Chapter 6

Microbial Growth

1

Growth

- **increase in cellular constituents that may result in:**
  - **increase in cell number**
    - **e.g., when microorganisms reproduce by budding or binary fission**
  - **increase in cell size**
    - **e.g., coenocytic microorganisms have nuclear divisions that are not accompanied by cell divisions**
- **microbiologists usually study population growth rather than growth of individual cells**

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The Growth Curve

- **observed when microorganisms are cultivated in batch culture**
  - **culture incubated in a closed vessel with a single batch of medium**
- **usually plotted as logarithm of cell number versus time**
- **usually has four distinct phases**

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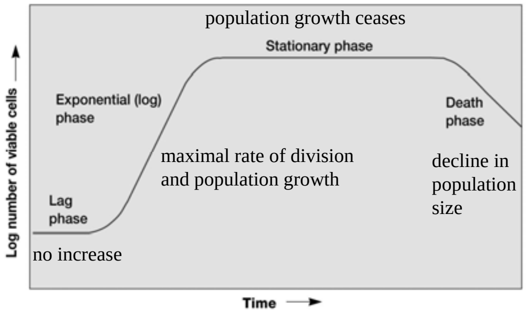

Figure 6.1

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Lag Phase

- **cell synthesizing new components**
  - **e.g., to replenish spent materials**
  - **e.g., to adapt to new medium or other conditions**
- **varies in length**
  - **in some cases can be very short or even absent**

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Exponential Phase

- **also called log phase**
- **rate of growth is constant**
- **population is most uniform in terms of chemical and physical properties during this phase**

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Table 6.1 An Example of Exponential Growth

Time[a]	Division Number	2^n	Population ($N_0 \times 2^n$)	$\log_{10} N_t$
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
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

[a]The hypothetical culture begins with one cell having a 20-minute generation time.

cells are dividing and doubling in number at regular intervals

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each individual cell divides at a slightly different time

curve rises smoothly rather than as discrete steps

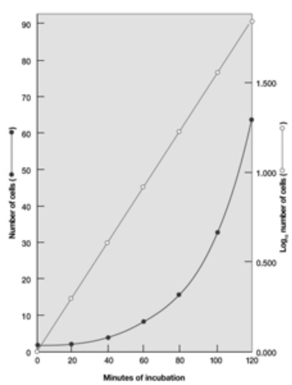

Figure 6.3

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Balanced growth

- **during log phase, cells exhibit balanced growth**
  - **cellular constituents manufactured at constant rates relative to each other**

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Unbalanced growth

- rates of synthesis of cell components vary relative to each other
- occurs under a variety of conditions
  - change in nutrient levels
    - shift-up (poor medium to rich medium)
    - shift-down (rich medium to poor medium)
  - change in environmental conditions

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Effect of nutrient concentration on growth

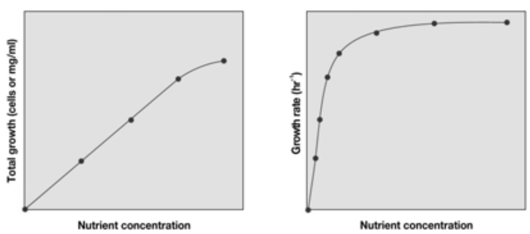

Figure 6.2

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Stationary Phase

- total number of viable cells remains constant
  - may occur because metabolically active cells stop reproducing
  - may occur because reproductive rate is balanced by death rate

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Possible reasons for entry into stationary phase

- **nutrient limitation**
- **limited oxygen availability**
- **toxic waste accumulation**
- **critical population density reached**

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Starvation responses

- **morphological changes**
  - **e.g., endospore formation**
- **decrease in size, protoplast shrinkage, and nucleoid condensation**
- **production of starvation proteins**
- **long-term survival**
- **increased virulence**

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Death Phase

- **cells dying, usually at exponential rate**
- **death**
  - **irreversible loss of ability to reproduce**
- **in some cases, death rate slows due to accumulation of resistant cells**

