylococcus aureus</i>	Prevent formation of fibrin clot.
6. Lecithinase	<i>Clostridium perfringenes</i>	Hydrolyse lecithin. destroys the integrity of the cytoplasmic membrane of many cells
7. Phospholipase	<i>Staphylococcus aureus</i>	Lyse red blood cells

# Enzymes

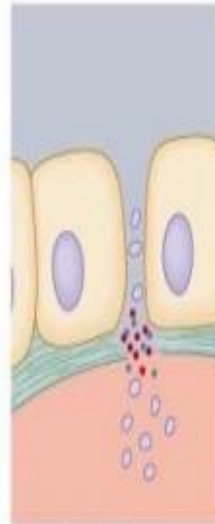
## Hyaluronidase and collagenase



Invasive bacteria reach epithelial surface

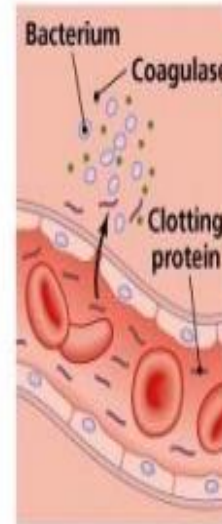


Bacteria produce hyaluronidase and collagenase



Bacteria invade deeper tissues

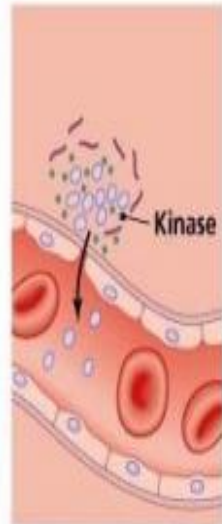
## Coagulase and kinase



Bacteria produce coagulase



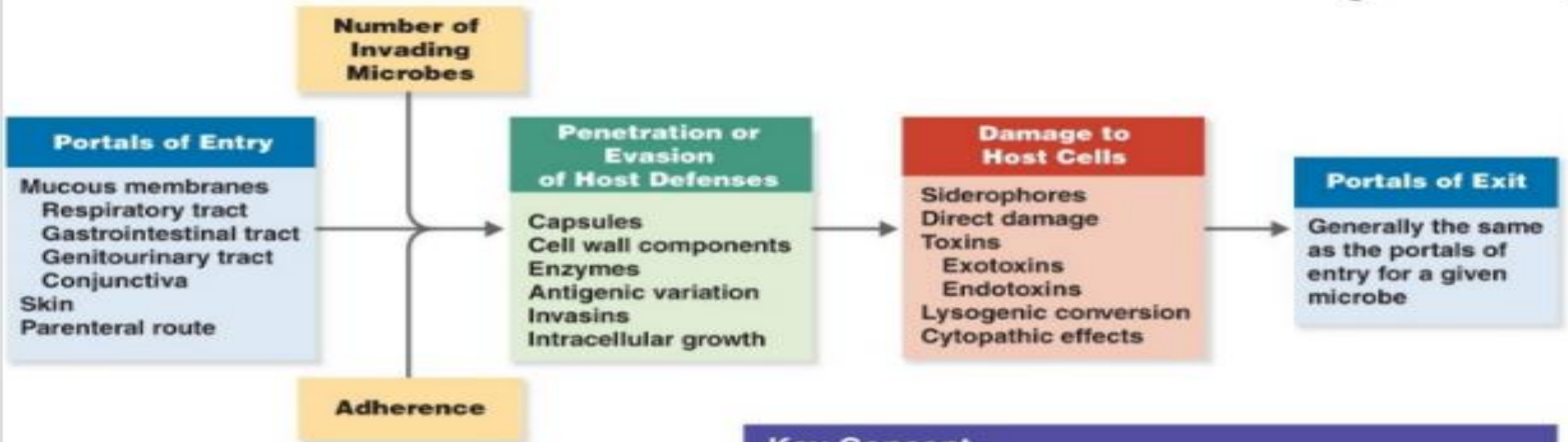
Clot forms



Bacteria later produce kinase, dissolving clot and releasing bacteria

# Objectives of lecture

## Microbial Mechanisms of Pathogenic



### Key Concept

Several factors are required for a microbe to cause disease. After entering the host, most pathogens adhere to host tissue, penetrate or evade host defenses, and damage host tissues. Pathogens usually leave the body via specific portals of exit, which are generally the same sites where they entered initially.



