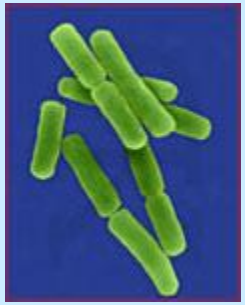
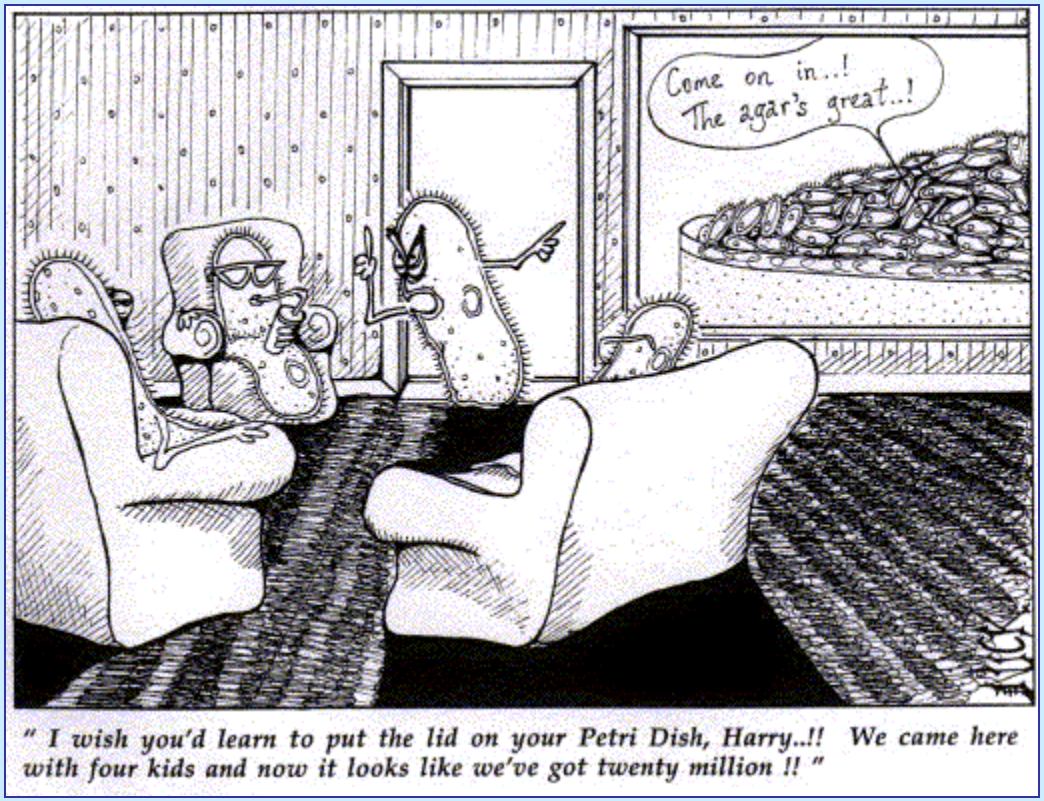
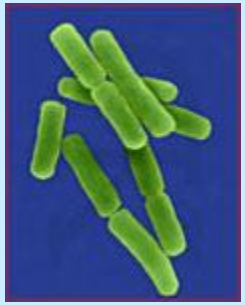




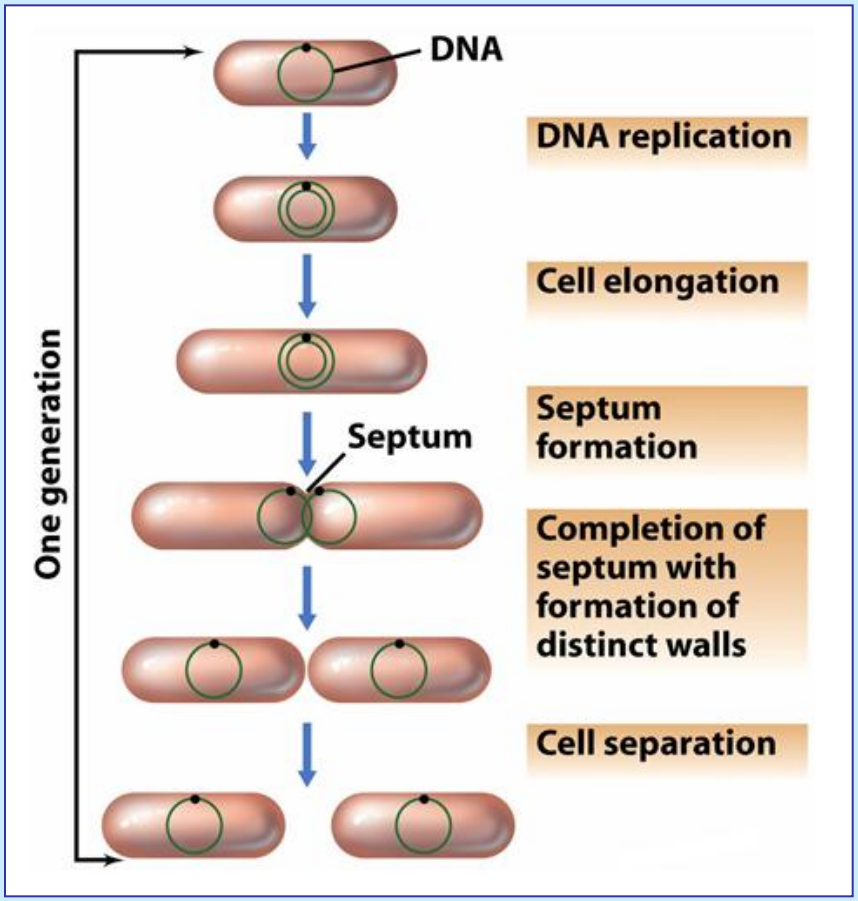
" I wish you'd learn to put the lid on your Petri Dish, Harry..!! We came here with four kids and now it looks like we've got twenty million !! "

MODALITA' DI CRESCITA

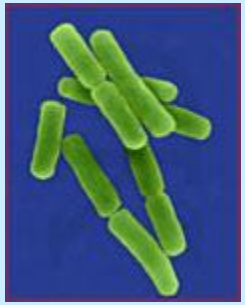
La crescita microbica



MODALITA' DI CRESCITA

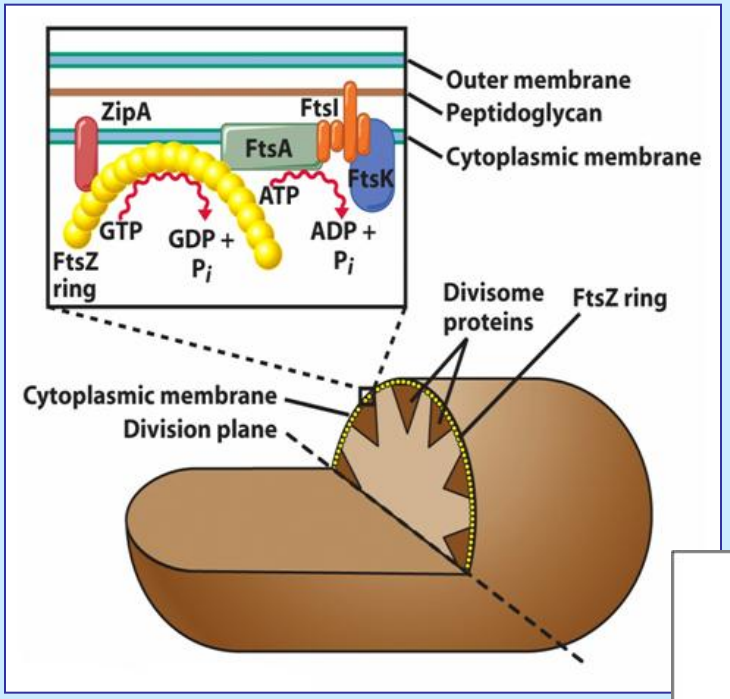


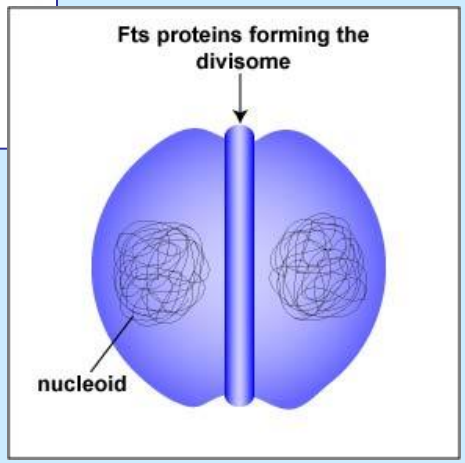
La crescita microbica

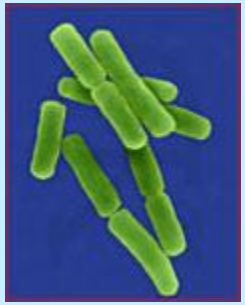



MODALITA' DI CRESCITA

DIVISOME PROTEINS





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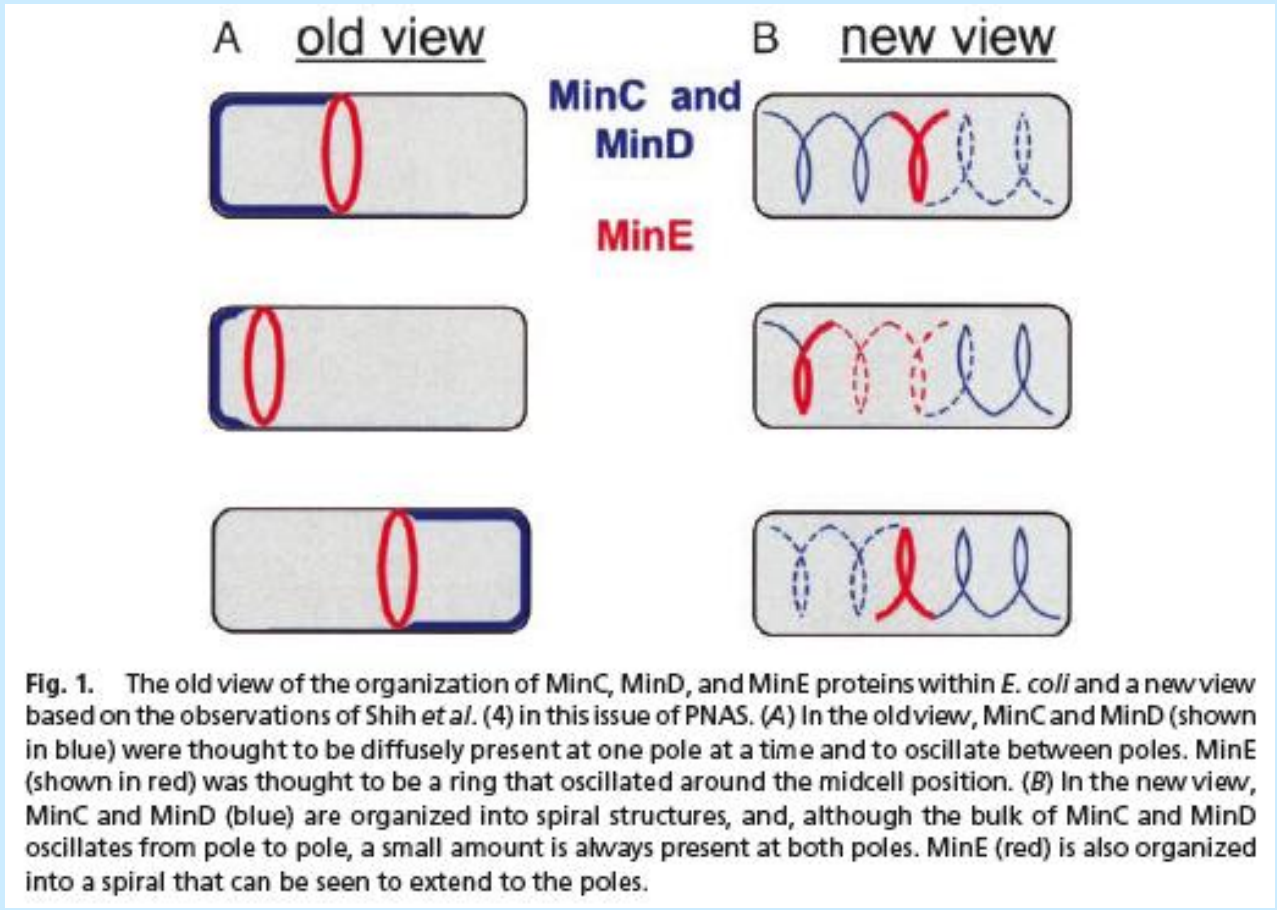
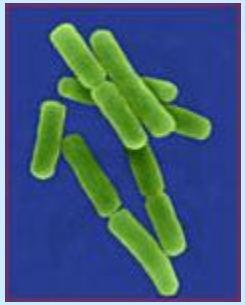
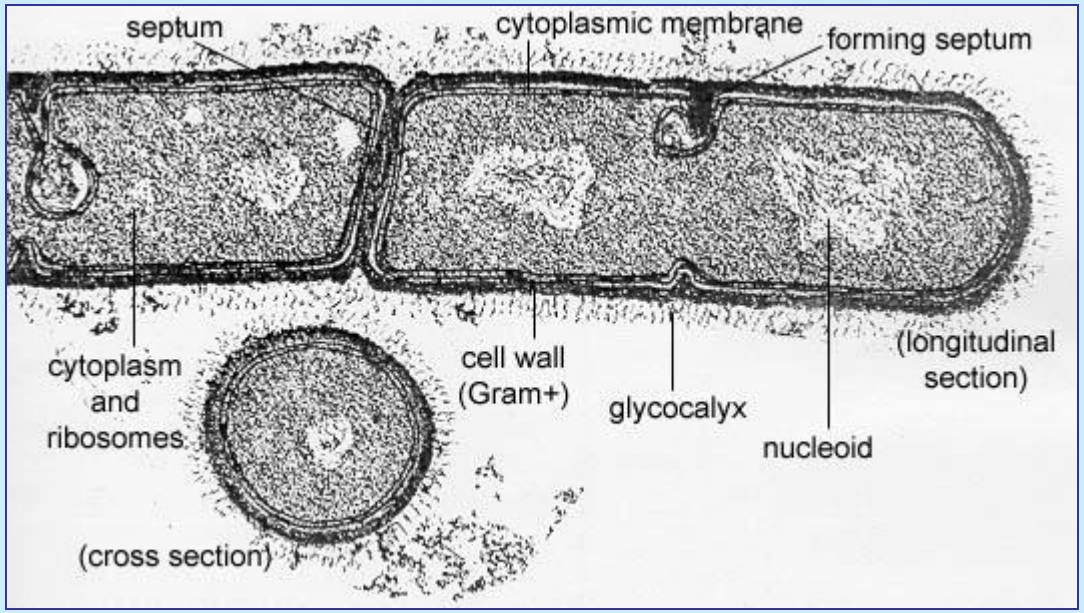


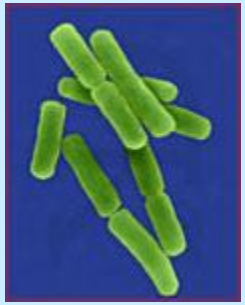
Fig. 1. The old view of the organization of MinC, MinD, and MinE proteins within *E. coli* and a new view based on the observations of Shih *et al*. (4) in this issue of PNAS. (*A*) In the old view, MinC and MinD (shown in blue) were thought to be diffusely present at one pole at a time and to oscillate between poles. MinE (shown in red) was thought to be a ring that oscillated around the midcell position. (*B*) In the new view, MinC and MinD (blue) are organized into spiral structures, and, although the bulk of MinC and MinD oscillates from pole to pole, a small amount is always present at both poles. MinE (red) is also organized into a spiral that can be seen to extend to the poles.



