Vliv barviv na růst MO

Cíl: Stanovit citlivost MO k barvivům (krystalové violeti)

Bakteriostatické působení barviv

- některá organická barviva mají bakteriostatický účinek na grampozitivní bakterie i v koncentracích, kdy gramnegativní bakterie ještě rostou → využití pro přípravu selektivních půd (např. stanovení koliformních bakterií)

- krystalová nebo gencianová violeť, malachitová zeleň, methylenová modř, trypaflavin aj.

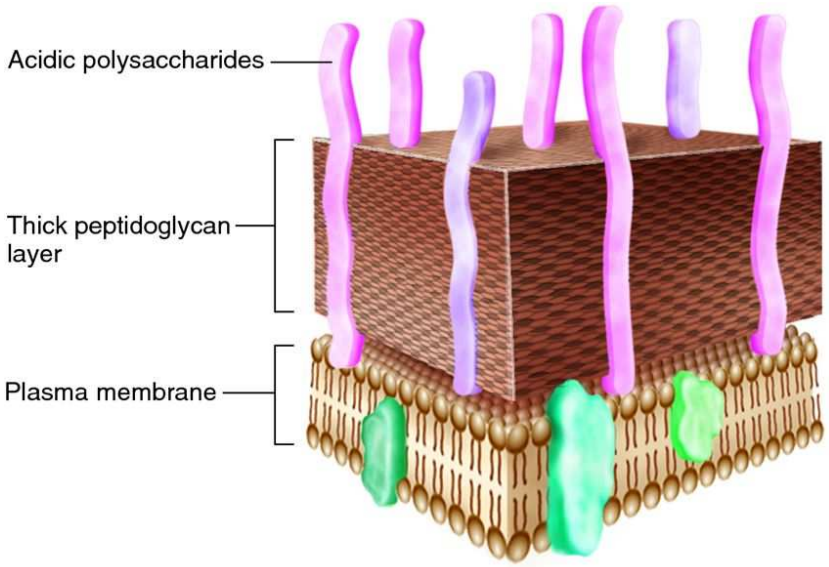
- selektivní pro streptokoky – akridinová oranž, etylová violeť, anilínová a trypanová modř