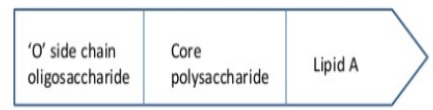
## ENDOTOXIN (PFEIFFER – 1893)

Toxic lipopolysaccharide components – gram negative bacteria

Exhibit profound biologic effect on the host

Released mainly during the cell lysis and also during multiplication

Basic structure:









Genus or Serotype antigens

Toxic moiety

Genus specific antigens

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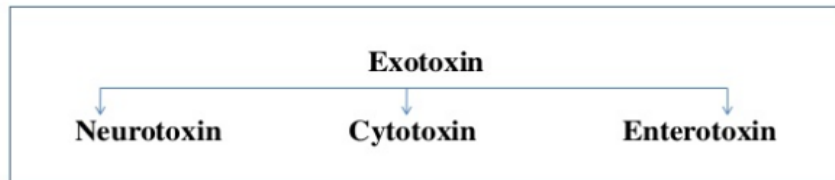
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- Gram positive and gram negative species - Soluble protein toxins released from viable bacteria during exponential growth phase
- Are excellent antigens that elicit specific antibodies called antitoxins
- Enter eukaryotic cells primarily through receptor mediated endocytosis

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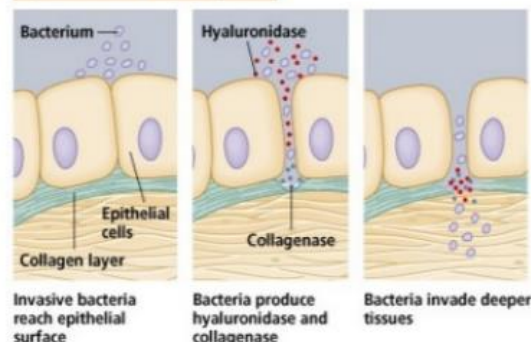
Bacterial virulence factors  
Ahmed Mohamed

# Enzymes

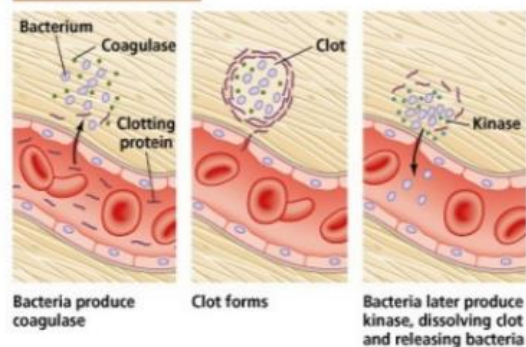
Excretion of certain pathogens to assist them in establishing infection and producing a disease.

There are virulence determinant enzymes that dissolve the glue between cells, thus allowing the bacteria to spread rapidly through the tissue.