MODALITA' DI CRESCITA

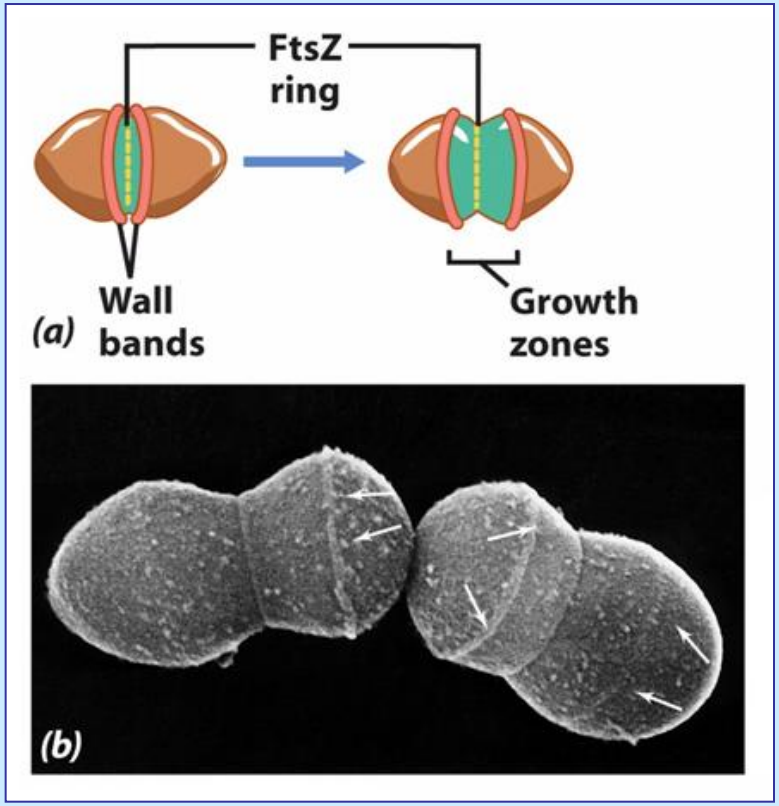
Scissione binaria di un batterio Gram positivo

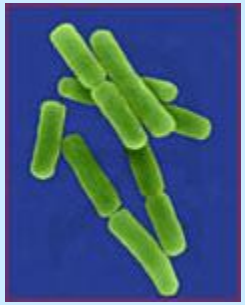




MODALITA' DI CRESCITA

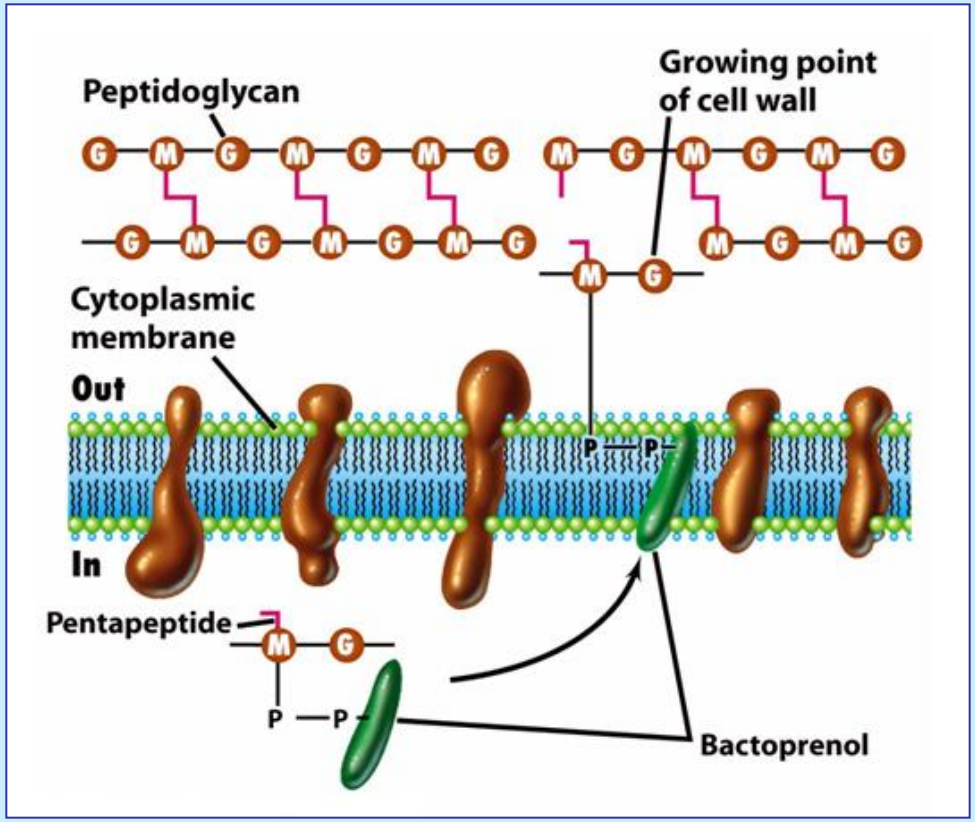
SINTESI DELLA PARETE CELLULARE IN UN BATTERIO Gram $^{+}$

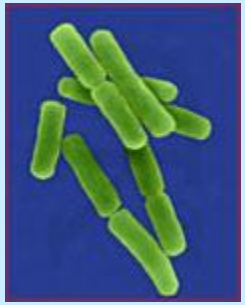




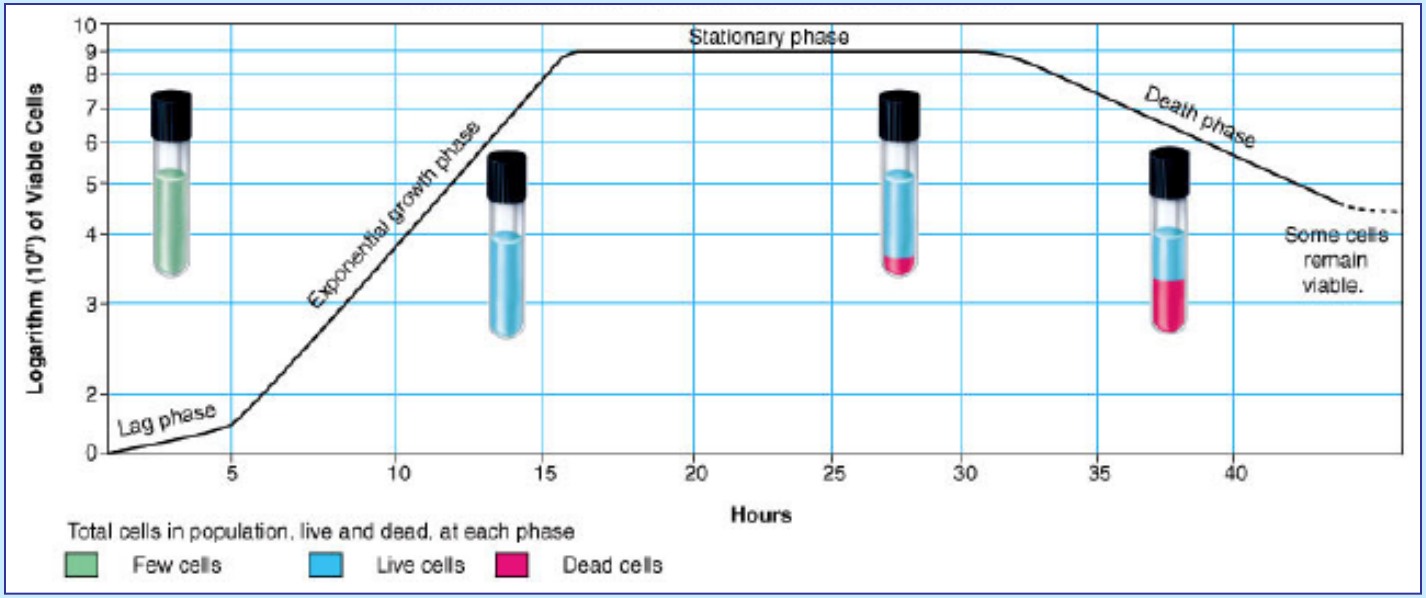
MODALITA' DI CRESCITA

SINTESI DEL PEPTIDOGLICANO

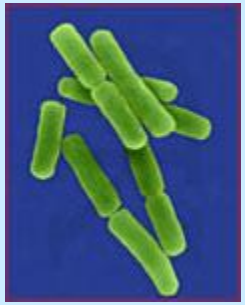




MODALITA' DI CRESCITA



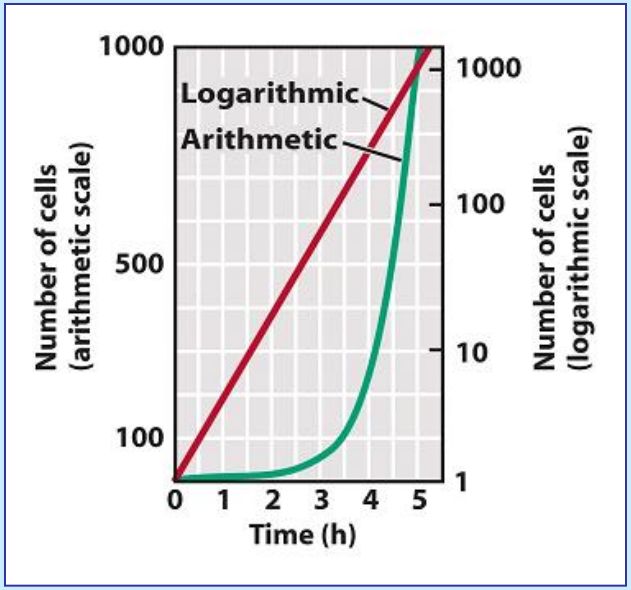
La crescita microbica



MODALITA' DI CRESCITA

IL TASSO DI CRESCITA DI UNA COLTURA BATTERICA

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 (2^8)
0.5	2	4.5	512 (2^9)
1	4	5	1,024 (2^{10})
1.5	8	5.5	2,048 (2^{11})
2	16	6	4,096 (2^{12})
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 (2^{19})



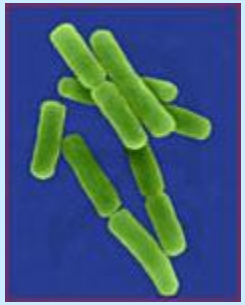
Equation for calculating population size over time:

$$N_f = (N_i)2^n$$

N_f is total number of cells in the population.

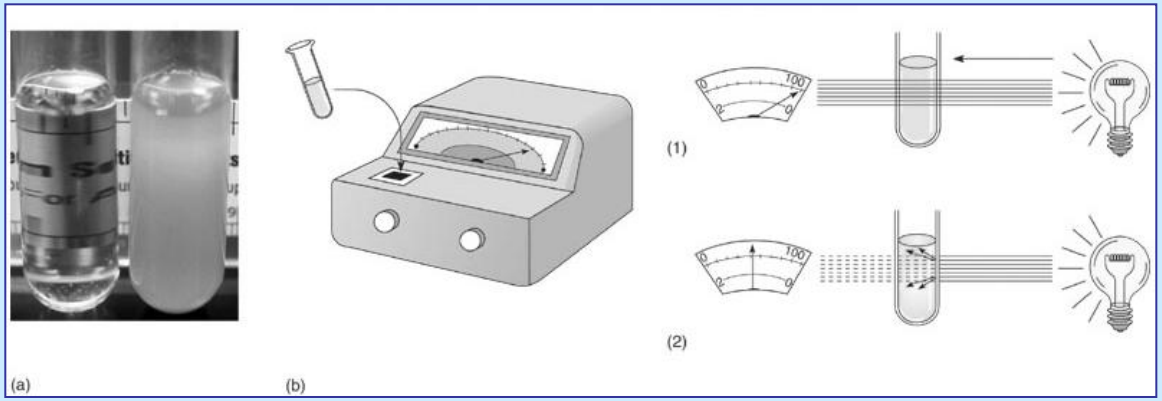
N_i is starting number of cells.