Buněčná stěna bakterií

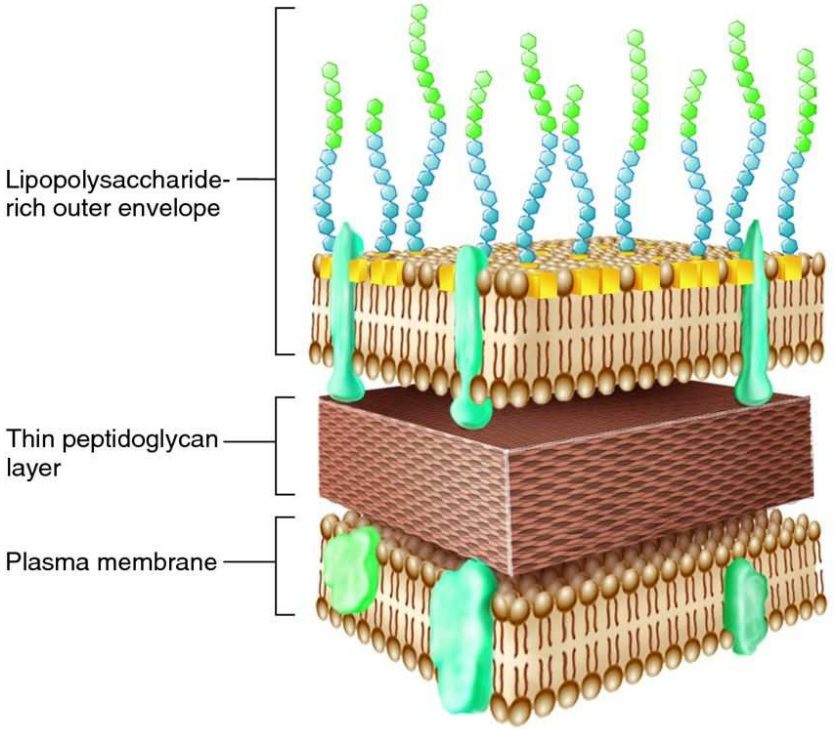
Rozdíly

Gram-pozitivní

Gram-negativní

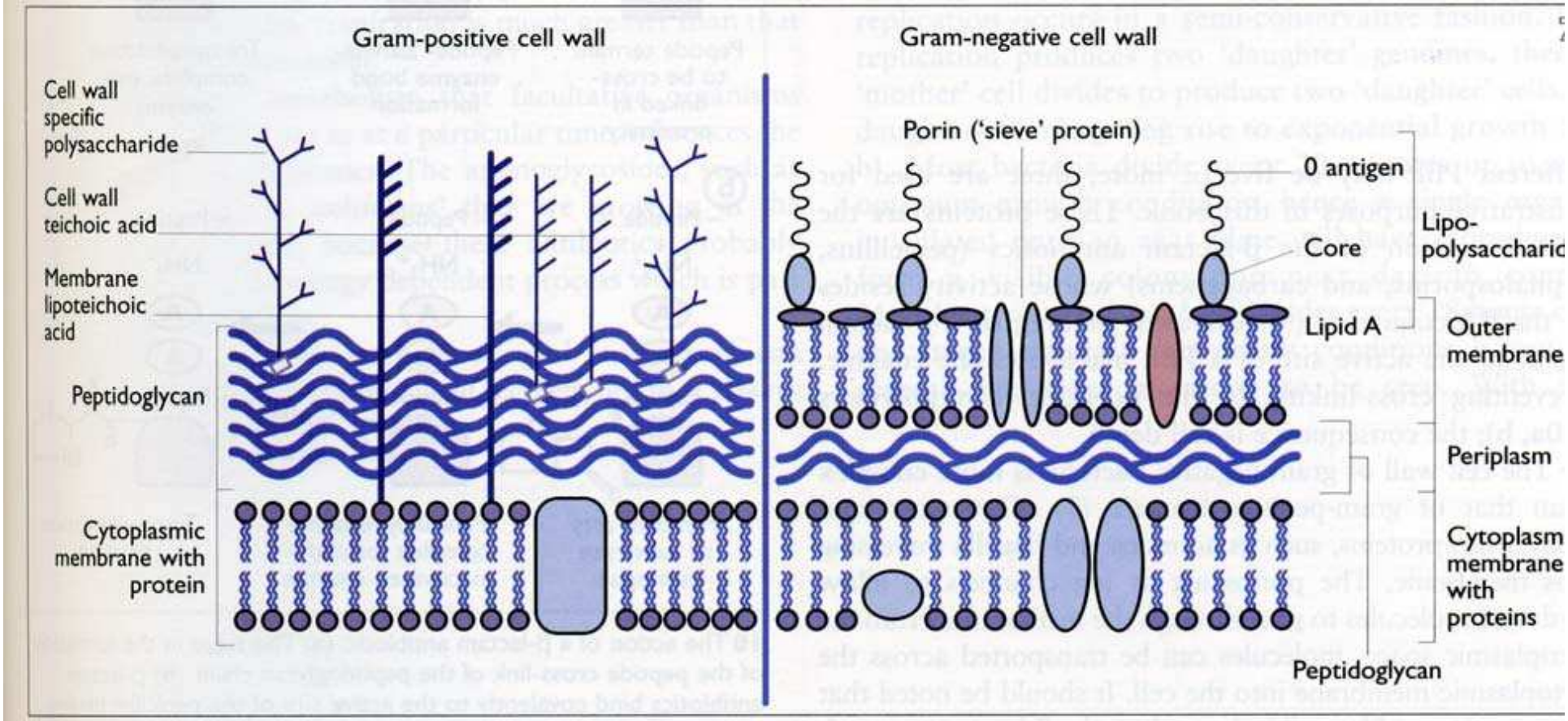


(a) Gram-positive: thick cell wall, no outer envelope



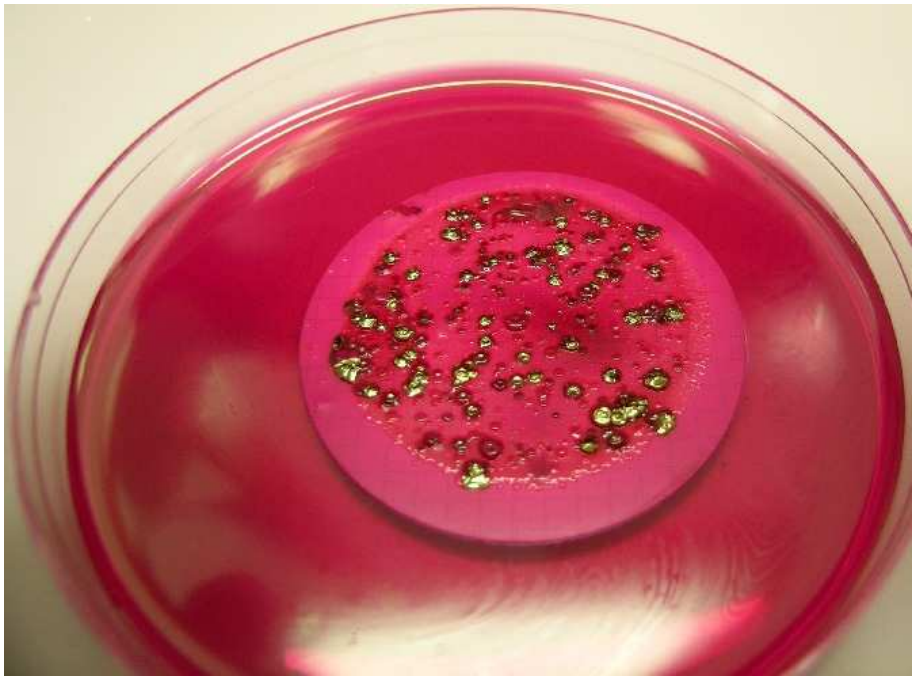
(b) Gram-negative: thinner cell wall, with outer envelope

Složení buněčné stěny bakterií



7 The structure of the cell wall of gram-positive and gram-negative bacteria.

- ENDO agar – bazický fuchsin



Fung a Miller (1973)

30 bakteriálních kmenů

42 barviv

TABLE 2. *Effect of acid and neutral dyes at 1:1,000 dilution on bacterial growth*[a]