Hyaluronidase and collagenase

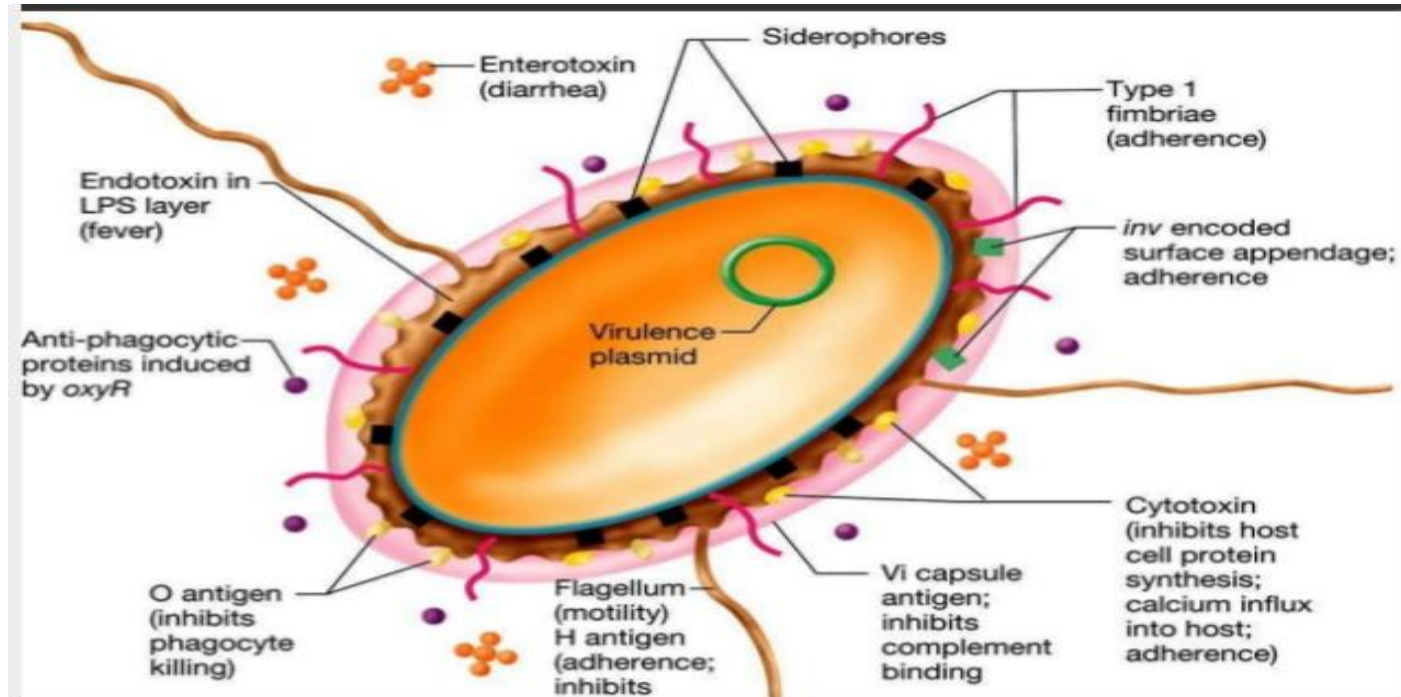


Coagulase and kinase



(a) Extracellular enzymes

# Bacterial virulence factors





# Portals of Entry

**TABLE 15.1** Portals of Entry for the Pathogens of Some Common Diseases

Portal of Entry	Pathogen*	Disease
<b>MUCOUS MEMBRANES</b>		
Respiratory tract	<i>Streptococcus pneumoniae</i>	Pneumococcal pneumonia
	<i>Mycobacterium tuberculosis</i> <sup>†</sup>	Tuberculosis
	<i>Bordetella pertussis</i>	Whooping cough (pertussis)
	Influenza virus ( <i>Influenzavirus</i> )	Influenza
	Measles virus ( <i>Morbillivirus</i> )	Measles (rubeola)
	Rubella virus ( <i>Rubivirus</i> )	German measles (rubella)
	Epstein-Barr virus ( <i>Lymphocryptovirus</i> )	Infectious mononucleosis
	Varicella-zoster virus ( <i>Varicellovirus</i> )	Chickenpox (varicella) (primary infection)
Gastrointestinal tract	<i>Histoplasma capsulatum</i> (fungus)	Histoplasmosis
	<i>Shigella</i> spp.	Shigellosis (bacillary dysentery)
	<i>Brucella</i> spp.	Brucellosis (undulant fever)
	<i>Vibrio cholerae</i>	Cholera
	<i>Salmonella enterica</i>	Salmonellosis
	<i>Salmonella typhi</i>	Typhoid fever
	Hepatitis A virus ( <i>Hepatovirus</i> )	Hepatitis A
	Mumps virus ( <i>Rubulavirus</i> )	Mumps
Genitourinary tract	<i>Trichinella spiralis</i> (helminth)	Trichinellosis
	<i>Neisseria gonorrhoeae</i>	Gonorrhea
	<i>Treponema pallidum</i>	Syphilis
	<i>Chlamydia trachomatis</i>	Nongonococcal urethritis
	Herpes simplex virus type 2	Herpes virus infections
	Human immunodeficiency virus (HIV) <sup>‡</sup>	AIDS
	<i>Candida albicans</i> (fungus)	Candidiasis
<b>SKIN OR PARENTERAL ROUTE</b>		
	<i>Clostridium perfringens</i>	Gas gangrene
	<i>Clostridium tetani</i>	Tetanus
	<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever
	Hepatitis B virus ( <i>Hepadnavirus</i> ) <sup>‡</sup>	Hepatitis B
	Rabiesvirus ( <i>Lyssavirus</i> )	Rabies
	<i>Plasmodium</i> spp. (protozoan)	Malaria