Exponent *n* denotes the number of generations

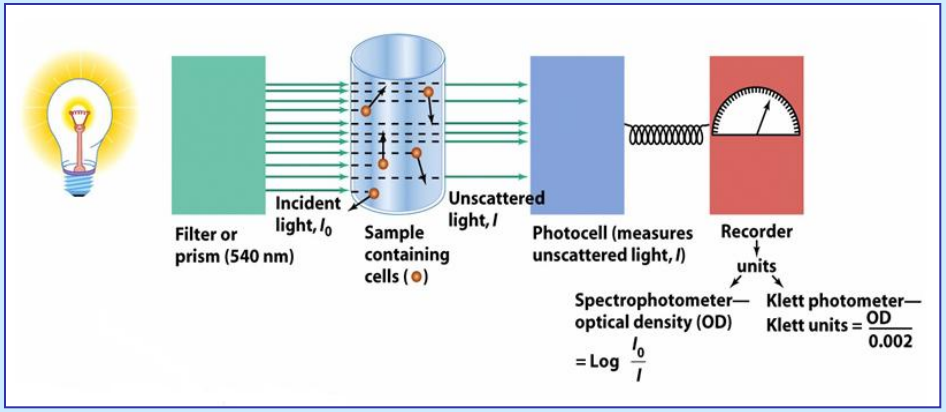


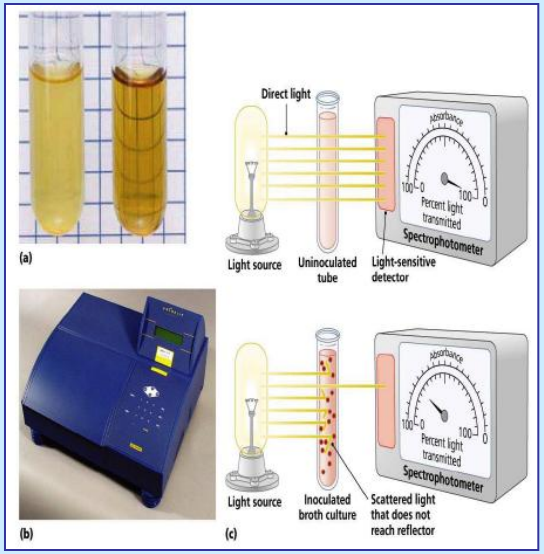
Conta delle cellule batteriche mediante turbidimetro (nefelometro)

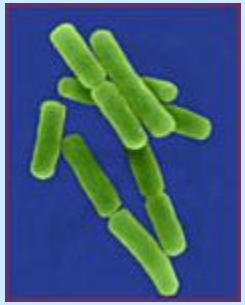
(OD = optical density; assorbanza espressa in NTU = nephelometric turbidity units)



STIMA DELLA CRESCITA



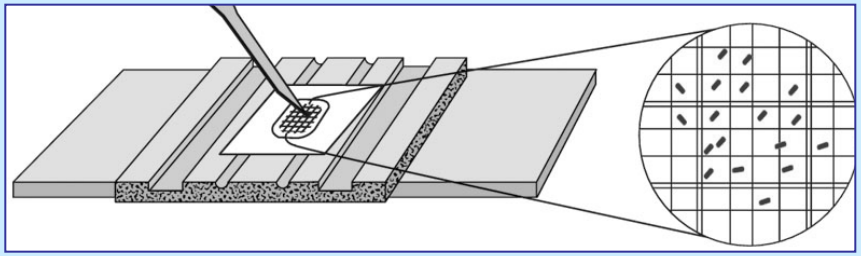


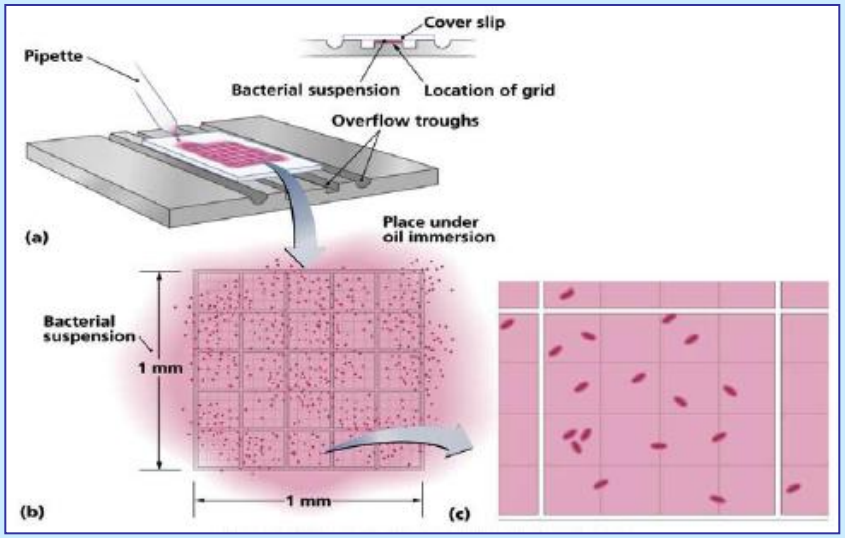


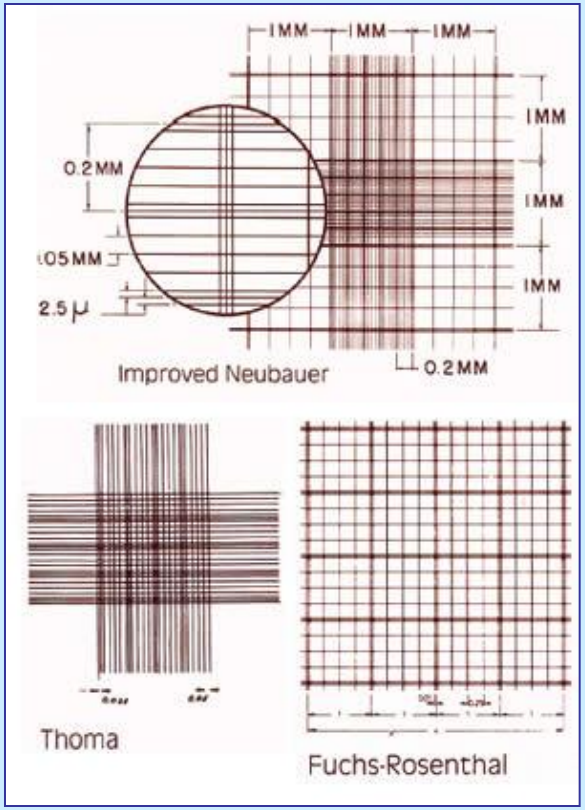
STIMA DELLA CRESCITA

Conta delle cellule batteriche mediante ematocitometro

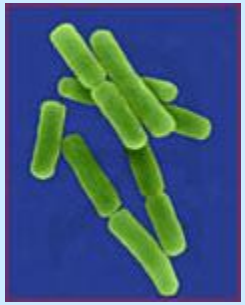
(*es.* Camera di Thoma modificata da Helber:
profondità 0.02 mm, superficie del quadrato centrale 0.0025 mm^2)





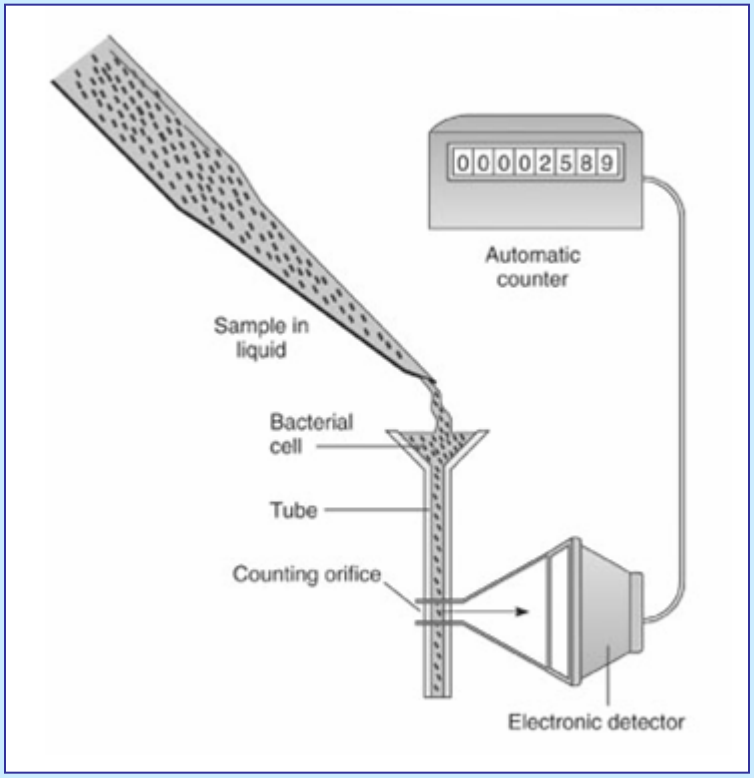


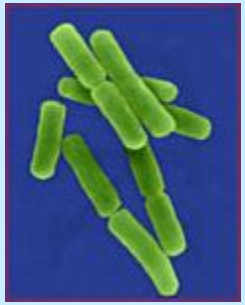
La crescita microbica



STIMA DELLA CRESCITA

Conta batterica mediante lettore automatico

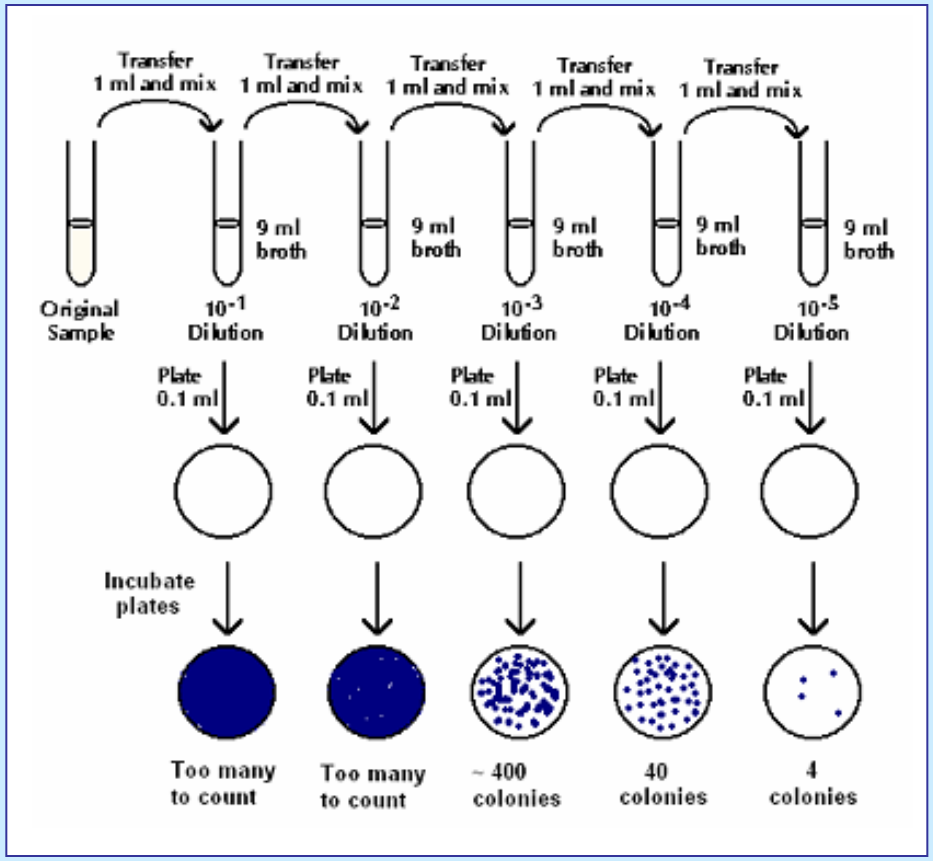




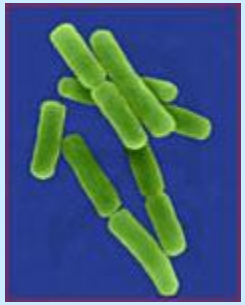
Conta microbica diretta
mediante il metodo del piastraggio delle sospensioni/diluizioni seriali

$$\frac{\text{CFU}}{\text{ml}} \text{ in original sample} = \frac{\text{\# colonies counted}}{(\text{dilution factor})(\text{volume plated, in ml})}$$