Organism	Acid dye medium																			Neutral dye medium		
	Brom thymol blue	*o*-Cresolphthalein	Eosine B	Chlorophenol red	Rose bengal	Brom cresol purple	Erythrosine B	Eosine Y	Cresol red	Phenol red	Methyl red	Resazurin	Trypan blue	Brom phenol blue	Nigrosine	Aniline blue WS	Acid fuchsin	Indigo tetrasulfonate	Thymolphthalein	Wright's stain	Jenners stain	Carmine
Gram-negative organisms	Positive	Positive	Positive																			
Alcaligenes faecalis				–	+	+			+		v											
Enterobacter aerogenes 11a				+	+	+			+		+											
Enterobacter aerogenes 11b				+	+	+			+		+											
Enterobacter cloacae				+	+	+			+		+											
Escherichia coli				+	–	v			+		v											
Proteus vulgaris				+	+	+			+		+											
Pseudomonas aeruginosa				+	+	+			+		+											
Salmonella typhimurium				+	+	+			+		+											
Salmonella typhosa				+	+	+			+		+											
Salmonella paratyphi				+	+	+			+		–											
Salmonella pullorum				+	+	+			+		+											
Salmonella thompson				+	+	+			+		+											
Shigella flexneri				+	+	+			v		–											
Serratia mercescens				+	+	+			+		+											
Gram-positive organisms	Negative	Negative	–	Negative	Negative	–	–	v	v	–	Negative	+	+	+	+	+	+	+	+	Positive	Positive	Positive
Bacillus cereus			–			–	–	–	v	–		+	+	+	+	+	+	+	+	–	–	+
Bacillus danicus			–			–	–	–	v	+		+	v	+	+	+	+	+	+	v	+	+
Bacillus polymyxa			–			–	–	–	v	–		–	+	+	+	+	+	+	+	–	+	+
Bacillus subtilis			–			–	v	–	v	–		–	+	+	+	+	+	+	+	–	+	+
Bacillus sulfidus			–			–	–	–	v	+		+	v	+	+	+	+	+	+	–	–	+
Gaffkya tetragena			–			–	–	–	v	–		–	v	+	+	+	+	+	+	+	+	+
Micrococcus rhodochrous			–			–	–	–	v	–		–	v	+	+	+	+	+	+	–	+	+
Sarcina lutea			–			–	–	–	v	–		+	v	+	+	+	+	+	+	+	+	+
Staphylococcus aureus 241b			–			v	–	v	v	v		+	+	+	+	+	+	+	+	–	+	+
Staphylococcus aureus 241c			+			–	–	–	v	+		+	+	+	+	+	+	+	+	v	+	+
Staphylococcus aureus 241f			–			v	–	v	v	v		+	+	+	+	+	+	+	+	–	–	+
Staphylococcus aureus 241g			–			–	–	v	v	v		–	+	+	+	+	+	+	+	–	–	+
Streptococcus bovis			–			v	v	–	v	v		+	+	+	+	+	+	+	+	v	–	+
Streptococcus faecalis			–			–	v	v	v	–		+	v	+	v	+	+	+	+	–	–	+
Streptococcus lactis			–			v	v	–	v	+		–	+	+	+	+	+	+	+	v	–	+
Streptococcus liquefaciens			–			v	v	–	v	+		+	+	+	+	+	+	+	+	v	–	+

[a] Symbols: – = Negative, + = positive (growth), v = variable. Data compiled from three to six replicate tests of each organisms on each dye. Data (24- and 48-hr) are essentially identical. All organisms grew on the basal medium. The dyes are arranged according to their apparent effectiveness in separating between gram negative and gram positive organisms at the lowest dilution of the dyes (1:1,000).

TABLE 3. *Effect of basic dyes at 1:1,000 dilution on bacterial growth*[a]