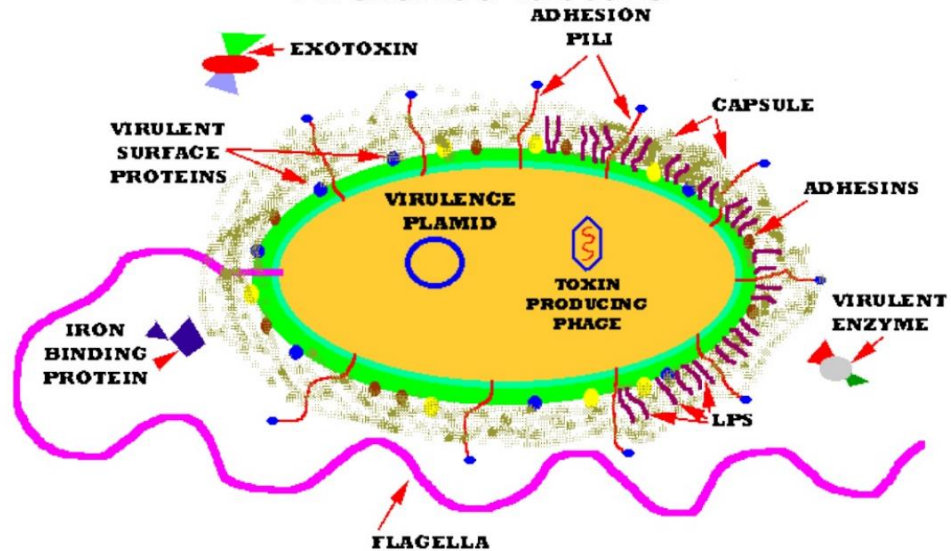
TYPES OF ADHESION	MECHANISM	EXAMPLES
Pillus adhesion  Fimbriae A) Mannose sensitive fimbriae  B) Mannose-resistant fimbriae	These are the main mechanism by which bacteria adhere to host cell. These are the fibers that extends from bacterial surface, mediate attachment of bacteria to specific receptor on host cell	<i>E.coli, Neisseria gonorrhoea, Vibrio cholerae.</i>

TYPES OF NON-PILLUS ADHESION	ORGANISMS INVOLVED
Haemagglutinin ( filamentous , mannose resistant, fibrillar)	<i>Bordetella pertusis, Helicobacter pylori, Salmonella typhimureum</i>
Biofilm	<i>CONS, Staphylococci, E.coli, Viridans group of streptococci</i>
Curli (surface protein)	<i>E. coli, Salmonella, Shigella</i>
Fibronectin	<i>Streptococcus pyogenes</i>
Exopolysaccharide	<i>Streptococcus mutans</i>

# Summary

## Virulence factors

Clip slide



الجامعة التقنية الشمالية  
المعهد التقني الموصل  
قسم : تقنيات البيئة والموارد المائية

المادة | علم الاحياء المجهرية  
دكتورة : مها النعيمي

2025 - 2024



وزارة التعليم العالي والبحث العلمي  
الجامعة التقنية الشمالية  
المعهد التقني / الموصل



# الحقية التعليمية

القسم العلمي: تقنيات البيئة والموارد المائية

اسم المقرر: الاحياء المجهرية في المياه

المرحلة / المستوى: الاول

الفصل الدراسي: الاول

السنة الدراسية: 2024 - 2025



عدد الساعات الاسبوعية				السنة الدراسية الاولى	الاحياء المجهرية في المياه (1)	باللغة العربية	سم المادة
عدد الوحدات	المجموع	عملي	نظري		Microbiology of water (1)	باللغة الانكليزية	
2	3	2	1	المستوى الاول الفصل الاول (الدروس الاجبارية)			لغة التدريس للمادة