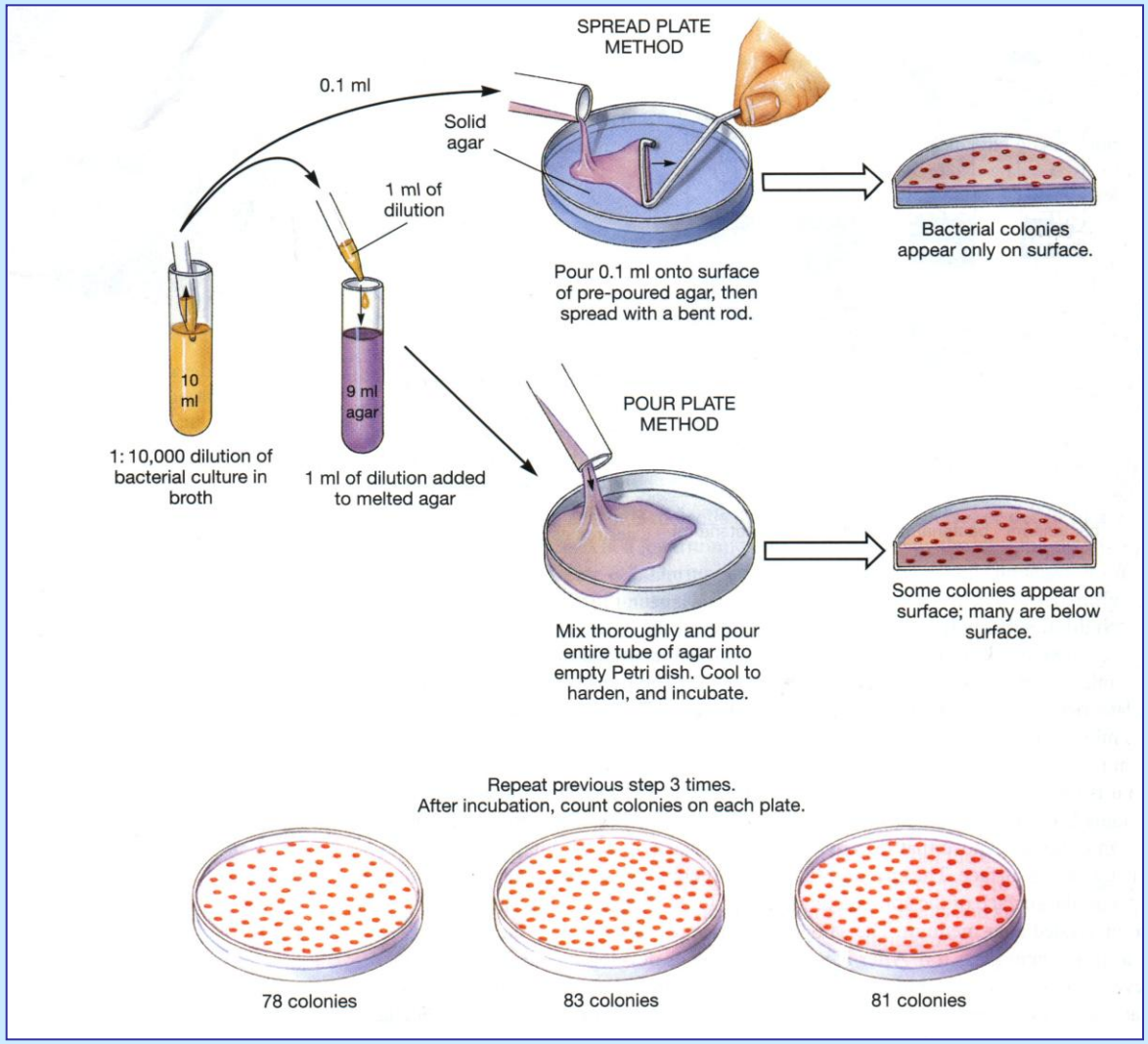
(CFU = colony forming units)



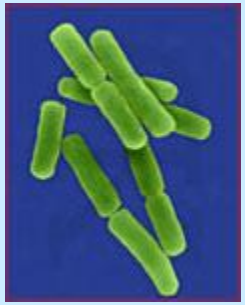
STIMA DELLA CRESCITA



CONTA MICROBICA SU PIASTRA

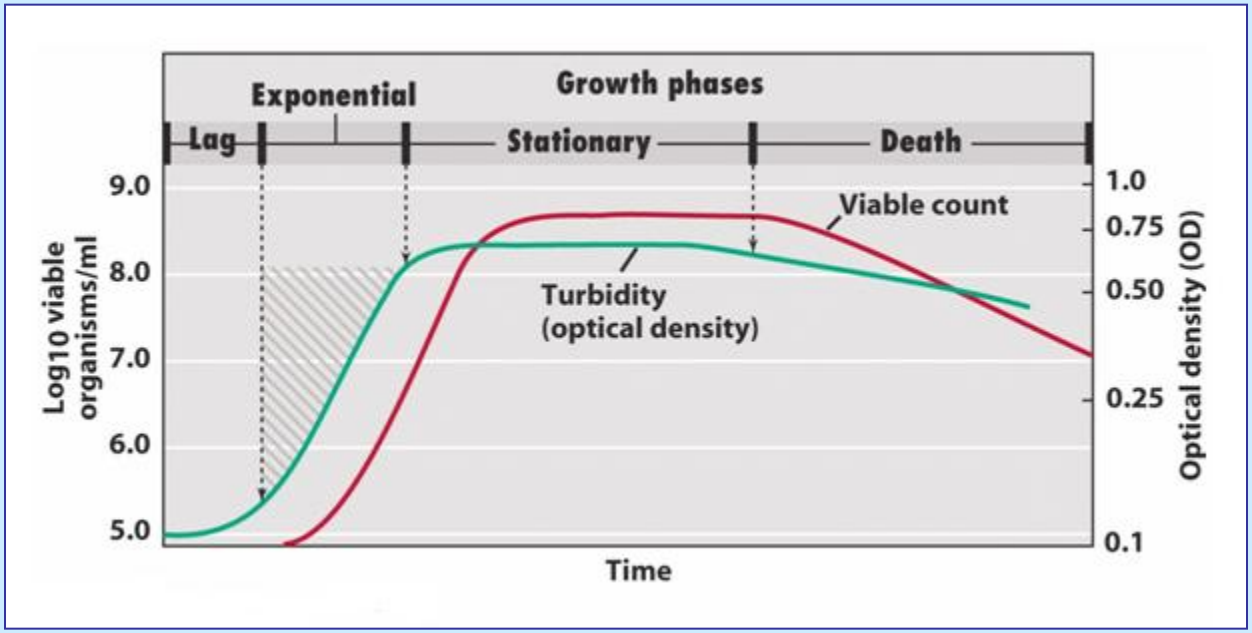


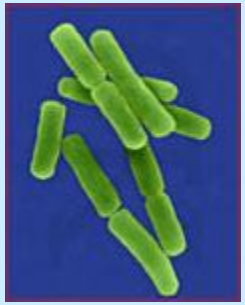
STIMA DELLA CRESCITA




STIMA DELLA CRESCITA

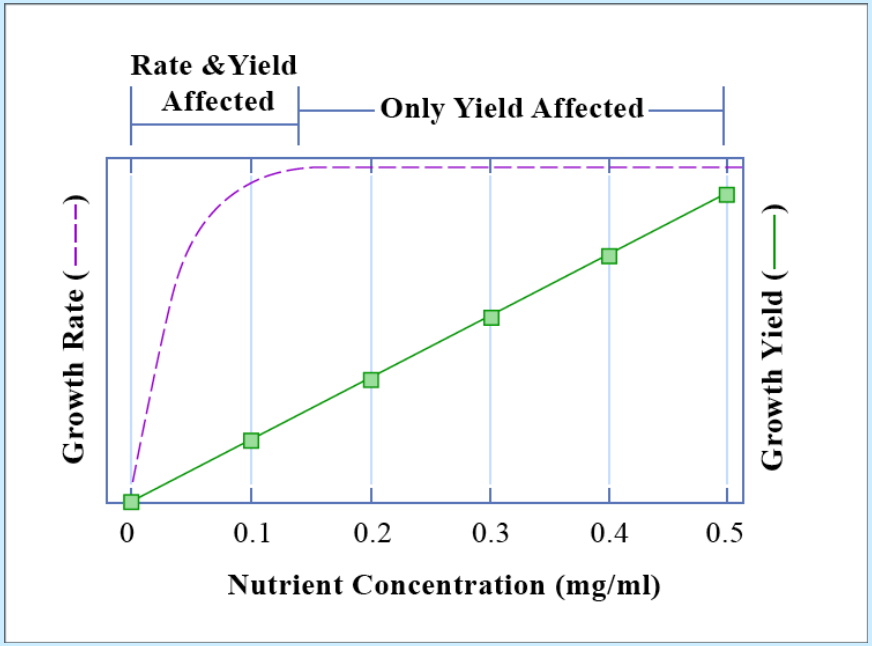
Raffronto della stima della crescita microbica mediante determinazione della densità ottica (nefelometria) e conta diretta su piastra

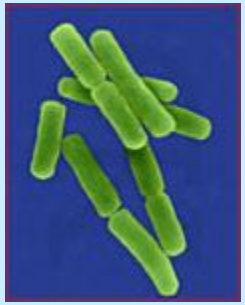




LA CRESCITA IN CONTINUO

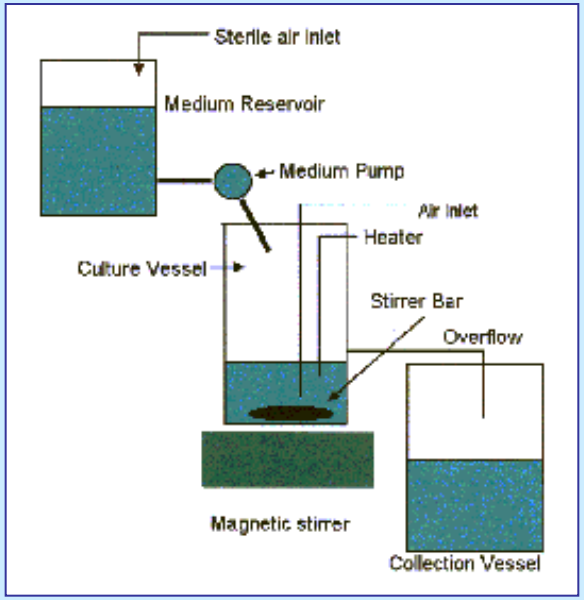
Effetto deli nutrienti sulla crescita microbica in coltura discontinua (batch culture)