Organism	Basic dye medium																		
	Janus Green	Methylene blue	Safranin O	Safranin Y	Basic fuchsin	Methyl green	Nile blue A	*p*-Rosaniline	Malachite green	Bismarck brown	Thionin	Thioflavine TG	Chrysoidine Y	Brilliant cresyl blue	Brilliant Green	Neutral red	Methyl violet B	Auromin O	Acriflavine	Crystal violet
Gram-negative organisms	Positive	Positive	Positive	Positive			Positive		Positive	Positive						Positive		Positive		
Alcaligenes faecalis					+	+		+			v	–	v	–	–					
Enterobacter aerogenes 11a					+	+		+			+	+	+	+	+					
Enterobacter aerogenes 11b					+	+		+			+	+	+	+	–					
Enterobacter cloacae					+	+		+			+	–	v	+	–					
Escherichia coli					+	+		+			+	v	+	+	–					
Proteus vulgaris					+	+	–	+			v	–	–	–	–					
Pseudomonas aeruginosa					+	+	+	+			+	+	+	+	+					
Salmonella typhimurium					+	+	–	+			+	v	v	–	–					
Salmonella typhosa					+	+	+	+			+	–	v	–	–					
Salmonella paratyphi					+	+	+	–			v	+	+	+	–					
Salmonella pullorum					+	+	+	+			v	–	v	–	–					
Salmonella thompson					+	+	+	+			–	+	+	+	–					
Shigella flexneri					–	+	–	–			–	–	v	–	–					
Serratia marcescens					+	+	–	+			–	+	+	+	+					
Gram-positive organisms	Negative	Negative	Negative	Negative												Negative		Negative		
Bacillus cereus					–	–	–	–	–	+	v					+		+		
Bacillus danicus					–	–	–	–	v	+	+					+		+		
Bacillus polymyxa					–	–	+	–	v	+	+					–		+		
Bacillus subtilis					–	–	–	–	–	v	–					+		+		
Bacillus sulfidus					–	–	–	–	–	v	–					+		+		
Gaffkya tetragena					–	–	–	–	–	v	–					+		+		
Micrococcus rhodochrous					–	–	–	–	–	–	v					+		+		
Sarcina lutea					–	–	–	–	–	v	–					+		+		
Staphylococcus aureus 241b					–	–	–	–	–	v	–					+		+		
Staphylococcus aureus 241c					–	–	–	–	–	v	–					+		+		
Staphylococcus aureus 241f					–	–	–	–	–	v	–					+		+		
Staphylococcus aureus 241g					–	–	–	–	–	v	–					+		+		
Streptococcus bovis					–	–	–	–	v	v	–					–		+		
Streptococcus faecalis					–	–	+	–	v	+	–					+		+		
Streptococcus lactis					–	–	–	–	–	–	–					v		+		
Streptococcus liquefaciens					–	–	+	–	v	+	–					v		+		

[a] Symbols and data presentation are the same as in legend to Table 2.

TABLE 5. *Differentiation of Salmonella and Shigella by basic fuchsin, p-rosaniline, and thioflavine TG media*

Medium[b]	Differentiation[a]														
	Salmonella											*Shigella*			
	cholerasuis	*typhimurium*	*paratyphi*	*pullorum*	*thompson*	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	*flexneri* Boyd	*flexneri* V	*flexneri* W	*sonnei*
Basal + BF 1:500	+	v	+	v	+	+	v	+	v	v	+	−	−	−	−
Basal + BF 1:750	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
Basal + BF 1:1,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
SS agar + BF 1:500	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−
Bril Green + BF 1:500	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
Bismuth sulf. + BF 1:500	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Basal + Rosa 1:500	+	−	+	−	+	+	−	+	+	+	+	−	−	−	−
Basal + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
Basal + Rosa 1:4,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SS agar + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
Bril Green + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+
Bismuth sulf. + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Basal + Thio 1:1,000	+	−	+	−	+	+	+	+	−	+	+	−	−	−	−
Basal + Thio 1:2,000	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−
Basal + Thio 1:3,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
SS Agar + Thio 1:2,000	+	−	+	−	+	+	−	+	−	+	+	−	−	−	−
Bril Green + Thio 1:2,000	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−
Bismuth sulf. + Thio 1:2,000	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−

[a] *Salmonella* isolates were obtained from a local hospital and were confirmed to be *Salmonella*. All known cultures were obtained from the Pennsylvania State University collection. Symbols: + = positive, − = negative, v = variable.

[b] Basal medium = tryptic soy agar (Difco), SS agar = Salmonella-Shigella agar (Difco), Bril Green = Brilliant Green agar (Difco), bismuth sulf. = bismuth sulfite agar (Difco). All organism grew in all 4 media without added dyes, except *Shigella sonnei* which did not grow on bismuth sulfite agar. BF = basic fuchsin, rosa = *p*-rosaniline, thio = thioflavine TG. Many other dilutions of dyes were tested. The ones reported here illustrate the critical differential dilutions.