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The Mathematics of Growth

- **generation (doubling) time**
  - **time required for the population to double in size**
- **mean growth rate constant**
  - **number of generations per unit time**
  - **usually expressed as generations per hour**

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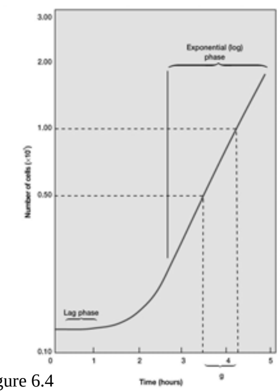

Figure 6.4

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Table 6.2 Generation Times for Selected Microorganisms

Microorganism	Temperature (°C)	Generation Time (Hours)
Bacteria		
Beneckea natriegens	37	0.16
Escherichia coli	40	0.35
Bacillus subtilis	40	0.43
Staphylococcus aureus	37	0.47
Pseudomonas aeruginosa	37	0.58
Clostridium botulinum	37	0.58
Rhodospirillum rubrum	25	4.6–5.3
Anabaena cylindrica	25	10.6
Mycobacterium tuberculosis	37	≈12
Treponema pallidum	37	33
Algae		
Scenedesmus quadricauda	25	5.9
Chlorella pyrenoidosa	25	7.75
Asterionella formosa	20	9.6
Euglena gracilis	25	10.9
Ceratium tripos	20	82.8
Protozoa		
Tetrahymena geleii	24	2.2–4.2
Leishmania donovani	26	10–12
Paramecium caudatum	26	10.4
Acanthamoeba castellanii	30	11–12
Giardia lamblia	37	18
Fungi		
Saccharomyces cerevisiae	30	2
Monilinia fruu	25	30

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Measurement of Microbial Growth

- can measure changes in number of cells in a population
- can measure changes in mass of population

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Measurement of Cell Numbers

- Direct cell counts
  - counting chambers
  - electronic counters
  - on membrane filters
- Viable cell counts
  - plating methods
  - membrane filtration methods

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Counting chambers

- easy, inexpensive, and quick
- useful for counting both eucaryotes and procaryotes
- cannot distinguish living from dead cells

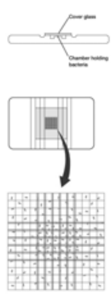

Figure 6.5

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Electronic counters

- microbial suspension forced through small orifice
- movement of microbe through orifice impacts electric current that flows through orifice
- instances of disruption of current are counted

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Electronic counters…

- cannot distinguish living from dead cells
- quick and easy to use
- useful for large microorganisms and blood cells, but not procaryotes

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Direct counts on membrane filters

- cells filtered through special membrane that provides dark background for observing cells
- cells are stained with fluorescent dyes
- useful for counting bacteria
- with certain dyes, can distinguish living from dead cells

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Plating methods

- measure number of viable cells
- population size is expressed as colony forming units (CFU)

plate dilutions of population on suitable solid medium
↓
count number of colonies
↓
calculate number of cells in population

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Plating methods…

- simple and sensitive
- widely used for viable counts of microorganisms in food, water, and soil
- inaccurate results obtained if cells clump together

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Membrane filtration methods

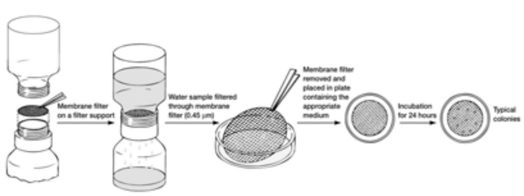

Figure 6.6

especially useful for analyzing aquatic samples

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Measurement of Cell Mass

- **dry weight**
  - **time consuming and not very sensitive**
- **quantity of a particular cell constituent**
  - **e.g., protein, DNA, ATP, or chlorophyll**
  - **useful if amount of substance in each cell is constant**
- **turbidometric measures (light scattering)**
  - **quick, easy, and sensitive**