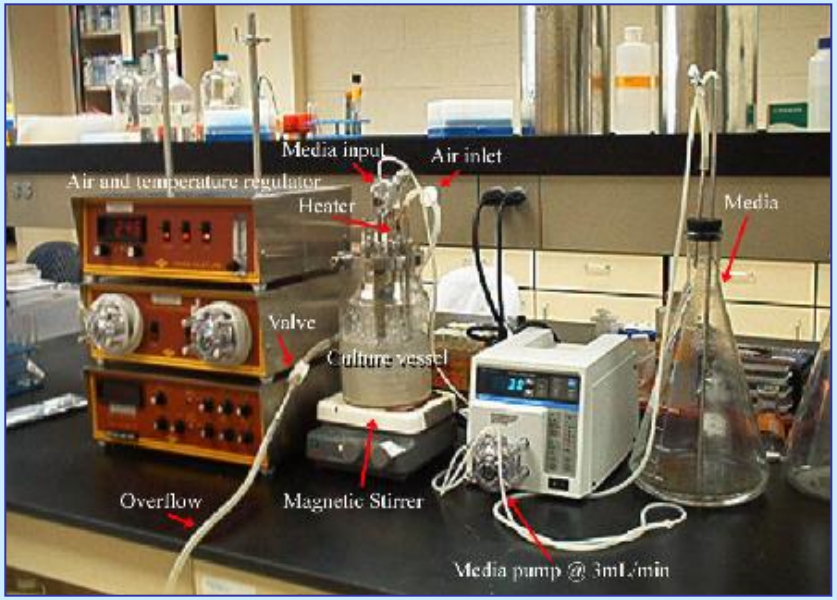


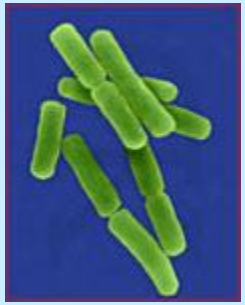



LA CRESCITA IN CONTINUO

IL CHEMOSTATO

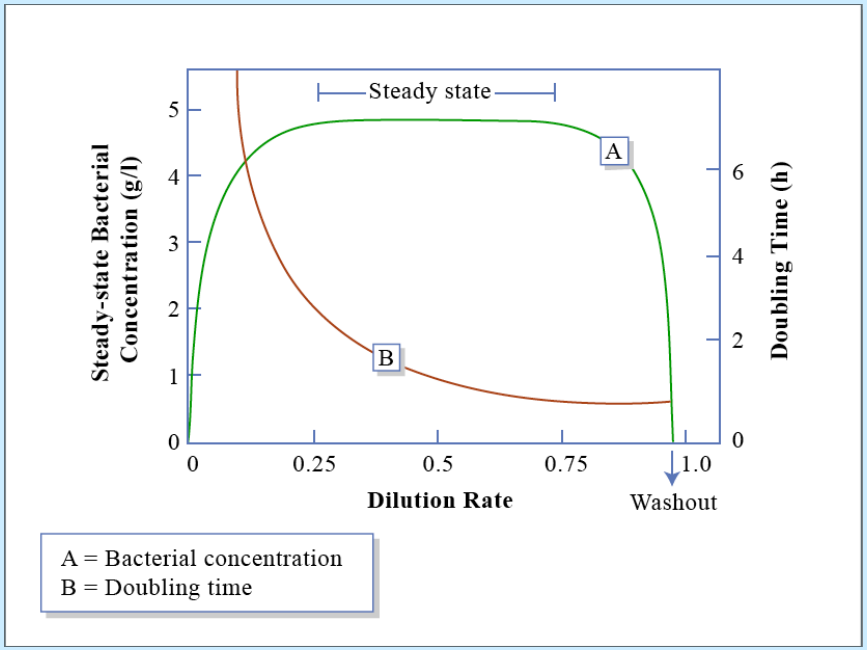


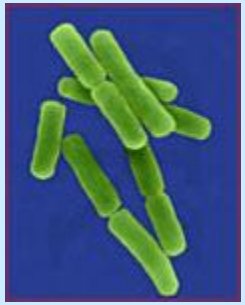





LA CRESCITA IN CONTINUO

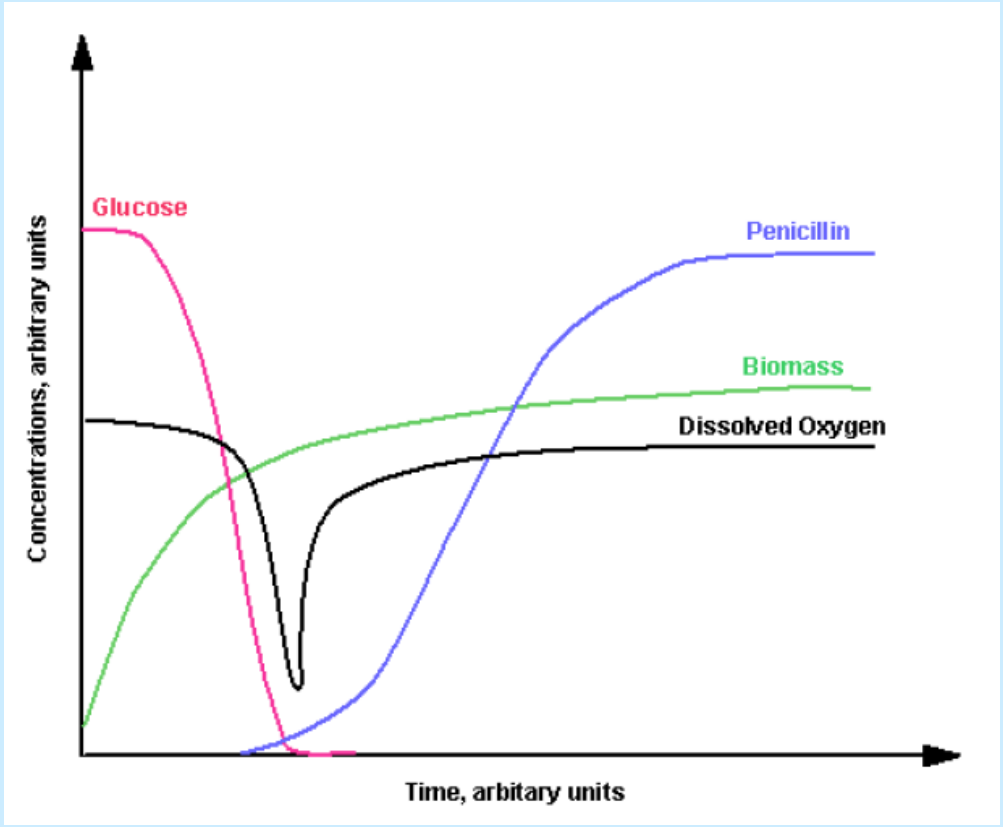
Caratteristiche dello stato stazionario in un chemostato

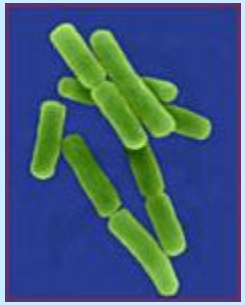




TROFOFASE / IDIOFASE

Crescita microbica con produzione di metaboliti secondari

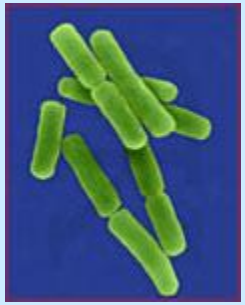




FATTORI CHE INFLUENZANO LA CRESCITA

What influences microbial growth?

- Nutrition
- Environment




FATTORI CHE INFLUENZANO LA CRESCITA

Nutrition

• Bacterial needs:

MAJOR ELEMENTS (C, H, O, N, P, S, K, Mg, Na, Ca, Fe)

• Essential elements that make up cell parts and macromolecules

• Source of C defines groups of bacteria:
– **Heterotrophs**
– **Autotrophs**

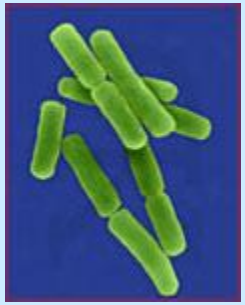
• Other elements can be derived from inorganic salts

TRACE ELEMENTS (B, Cr, Co, Cu, Mn, Mo, Ni, Se, W, V, Zn)

• Elements needed in minute amounts, usually for enzyme functioning

GROWTH FACTORS (vitamins, amino acids, etc.)

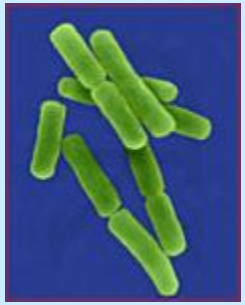
Organic compounds required in small amounts that microbes can not synthesised by themselves




FATTORI CHE INFLUENZANO LA CRESCITA

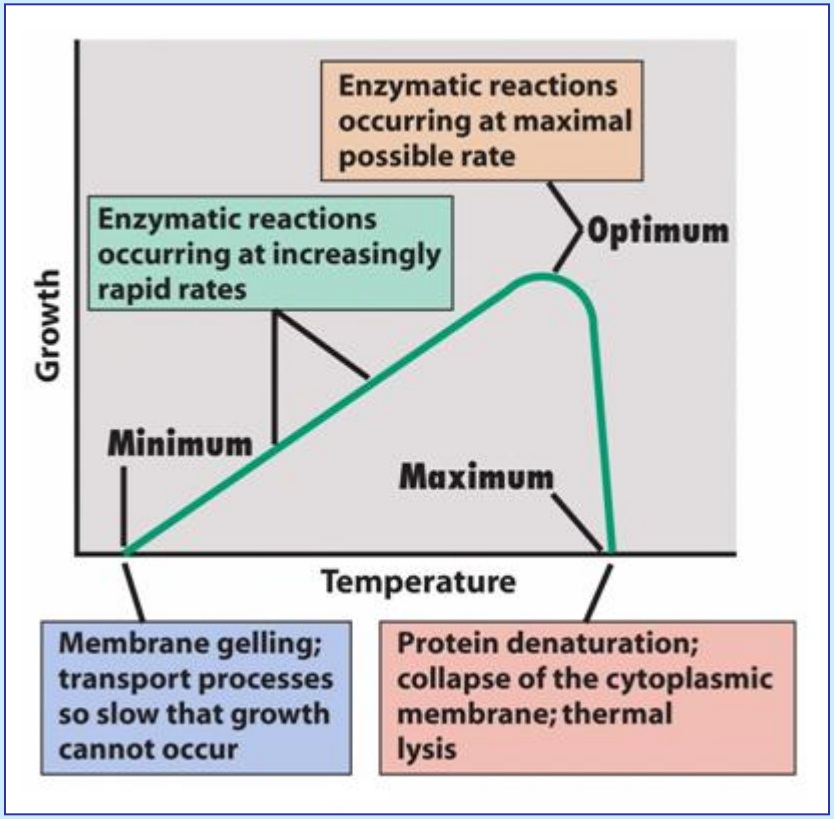
Environment

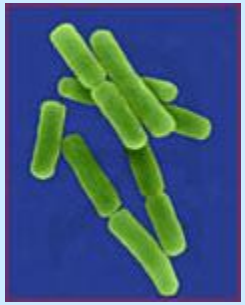
- Temperature
- pH
- Oxygen concentration
- Salinity




FATTORI CHE INFLUENZANO LA CRESCITA

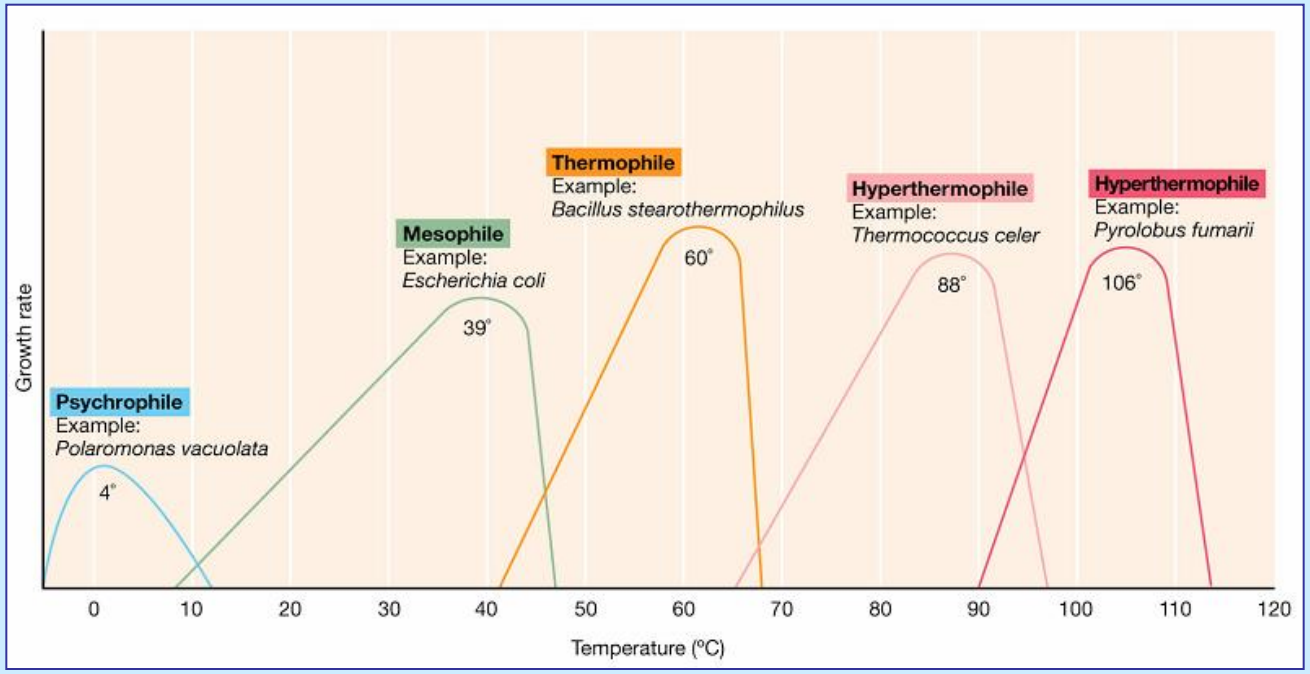
Effetto della temperatura sul tasso di crescita e conseguenze a livello molecolare per la cellula microbica



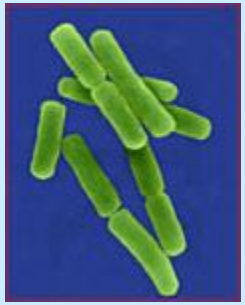


FATTORI CHE INFLUENZANO LA CRESCITA

OPTIMA di temperatura per i microorganismi



FATTORI CHE INFLUENZANO LA CRESCITA

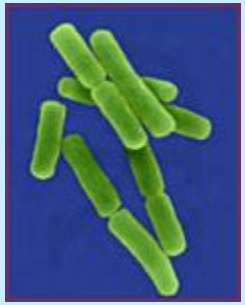


FATTORI CHE INFLUENZANO LA CRESCITA

Table 6.1 Presently known upper temperature limits for growth of living organisms

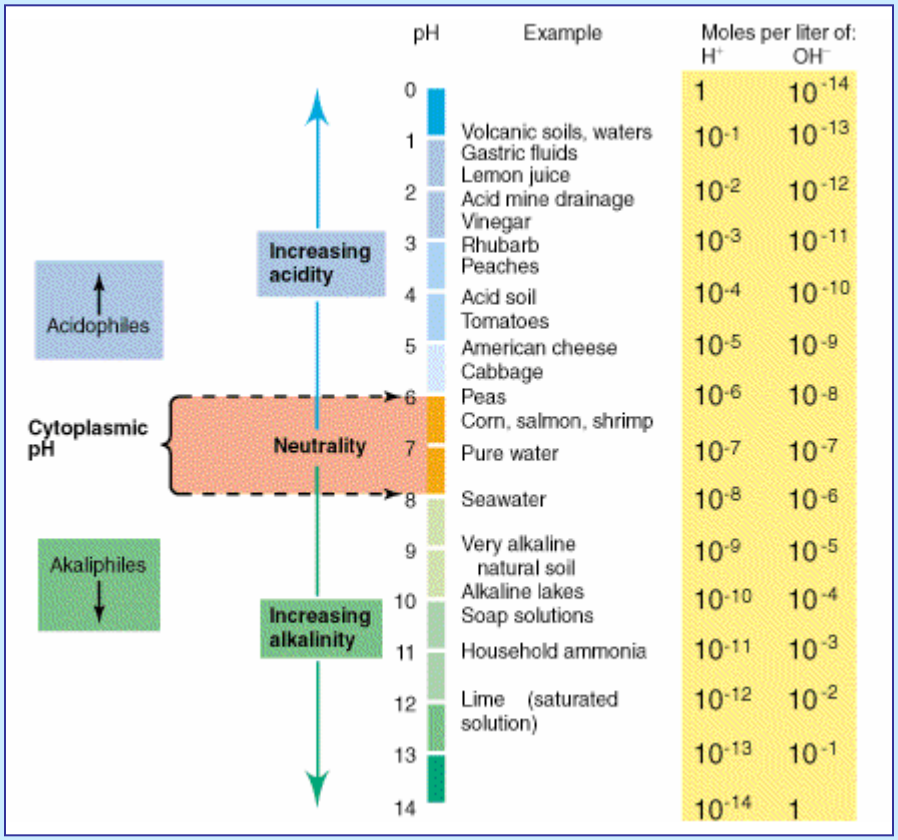
Group	Upper temperature limits (°C)
Animals	
Fish and other aquatic vertebrates	38
Insects	45–50
Ostracods (crustaceans)	49–50
Plants	
Vascular plants	45
Mosses	50
Eukaryotic microorganisms	
Protozoa	56
Algae	55–60
Fungi	60–62
Prokaryotes	
Bacteria	
Cyanobacteria	70–74
Anoxygenic phototrophs	70–73
Chemoorganotrophic/chemolithotrophic *Bacteria*	95
Archaea	
Chemoorganotrophic/chemolithotrophic *Archaea*	113[a]

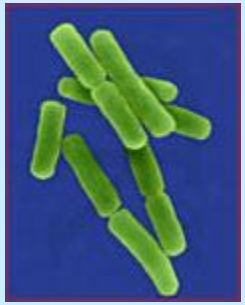
[a] The upper temperature limit for growth of the organism *Pyrolobus fumarii*. Related species of *Pyrodictium* may be able to grow up to as high as 121°C.