Type.	Bacteria tested.	Hoffman violet. 1:50,000	Hoffman violet. 1:100,000	Hoffman violet. 1:500,000	Crystal violet. 1:50,000	Crystal violet. 1:100,000	Crystal violet. 1:500,000	Dahlia. 1:50,000	Dahlia. 1:100,000	Dahlia. 1:500,000	Fuchsin. 1:50,000	Fuchsin. 1:100,000	Fuchsin. 1:500,000	Rosanalin violet. 1:50,000	Rosanalin violet. 1:100,000	Rosanalin violet. 1:500,000	Cresylechtviolett. 1:50,000	Cresylechtviolett. 1:100,000	Cresylechtviolett. 1:500,000	Anilin violet. Gentian B. 1:50,000	Anilin violet. Gentian B. 1:100,000	Anilin violet. Gentian B. 1:500,000	Naphthylamin blue R. 1:50,000	Naphthylamin blue R. 1:100,000	Naphthylamin blue R. 1:500,000	Bleu de Lyon. 1:50,000	Bleu de Lyon. 1:100,000	Bleu de Lyon. 1:500,000	Säureviolett (Kühne). 1:50,000	Säureviolett (Kühne). 1:100,000	Säureviolett (Kühne). 1:500,000	Methylviolet 6 B. 1:50,000	Methylviolet 6 B. 1:100,000	Methylviolet 6 B. 1:500,000
Gram-positive	Staphylococcus aureus	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–*	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	–	–	–
	Streptococcus (green)	–	–	+	–	×	+	–*	×	±	×	±	+	–	±	+	+	+	+	–	×	+	+	+	+	+	+	+	+	+	+	+	–	±	+
	Streptococcus (hemolytic)	–	–	±	–	–	±	–	–	+	–	–*	+	–	–	±	±	±	+	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+
	Streptococcus mucosus capsulatus	–	–	+	–	–	±	..	..	..	–	×	+	–	–	+	±	±	+	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+
	Pneumococcus	–	–	±	–	–	+	–	–*	+	–	±	+	–	–	+	–	–	+	–	×	+	+	+	+	+	+	+	+	+	+	–	–*	+
	B. diphtheriæ	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	×	–	–	–	±	±	+	+	+	+	+	+	+	–	–	+
	Diphtheroid bacilli (Cameron)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+
	Diphtheroid bacilli (Cunningham)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	..	..	..	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+
	B. hoffmanni	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±	–	–	–	–	±	+	+	+	+	+	+	+	–	–	+
	B. subtilis	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	–	–	+
	B. lactimorbi	–	–	–	–	–	–	–	–	–	–	–	+	–	–	±	×	±	+	–	–	–	+	+	+	+	+	+	+	+	+	+	–	–	+
	B. anthracis	–	–	–	–	–	–	–	–	–	–	–*	+	–	–	–	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	–	–	+
Gram-negative	Cholera	–	–	+	–	–	+	–	×	+	+	+	+	–	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	×	+
	Vibrio (indol-positive)	–	–*	+	–	–	+	×	±	+	+	+	+	–	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	×	+
	Vibrio (indol-negative)	–	–	+	–	–*	+	×	±	+	+	+	+	–	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–*	+	+
	B. proteus	±	+	+	–*	×	+	±	+	+	+	+	+	+	+	+	±	+	+	±	+	+	–	±	+	+	+	+	+	+	+	+	–*	–*	×
	B. pyocyaneus	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Shiga)	–*	×	+	–*	±	+	×	±	+	–	–*	+	–*	±	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	±	+	+
	B. dysenteriæ (Flexner)	×	±	+	–	×	+	–*	±	+	±	±	+	–*	±	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	×	±	+
	B. dysenteriæ (Mt. Desert)	±	+	+	–	±	+	+	+	+	×	±	+	±	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+
	B. typhosus	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..	..	..	+	+	+	+	+	+	+	+	+	+	±	±	+
	B. paratyphosus, A	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. paratyphosus, B	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. enteriditis (Gaertner)	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. coli communis	±	±	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. coli communior	±	+	+	±	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Friedländer's bacillus	±	+	+	×	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. lactis aerogenes	+	+	+	±	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+
	B. ozænæ	×	±	+	–	–	+	..	..	..	+	+	+	×	±	+	+	+	+	–*	+	+	+	+	+	+	+	+	+	+	+	+	×	±	+
	B. pullorum	+	+	+	×	±	+	..	..	..	±	+	+	..	..	..	+	+	+	+	+	+	..	..	..	+	+	+	+	+	+	+	+	+	+