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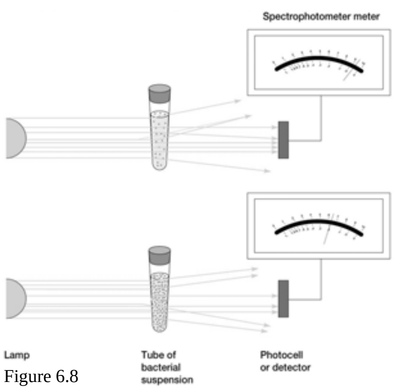

more cells
↓
more light scattered
↓
less light detected

Figure 6.8

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The Continuous Culture of Microorganisms

- **growth in an open system**
  - **continual provision of nutrients**
  - **continual removal of wastes**
- **maintains cells in log phase at a constant biomass concentration for extended periods**
- **achieved using a continuous culture system**

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The Chemostat

- **rate of incoming medium = rate of removal of medium from vessel**
- **an essential nutrient is in limiting quantities**

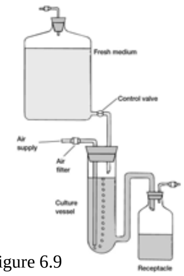

Figure 6.9

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Dilution rate and microbial growth

dilution rate – rate at which medium flows through vessel relative to vessel size

note: cell density maintained at wide range of dilution rates and chemostat operates best at low dilution rate

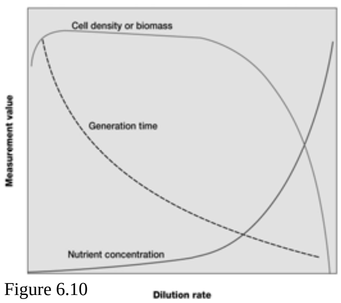

Figure 6.10

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The Turbidostat

- **regulates the flow rate of media through vessel to maintain a predetermined turbidity or cell density**
- **dilution rate varies**
- **no limiting nutrient**
- **turbidostat operates best at high dilution rates**

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Importance of continuous culture methods

- constant supply of cells in exponential phase growing at a known rate
- study of microbial growth at very low nutrient concentrations, close to those present in natural environment
- study of interactions of microbes under conditions resembling those in aquatic environments
- food and industrial microbiology

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The Influence of Environmental Factors on Growth

- most organisms grow in fairly moderate environmental conditions
- extremophiles
  - grow under harsh conditions that would kill most other organisms

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Solutes and Water Activity

- water activity (a_w)
  - amount of water available to organisms
  - reduced by interaction with solute molecules (osmotic effect)

    higher [solute] $\Rightarrow$ lower a_w
  - reduced by adsorption to surfaces (matric effect)

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Table 6.4 Approximate Lower a_w Limits for Microbial Growth

Water Activity	Environment	Bacteria	Fungi	Algae
1.00—Pure water	Blood; Plant wilt; Seawater { Vegetables, meat, fruit	Most gram-negative nonhalophiles		
0.95	Bread	Most gram-positive rods	*Basidiomycetes*	Most algae
0.90	Ham	Most cocci, *Bacillus*	*Fusarium*; *Mucor, Rhizopus*; Ascomycetous yeasts	
0.85	Salami	*Staphylococcus*	*Saccharomyces rouxii* (in salt)	
0.80	Preserves		*Penicillium*	
0.75	Salt lakes; Salted fish	*Halobacterium*; *Actinospora*	*Aspergillus*	*Dunaliella*
0.70	Cereals, candy, dried fruit		*Aspergillus*	
0.60	Chocolate; Honey; Dried milk		*Saccharomyces rouxii* (in sugars); *Xeromyces bisporus*	
0.55—DNA disordered				

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Osmotolerant organisms

- **grow over wide ranges of water activity**
- **many use compatible solutes to increase their internal osmotic concentration**
  - **solutes that are compatible with metabolism and growth**
- **some have proteins and membranes that require high solute concentrations for stability and activity**

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Effects of NaCl on microbial growth

- **halophiles**
  - **grow optimally at >0.2 M**
- **extreme halophiles**
  - **require >2 M**