FATTORI CHE INFLUENZANO LA CRESCITA

OPTIMA di pH per i microorganismi





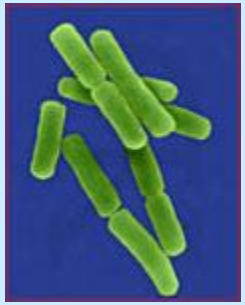

FATTORI CHE INFLUENZANO LA CRESCITA

Table 6.4 Oxygen relationships of microorganisms

Group	Relationship to O_2	Type of metabolism	Example[a]	Habitat[b]
Aerobes				
Obligate	Required	Aerobic respiration	*Micrococcus luteus* (B)	Skin, dust
Facultative	Not required, but growth better with O_2	Aerobic respiration, anaerobic respiration, fermentation	*Escherichia coli* (B)	Mammalian large intestine
Microaerophilic	Required but at levels lower than atmospheric	Aerobic respiration	*Spirillum volutans* (B)	Lake water
Anaerobes				
Aerotolerant	Not required, and growth no better when O_2 present	Fermentation	*Streptococcus pyogenes* (B)	Upper respiratory tract
Obligate	Harmful or lethal	Fermentation or anaerobic respiration	*Methanobacterium formicicum* (A)	Sewage sludge digestors, anoxic lake sediments

[a] Letters in parentheses indicate phylogenetic status (B, *Bacteria*; A, *Archaea*). Representatives of either domain of prokaryotes are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.

[b] Listed are typical habitats of the example organism.



Microorganismi e O_2

L'ossigeno molecolare è un gas che risulta estremamente tossico nei confronti dei sistemi biologici i quali non abbiano sviluppato meccanismi di difesa nei confronti dello stesso.

Bisogna ricordare che l'origine e lo sviluppo delle prime forme di vita sulla Terra è avvenuto in condizioni strettamente anaerobiche. Solo in un secondo momento, con l'avvento della fotosintesi ossigenica, l'atmosfera si è progressivamente arricchita di O_2. Ciò ha spinto l'evoluzione nel senso di organismi capaci di sopravvivere in presenza di questo gas, con indubbi vantaggi sul piano energetico-metabolico per i medesimi.

Al tempo stesso, lo sfruttamento di questi vantaggi ha richiesto l'adozione di adeguati sistemi di difesa contro gli intermedi (veri responsabili della tossicità, dal momento che ossidano molecole biologiche chiave come i lipidi di membrana!) quali lo ione superossido (O_2^-), il perossido di idrogeno (H_2O_2) ed il radicale idrossile ($HO\cdot$) che si formano allorquando l'O_2 viene ridotto – nella respirazione aerobica - ad H_2O.

La difesa nei confronti della tossicità legata alla presenza di ossigeno molecolare include meccanismi che limitino il più possibile la produzione delle specie chimiche intermedie oppure eliminino questi intermedi allorché inevitabilmente prodotti.

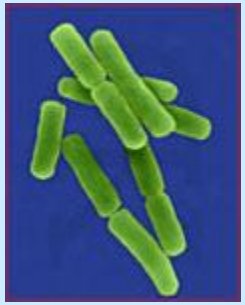
I microorganismi in grado di fronteggiare la presenza di O_2 devono quindi disporre di sistemi enzimatici quali ***a)*** **superossido-dismutasi** che catalizza la seguente reazione:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

e ***b)*** **catalasi e perossidasi** che invece catalizzano la reazione seguente:

$$H_2O_2 + H_2O_2 \text{-----> } 2H_2O + O_2$$

(la catalasi non richiede NADH, mentre la perossidasi sì)



SULLA BASE DELLA RISPOSTA ALLA PRESENZA DI O_2, SI DISTINGUONO TRE CATEGORIE DI MICROORGANISMI

Categoria 1

AEROBI

Usano O_2 e posseggono gli enzimi per detossificare gli intermedi

Categoria 2

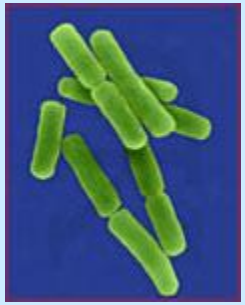
ANAEROBI OSSIGENO-TOLLERANTI

Non usano l'O_2 ma sono in grado di detossificare gli intermedi

Categoria 3

ANAEROBI OBBLIGATI

Non usano l'O_2 e sono incapaci di detossificare le specie chimiche intermedie



Classificazione dei microorganismi sulla base della richiesta di ossigeno

Categoria 1

A) **AEROBI OBBLIGATI** — muoiono in assenza di ossigeno molecolare (O_2); non hanno capacità di metabolismo fermentativo

B) **MICROAEROFILI** — crescono in maniera ottimale a concentrazioni di O_2 più basse (5-10 % fino a 15%) rispetto al livello di ossigeno atmosferico (20 -21%)

C) **CAPNOFILI** — crescono in presenza di concentrazioni di O_2 ridotte (intorno al 15%) ed elevate concentrazioni di CO_2 (intorno al 5%)

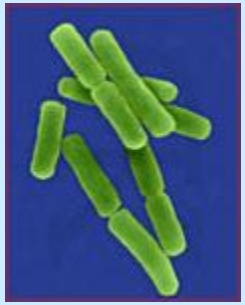
D) **ANAEROBI FACOLTATIVI** — crescono meglio in presenza di ossigeno; se assente, possono fermentare o adottare la respirazione anaerobica: in tali condizioni crescono più lentamente

Categoria 2

E) **ANAEROBI AEROTOLLERANTI** — fermentano sia in condizioni aerobiche che anaerobiche: l'O_2 non li uccide (*es*. *Streptococcus lactis*)

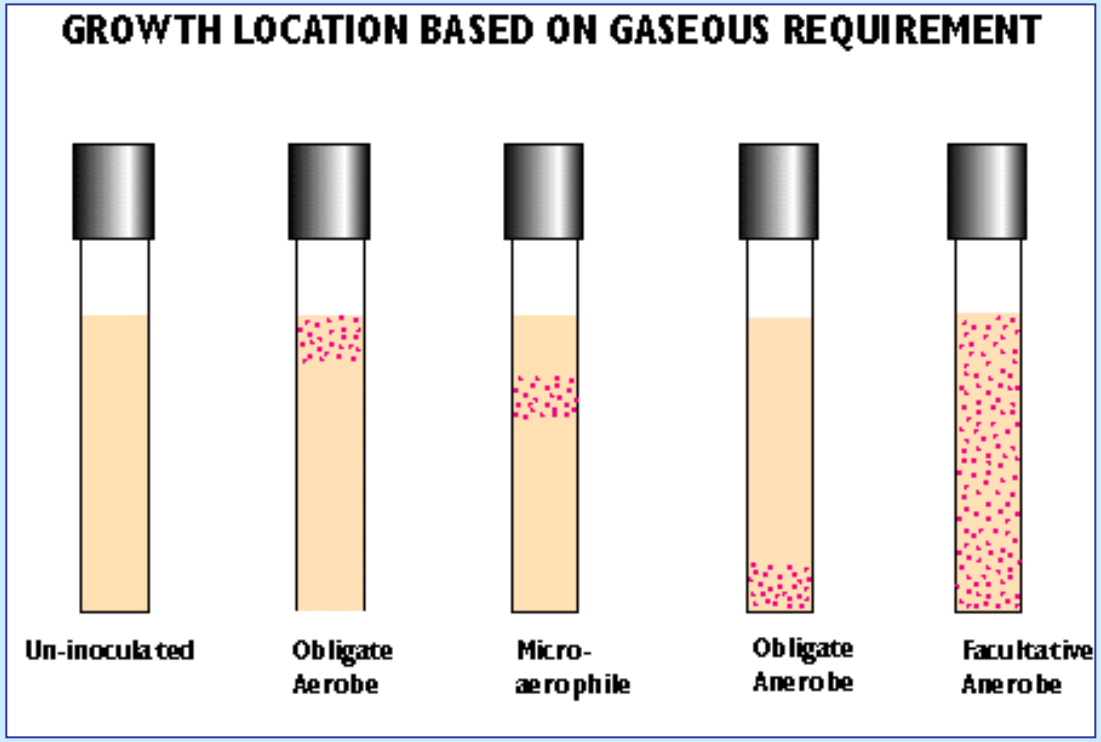
Categoria 3

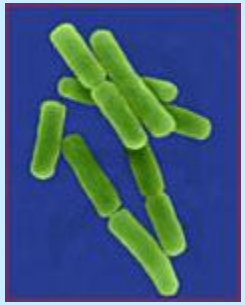
F) **ANAEROBI OBBLIGATI** — sono uccisi dalla presenza di ossigeno molecolare; possono avere metabolismo fermentativo ovvero adottare la respirazione anaerobica



FATTORI CHE INFLUENZANO LA CRESCITA

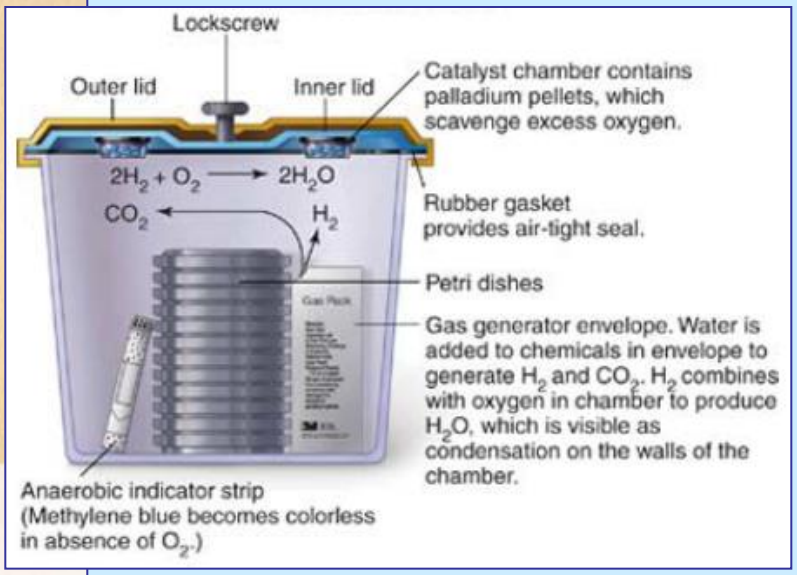
CRESCITA BATTERICA SULLA BASE DELLA RICHIESTA DI OSSIGENO

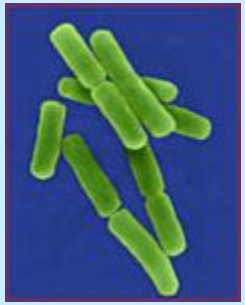





FATTORI CHE INFLUENZANO LA CRESCITA

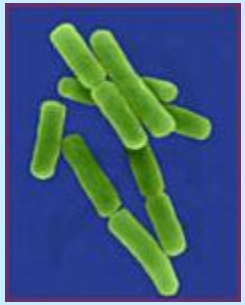
Sistema Gas-Pack per colture microbiche in anaerobiosi






FATTORI CHE INFLUENZANO LA CRESCITA

Anaerobic glove box




FATTORI CHE INFLUENZANO LA CRESCITA

Toxic Forms of Oxygen and Detoxifying Enzymes

Hydrogen peroxide

***(a)* Catalase:**

$$H_2O_2 + H_2O_2 \rightarrow 2\,H_2O + O_2$$

***(b)* Peroxidase:**

$$H_2O_2 + NADH + H^+ \rightarrow 2\,H_2O + NAD^+$$

Superoxide

***(c)* Superoxide dismutase:**

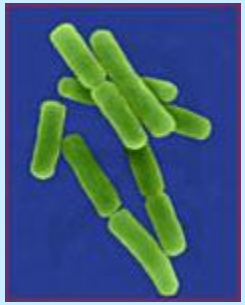
$$O_2^- + O_2^- + 2\,H^+ \rightarrow H_2O_2 + O_2$$

***(d)* Superoxide dismutase/catalase in combination:**

$$4\,O_2^- + 4\,H^+ \rightarrow 2\,H_2O + 3\,O_2$$

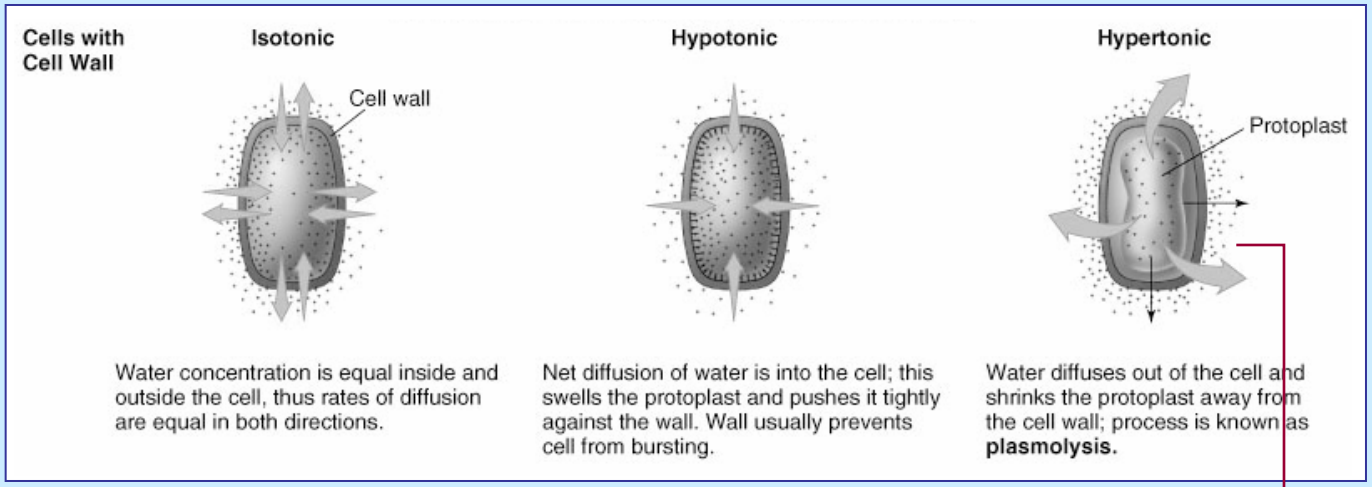
***(e)* Superoxide reductase:**

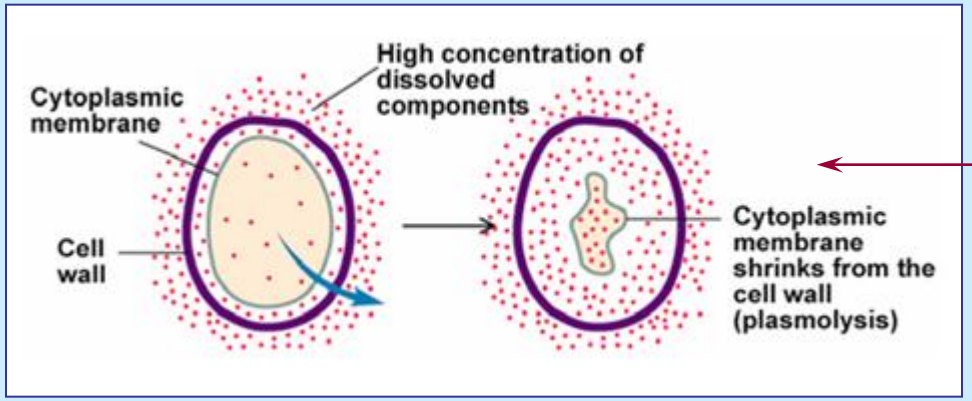
$$O_2^- + 2\,H^+ + \text{cyt } c_{reduced} \rightarrow H_2O_2 + \text{cyt } c_{oxidized}$$