#### المفردات النظرية

الأسبوع	تفاصيل المفردات
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1	مقدمه في علم الأحياء المجهرية : التعريف - الأهمية - صفات وأقسام الأحياء المجهرية بصورة عامه العلاقة بين الأحياء المجهرية وأشكال الحياة الأخرى وانتشار الأحياء المجهرية في الطبيعة
2	نظام تسمية وتصنيف الأحياء المجهرية : الطريقة العامة التي سميت بها الأحياء المجهرية باستخدام نظام التسمية الثنائية وأعضاء البكتريا - الأحياء الشائعة للبكتريا تصنيف الأحياء المجهرية بصورة عامة وحسب طبقات التصنيف ( نوع / جنس الى أخرى 00 )
3-4	البكتريا ، تعريفها ، صفاتها الظاهرية - انتشارها في الطبيعة تركيب خلية البكتريا - نمو وتكاثر البكتريا - معدل النمو - منحنى النمو البكتيري 0
5-6	العوامل التي تؤثر على النمو / إنبات البكتريا في ظروف المختبر / الاحتياجات المختلفة للإنبات / المزارع النقية أهميتها وطرق عزلها / تقسيم البكتريا
7	السيطرة على الأحياء المجهرية
8	السيطرة على الأحياء المجهرية بالطرق الفيزيائية
9	السيطرة على الأحياء المجهرية بالطرق الكيميائية
10	العفن : انتشاره في الطبيعة / الأهمية - صفاته الظاهرية - التكاثر بعض الاعفان المهمة
11-12	الخمائر : التعريف - انتشارها في الطبيعة- صفاته الظاهرية- التركيب الخلوي - التكاثر- بعض أنواع المهمة
13-14	الطحالب والاشنات : تعريفها / انتشارها في الطبيعة / صفاتها تكاثرها / الصفات في الطحالب / الأهمية الاقتصادية للطحالب
15	الابتدائيات : صفاتها / تقسيمها / تركيبها الخلوي - تكاثرها - أهم الأنواع المرضية في المياه

#### المفردات العملية

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عدد الساعات الاسبوعية				السنة الدراسية الاولى	الاحياء المجهرية (2) في المياه	باللغة العربية	اسم المادة
عدد الوحدات	المجموع	عملي	نظري		Microbiology of Water (2)	باللغة الانكليزية	
2	3	2	1	المستوى الاول الفصل الثاني (الدروس الاجبارية)			لغة التدريس للمادة

المفردات النظرية

الأسبوع	تفاصيل المفردات
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2 - 1	الفايروسات : التعريف - صفاتها - تركيبها - طرق تصنيفها - التناسخ في الفايروسات بعض الفايروسات المهمة في المياه
3	أهم الأمراض التي تسببها الأحياء المجهرية في المياه -التعريف- أهم مسببات الأمراض - التعريف الأمراض التي تسببها الأحياء المجهرية وطرق انتقالها
4	التعريف بالأمراض الميكروبيه المهمة التي قد تنتقل عن طريق المياه - الأمراض التي تسببها الكائنات الحية الأخرى
5	أهم الأمراض التي تسببها الأحياء المجهرية في المياه : التعريف - أهم مسببات الأمراض - التعريف بالأمراض التي تسببها الأحياء المجهرية وطرق انتقالها - التعريف بالأمراض التي تسببها الأحياء المجهرية وطرق انتقالها - التعريف الأمراض الميكروبية المهمة التي قد تنتقل عن طريق المياه - الأمراض التي تسببها الكائنات الحية الأخرى
8 - 7 - 6	الأحياء المجهرية المستخدمة كدليل على تلوث المياه أهم الفحوصات المختبرية المستخدمة لتحديد صلاحية المياه من الناحية الصحية
10 - 9	الأحياء المجهرية في البيئات المائية : تعريف الطالب بأهمية البيئة بشكل عام والبيئات المائية بشكل خاص 0 مصادر علاقاتها بالأحياء المجهرية في الأنواع المختلفة من المياه - أهمية الأحياء المجهرية في البيئات المائية
12 - 11	الأحياء المجهرية ومياه الشرب : طرق تصفية - تأثير المعاملات المختلفة على الأحياء المجهرية - المواصفات القياسية الميكروبيولوجيه لمياه الشرب
13	فحوصات تطبيقية على تلوث آلاماه
14	الفحوصات البكتريولوجيه الروتينيه للماء الخام وماء مشاريع التصفية - والمياه في الشبكة ومياه الصرف الصحي
15	فحوصات على البكتريا في مجموعة بكتريا القولون

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5 - 4 - 3	العدد الكلي القياسي لبكتيريا ماء الشرب SPC
7 - 6	العدد الكلي القياسي لبكتيريا ماء المجاري
9 - 8	الكشف عن بكتيريا القولون في مياه الشرب
11 - 10	الكشف عن بكتيريا القولون في مياه المجاري
13 - 12	الكشف عن بكتيريا القولون البرازية في مياه الشرب
15 - 14	الكشف عن بكتيريا القولون البرازية في مياه الشرب