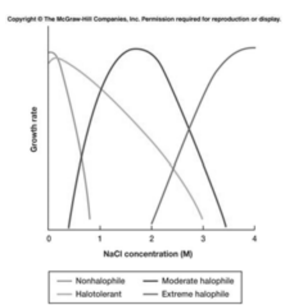

Figure 6.11

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pH

- negative logarithm of the hydrogen ion concentration

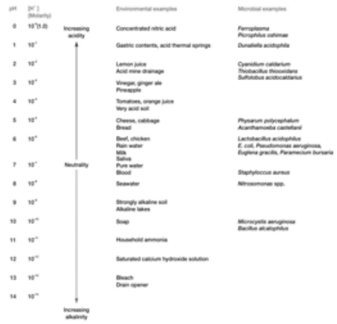

Figure 6.12

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pH

- acidophiles
  - growth optimum between pH 0 and pH 5.5
- neutrophiles
  - growth optimum between pH 5.5 and pH 7
- alkalophiles
  - growth optimum between pH8.5 and pH 11.5

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pH

- most acidophiles and alkalophiles maintain an internal pH near neutrality
  - some use proton/ion exchange mechanisms to do so
- some synthesize proteins that provide protection
  - e.g., acid-shock proteins
- many microorganisms change pH of their habitat by producing acidic or basic waste products
  - most media contain buffers to prevent growth inhibition

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Temperature

- **organisms exhibit distinct cardinal growth temperatures**
  - **minimal**
  - **maximal**
  - **optimal**

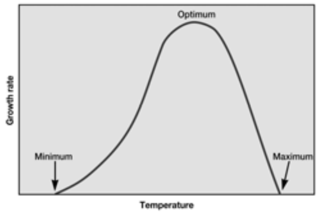

Figure 6.13

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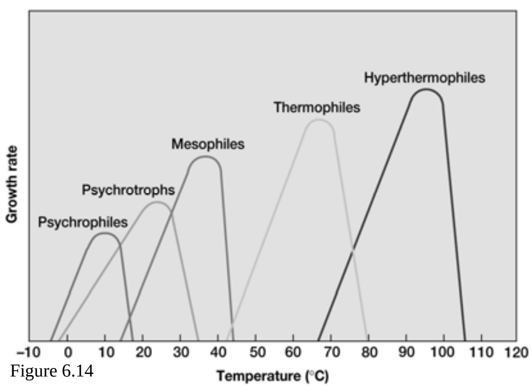

Figure 6.14

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Adaptations of thermophiles

- **protein structure stabilized by a variety of means**
  - **e.g., more H bonds**
  - **e.g., more proline**
  - **e.g., chaperones**
- **histone-like proteins stabilize DNA**
- **membrane stabilized by variety of means**
  - **e.g., more saturated, more branched and higher molecular weight lipids**
  - **e.g., ether linkages (archaeal membranes)**

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Oxygen Concentration

need oxygen | prefer oxygen | ignore oxygen | oxygen is toxic | < 2 – 10% oxygen

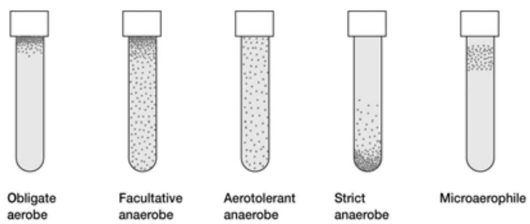

Figure 6.15

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Basis of different oxygen sensitivities

- **oxygen easily reduced to toxic products**
  - **superoxide radical**
  - **hydrogen peroxide**
  - **hydroxyl radical**
- **aerobes produce protective enzymes**
  - **superoxide dismutase (SOD)**
  - **catalase**