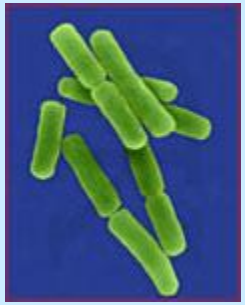

FATTORI CHE INFLUENZANO LA CRESCITA

BATTERI ED OSMOLARITA'



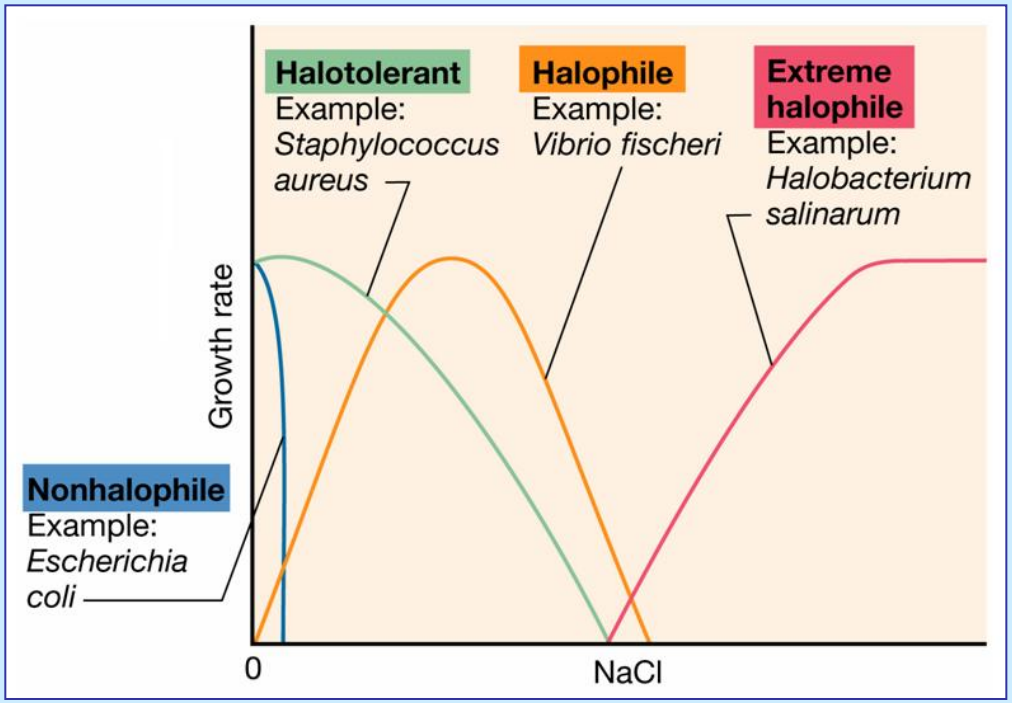


- Hypotonic
- Isotonic (0.85% NaCl)
- Hypertonic



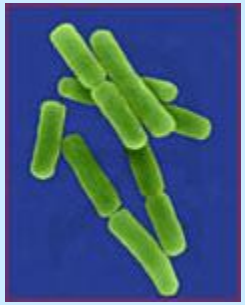
FATTORI CHE INFLUENZANO LA CRESCITA

BATTERI E SALINITA' DEL MEZZO DI CRESCITA



Halophiles require elevated salt concentrations to grow; often require 0.2 M ionic strength or greater and may some may grow at 1 M or greater; example, *Halobacterium.*

Osmotolerant (halotolerant) organisms grow over a wide range of salt concentrations or ionic strengths; for example, *Staphylococcus aureus.*



FATTORI CHE INFLUENZANO LA CRESCITA

MOISTURE/WATER ACTIVITY

Water activity is a measure of how efficiently the water present can take part in a chemical (physical) reaction. If half the water is so tightly bound to a protein molecule that it could not take part in a hydrolysis reaction the overall water activity would be reduced. Water activity (a_w) is defined as

$$a_w = p/p_o$$

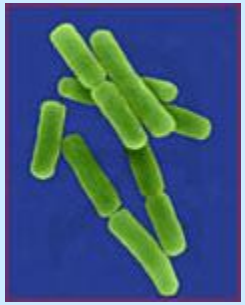
where p and p_o are respectively the partial pressure of water at suface of the matrix of interest and that above pure water under identical environmental conditions respectively (*i.e.* temperature).

Water activity is sometimes defined as "free", "unbound", or "available water" in a system. The values of a_w range from 0.0 (water completely bound) to 1.0 (water completely available).

Equilibrium relative humidity (ERH) describes the amount of water in the air surrounding the matrix of reference when in equilibrium with water in the matrix. It is expressed as follows:

$a_w = p/p_o = ERH\ (\%) / 100$ thus, simply $RH\ (\%) = 100 \times a_w$

Water activity (a_w) or equilibrium relative humidity (ERH) quantifies the active part of the moisture content or 'free water' as opposed to the total moisture content which also includes 'bound water'.

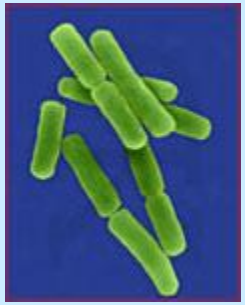



FATTORI CHE INFLUENZANO LA CRESCITA

Table 6.2 Water activity of several substances

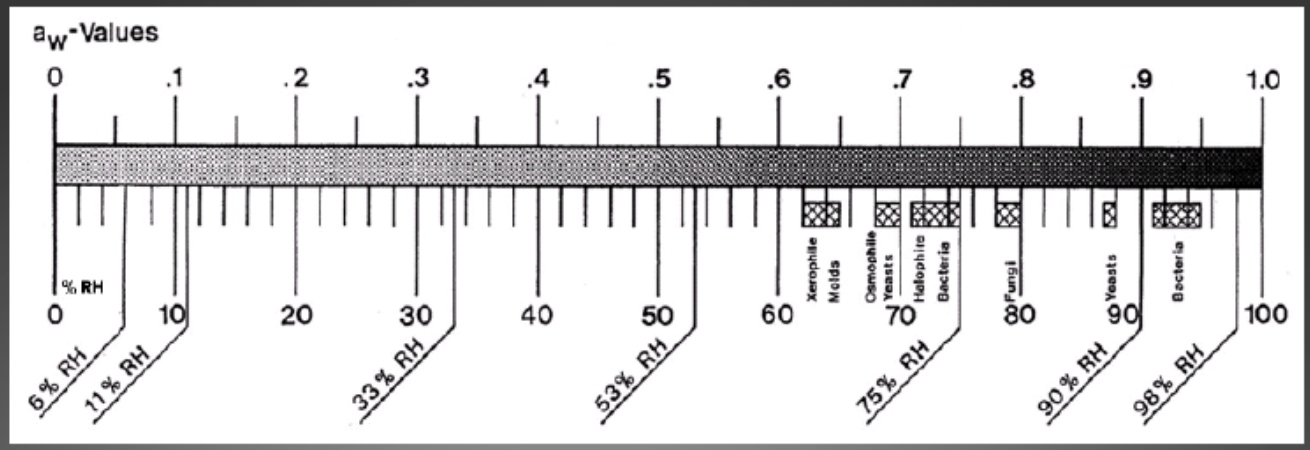
Water activity (a_w)	Material	Example organisms[a]
1.000	Pure water	*Caulobacter, Spirillum*
0.995	Human blood	*Streptococcus, Escherichia*
0.980	Seawater	*Pseudomonas, Vibrio*
0.950	Bread	Most gram-positive rods
0.900	Maple syrup, ham	Gram-positive cocci such as *Staphylococcus*
0.850	Salami	*Saccharomyces rouxii* (yeast)
0.800	Fruit cake, jams	*Saccharomyces bailii, Penicillium* (fungus)
0.750	Salt lakes, salted fish	*Halobacterium, Halococcus*
0.700	Cereals, candy, dried fruit	*Xeromyces bisporus* and other xerophilic fungi

[a] Selected examples of prokaryotes or fungi capable of growth in culture media adjusted to the stated water activity.

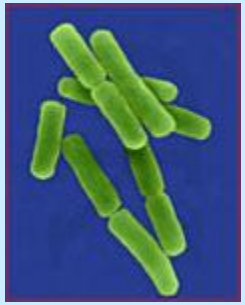



FATTORI CHE INFLUENZANO LA CRESCITA

MOISTURE/WATER ACTIVITY



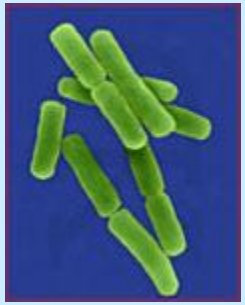
Growth limits of different microorganisms with respect to the water activity/relative humidity ranges.




FATTORI CHE INFLUENZANO LA CRESCITA

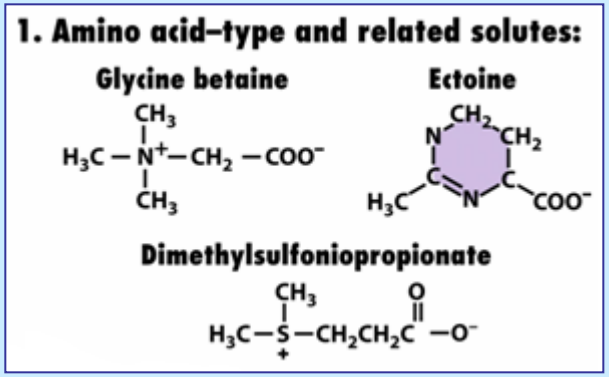
Table 6.3 Compatible solutes of microorganisms

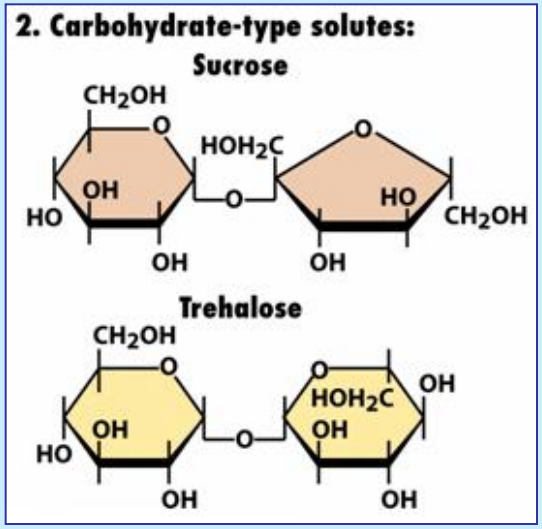
Organism	Major solute(s) accumulated	Minimum a_w for growth
Bacteria, nonphototrophic	Glycine betaine, proline (mainly gram-positive), glutamate (mainly gram-negative)	0.97–0.90
Freshwater cyanobacteria	Sucrose, trehalose	0.98
Marine cyanobacteria	α-Glucosylglycerol	0.92
Marine algae	Mannitol, various glycosides, proline, dimethylsulfoniopropionate	0.92
Salt lake cyanobacteria	Glycine betaine	0.90–0.75
Halophilic anoxygenic phototrophic *Bacteria* (*Ectothiorhodospira*/*Halorhodospira* and *Rhodovibrio* species)	Glycine betaine, ectoine, trehalose	0.90–0.75
Extremely halophilic *Archaea* (for example, *Halobacterium*) and some *Bacteria* (for example, *Haloanaerobium*)	KCl	0.75
Dunaliella (halophilic green alga)	Glycerol	0.75
Xerophilic yeasts	Glycerol	0.83–0.62
Xerophilic filamentous fungi	Glycerol	0.72–0.61

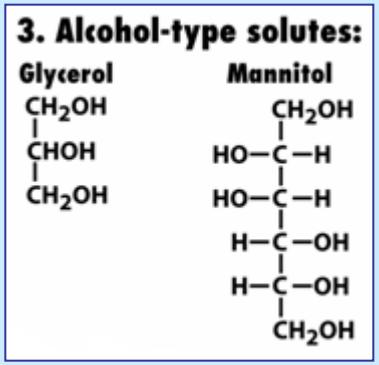



FATTORI CHE INFLUENZANO LA CRESCITA

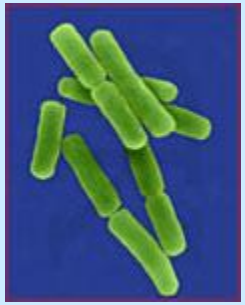
Struttura di alcuni soluti ricorrenti comunemente nei microorganismi