+ = growth like control; ± = restrained growth; × = markedly restrained growth; – = no growth; –* = some growth developed later.

Krumwiede a Pratt (1914)
Vliv anilínových barviv na bakterie

Growth of Dysentery Bacilli on Fuchsin Agar.

Culture.	Mannite fermentation.	Time of incubation and dilution of dye.										
		18 to 24 hours.				48 hours.				72 hours.		
		25,000	50,000	100,000	150,000	25,000	50,000	100,000	150,000	25,000	50,000	100,000
Shiga, Gay-Shiga, Shiga	−	−	−	−	− or ×	−	−	s.c. or ×	±	−	s.c.	±
Kruse, Kruse	−	−	−	− or s.c.	− or ×	−	−	s.c. or ×	±	−	s.c. or ×	±
Kruse, Shiga	−	−	−	− or ×	×	−	−	× or ±	±	−	×	+
Shiga	−	−	−	− or ×	±	−	− or s.c.	× or ±	±	−	±	+
Torrey 21	−	−	−	× or ±	±	−	× or ±	+	+	− or 1 c.	× or ±	+
Cruz 1	−	−	− or ×	± or +	+	− or ×	±	+	+	− or ×	±	+
Sydenham	+	−	− or ×	± or +	+	−	× or ±	+	+	−	±	+
Torrey 4, Torrey 5–6, Hiss Y	+	−	×	± or +	+	s.c. or ×	± or +	+	+	× or ±	±	+
Hiss Y, Flexner-Harris, Medburg, Ferra-Hiss, Torrey 16–3, Torrey 32, Y, Baltimore, Strong, Flexner, Mt. Desert	+	×	± or +	± or +	+	× or ±	± or +	+	+	±	± or +	+
Strong, Gay-Flexner, Silk, Baltimore, Flexner, Gray, Torrey 3, Torrey 41, Flexner-Harris	+	±	± or +	+	+	± or +	+	+	+	± or +	+	+
Gray B, Hiss 48	+	+	+	+	+	+	+	+	+	+	+	+

− = no growth; 1 c. = colony; s.c. = several colonies; × = very slight growth; ± = evident restraint; + = practically like control.

Bakteriostatické působení roztoku krystalové violeti

Potřeby :

- 1% vodný roztok krystalové violeti (nezaměňovat s roztokem označeným Gram I)
- bujonové kultury *Bacillus subtilis*
  Enterobacter aerogenes
  Escherichia coli
  Micrococcus luteus
- 4 sterilní zkumavky, sterilní destilovaná voda a pipety
- 4 sterilní Petriho misky
- 4 zkumavky s 18 ml MPA

Postup:

- aseptická práce!!!

- ředění roztoku krystalové violeti:

  1x (neředěný vzorek),10x, 100x a 1000x
- rozehřát 4 zkumavky s MPA ve vodní lázni
- pipetovat roztok violeti do sterilní misky a přelít MPA
- po ztuhnutí rozdělit fixem dno misek na čtvrtiny
- očkovat „hádkem“ 4 kmeny bakterií
- inkubace při 37°C 48 hod

- odečet růstu bakterií
- výsledky zapsat do tabulky
- srovnat růst gram-negativních bakterií s gram-pozitivními

Ředění krystalové violeti