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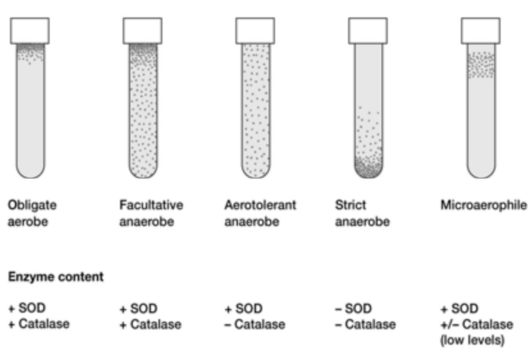

Figure 6.14

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Pressure

- **barotolerant organisms**
  - **adversely affected by increased pressure, but not as severely as nontolerant organisms**
- **barophilic organisms**
  - **require or grow more rapidly in the presence of increased pressure**

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Radiation

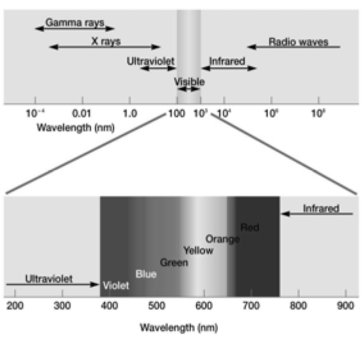

Figure 6.18

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Radiation damage

- **ionizing radiation**
  - **x rays and gamma rays**
  - **mutations → death**
  - **disrupts chemical structure of many molecules, including DNA**
    - **damage may be repaired by DNA repair mechanisms**

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Radiation damage…

- **ultraviolet (UV) radiation**
  - **mutations → death**
  - **causes formation of thymine dimers in DNA**
  - **DNA damage can be repaired by two mechanisms**
    - **photoreactivation – dimers split in presence of light**
    - **dark reactivation – dimers excised and replaced in absence of light**

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Radiation damage…

- **visible light**
  - **at high intensities generates singlet oxygen ($^1O_2$)**
    - **powerful oxidizing agent**
  - **carotenoid pigments**
    - **protect many light-exposed microorganisms from photooxidation**

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Microbial Growth in Natural Environments

- **microbial environments are complex, constantly changing, and may expose a microorganism to overlapping gradients of nutrients and environmental factors**

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Growth Limitation by Environmental Factors

- **Leibig's law of the minimum**
  - **total biomass of organism determined by nutrient present at lowest concentration**
- **Shelford's law of tolerance**
  - **above or below certain environmental limits, a microorganism will not grow, regardless of the nutrient supply**

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Responses to low nutrient levels

- **oligotrophic environments**
- **morphological changes to increase surface area and ability to absorb nutrients**
- **mechanisms to sequester certain nutrients**

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Counting Viable but Nonculturable Vegetative Procaryotes

- **stressed microorganisms can temporarily lose ability to grow using normal cultivation methods**
- **microscopic and isotopic methods for counting viable but nonculturable cells have been developed**

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Quorum Sensing and Microbial Populations

- quorum sensing
  - microbial communication and cooperation
  - involves secretion and detection of chemical signals

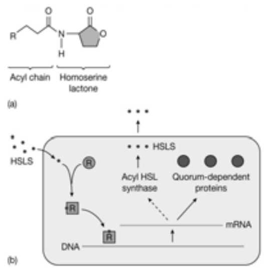

Figure 6.20

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Processes sensitive to quorum sensing: gram-negative bacteria

- **bioluminescence (*Vibrio fischeri*)**
- **synthesis and release of virulence factors (*Pseudomonas aeruginosa*)**
- **conjugation (*Agrobacterium tumefaciens*)**
- **antibiotic production (*Erwinia carotovora, Pseudomonas aureofaciens*)**
- **biofilm production (*P. aeruginosa*)**

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Quorum sensing: gram-positive bacteria

- **often mediated by oligopeptide pheromone**
- **processes impacted by quorum sensing:**
  - **mating (*Enterococcus faecalis*)**
  - **transformation competence (*Streptococcus pneumoniae*)**
  - **sporulation (*Bacillus subtilis*)**
  - **production of virulence factors (*Staphylococcus aureus*)**
  - **development of aerial mycelia (*Streptomyces griseus*)**
  - **antibiotic production (*S. griseus*)**

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