La crescita microbica

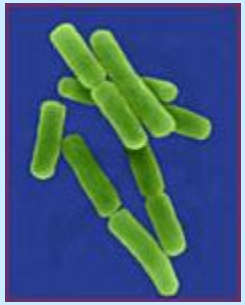


TERRENI DI CRESCITA

Esempio di mezzo colturale definito (defined medium)

A Defined Synthetic Medium for Growing *Proteus vulgaris*

Ingredient	Amount	Ingredient	Amount
Water	1 liter	K_2HPO_4	1 g
$MgSO_4 \cdot 7H_2O$	200 mg	$FeSO_4 \cdot 7H_2O$	10 mg
$CaCl_2$	10 mg	Glucose	5 g
NH_4Cl	1 g	Nicotinic acid	0.1 mg
Trace elements (Mn, Mo, Cu, Co, Zn as inorganic salts, known quantities of 0.02–0.5 mg each)			

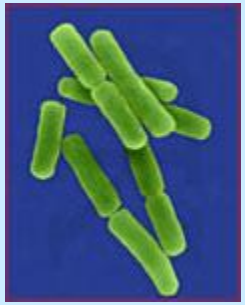



TERRENI DI CRESCITA

Esempio di mezzo colturale sintetico per la crescita di un microorganismo particolarmente esigente (fastidioso)

A Defined Synthetic Medium for Growing the Fastidious Bacterium *Leuconostoc mesenteroides*

Ingredient	Amount	Ingredient	Amount
Water	1 liter		
Energy Source			
Glucose	25 g		
Nitrogen Source			
NH_4Cl	3 g		
Minerals			
KH_2PO_4	600 mg	$FeSO_4 \cdot 7H_2O$	10 mg
K_2HPO_4	600 mg	$MnSO_4 \cdot 4H_2O$	20 mg
$MgSO_4 \cdot 7H_2O$	200 mg	NaCl	10 mg
Organic Acid			
Sodium acetate	20 g		
Amino Acids			
DL-α-Alanine	200 mg	L-Lysine · HCl	250 mg
L-Arginine	242 mg	DL-Methionine	100 mg
L-Asparagine	400 mg	DL-Phenylalanine	100 mg
L-Aspartic acid	100 mg	L-Proline	100 mg
L-Cysteine	50 mg	DL-Serine	50 mg
L-Glutamic acid	300 mg	DL-Threonine	200 mg
Glycine	100 mg	DL-Tryptophan	40 mg
L-Histidine · HCl	62 mg	L-Tyrosine	100 mg
DL-Isoleucine	250 mg	DL-Valine	250 mg
DL-Leucine	250 mg		
Purines and Pyrimidines			
Adenine sulfate · H_2O	10 mg	Uracil	10 mg
Guanine · HCl · $2H_2O$	10 mg	Xanthine · HCl	10 mg
Vitamins			
Thiamine · HCl	0.5 mg	Riboflavin	0.5 mg
Pyridoxine · HCl	1.0 mg	Nicotinic acid	1.0 mg
Pyridoxamine · HCl	0.3 mg	p-Aminobenzoic acid	0.1 mg
Pyridoxal · HCl	0.3 mg	Biotin	0.001 mg
Calcium pantothenate	0.5 mg	Folic acid	0.01 mg



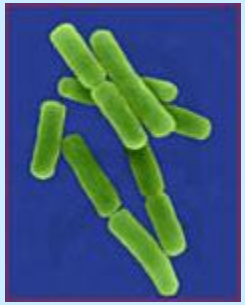
TERRENI DI CRESCITA

Esempio di mezzo colturale complesso (rich medium)

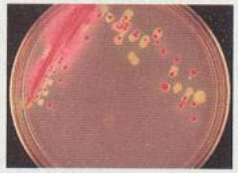
A Complex Medium Suitable for Many Heterotrophic Organisms

Nutrient Broth Ingredient	Amount
Water	1 liter
Peptone	5 g
Beef extract	3 g
NaCl	8 g
Solidified Medium	
Agar	15 g

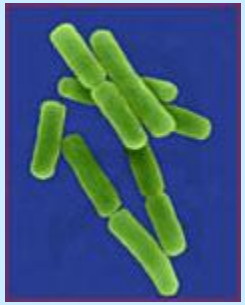
Above ingredients in amounts specified.



TERRENI SELETTIVI E MEZZI DIFFERENZIALI

Eosin methylene blue agar (EMB)		Gram-negative enterics (Enterobacteriaceae)	*Selective* Medium partially inhibits Gram-positive bacteria. *Differential* Eosin and methylene blue differentiate among organisms: *Escherichia coli* colonies are purple and typically have a metallic green sheen; *Enterobacter aerogenes* colonies are pink, indicating that they ferment lactose; and colonies of other organisms are colorless, indicating they do not ferment lactose.
MacConkey agar		Gram-negative enterics	*Selective* Crystal violet and bile salts inhibit Gram-positive bacteria. *Differential* Lactose and the pH indicator neutral red (red when acidic) identify lactose fermenters as red colonies and nonfermenters as light pink. Most intestinal pathogens are nonfermenters and hence do not produce acid.

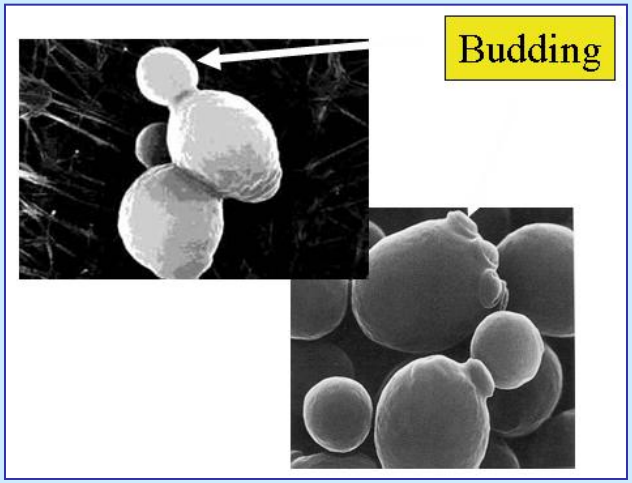
TERRENI DI CRESCITA

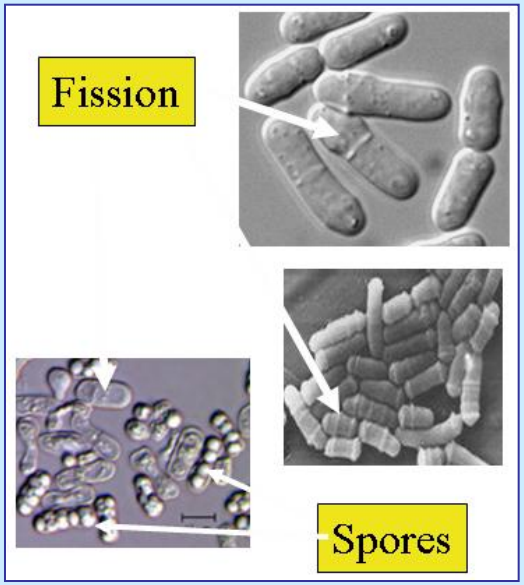



LA CRESCITA FUNGINA

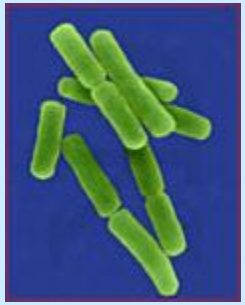
MODALITA' DI REPLICAZIONE NEI LIEVITI

Saccharomyces cerevisiae



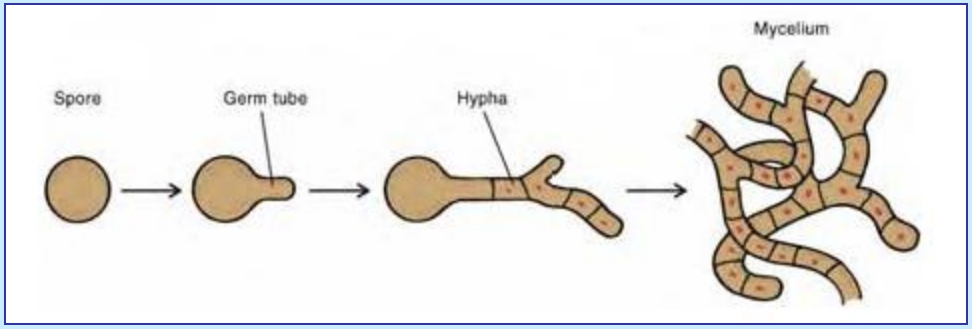


Schizosaccharomyces pmbe

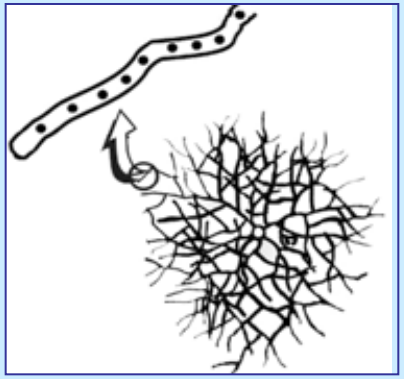


LA CRESCITA FUNGINA

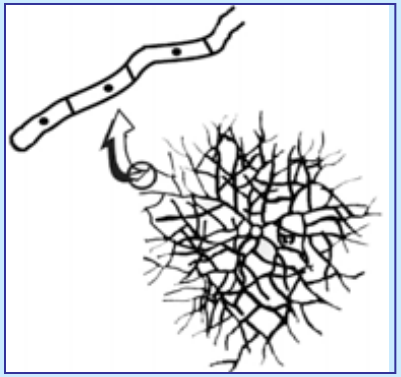
MODALITA' DI CRESCITA DEI FUNGHI FILAMENTOSI



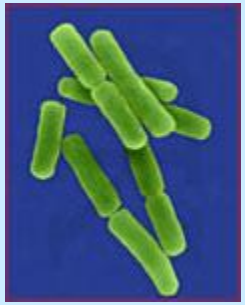
Modalità della crescita vegetativa
di un fungo filamentoso



Ife non settate (cenocitiche)
(es. *Zigomycota*)

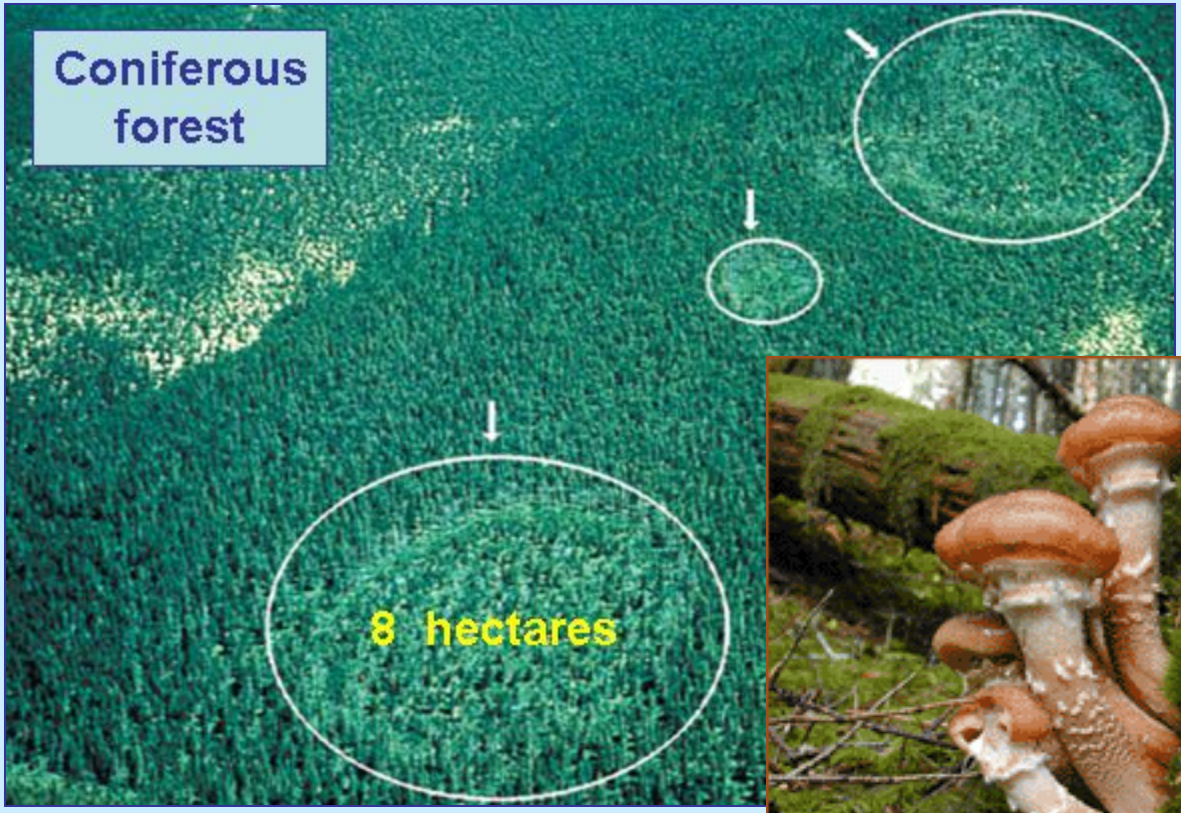


Ife settate




LA CRESCITA FUNGINA

CRESCITA FUNGINA INDETERMINATA



Armillaria ostoyae
(Malheur National Forest, Oregon, USA)