KMBF01 - Lab report nr. 3

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Laboratory exercise 4 - Bacteriacidy and bacteriostasis

Introduction

The aim of this lab is to determine the Minimum Inhibitory Concentration (MIC) and Minimum Lethal concentration (MLC) of chlorohexidine on *E. coli* and *S. epidermidis*. This was achieved by utilizing the serial dilution method.

Method

The epindorf tubes for each bacterial strain and following agar plates were prepared and incubated as per lab instructions. The resulting concentrations of chlorohexidine can be seen in Table 1.

Table 1: Table showing the tested concentrations of chlorohexadine.

Epindorf tube nr.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Concentration Chlorohexadinec / $\mu g \cdot mL^{-1}$	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0

Results

E. coli

Results given by Table 2 shows that tubes 10-8 contained culture growth while tubes 7-1 contained none. The MIC of Chlorohexadine on E. coli can therefore be estimated to 1.6-0.8 μ g/ml. Sections 1-4 on the agar plate displayed no growth of culture, giving an MLC of 12.5 μg/ml.

S. epidermidis

Tube 10 was the only tube showing culture growth, giving an estimated MIC of Chlorohexadine on S. epidermis of <0.4 μg/ml. Sections 1-3 on the agar plate displayed no growth of culture, giving an MLC of 25 μg/ml.

Table 2: Results from agar plate and epindorf dilution tests for *E. coli* and *S. epidermidis*.
G and NG indicates Growth and No Growth respectively.
*- Very few colonies, counted as NG.

Sample nr.	1	2	3	4	5	6	7	8	9	10
Concentration chlorohexidine / $\frac{\mu g}{ml}$	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
E. coli agar	NG	NG	NG	NG*	G	G	G	G	G	G
S. epidermidis agar	NG	NG	NG	G	G	G	G	G	G	G
E. coli epindorf	NG	NG	NG	NG	NG	NG	NG	G	G	G
S. epidermidis epindorf	NG	NG	NG	NG	NG	NG	NG	NG	NG	G

Discussion

Chlorohexidine is an microbial agent frequently used as a topical antiseptic. It is a cation-active substance that binds to negatively charged cell surfaces, compromising the cell membrane causing leakage and cell death. Gram-positive cells are more negatively charged than Gram-negative and are therefore more sensitive to chlorohexidine [1].

The results generally support the assumed model - chlorohexidine is static (causes reversible growth inhibition) to *E. coli* and *S. epidermidis* at relatively low concentrations and is cidal (causes cell death) at higher concentrations. The Gram-positive *S. epidermidis* shows higher static sensitivity to chrolohexidine, as expected given chlorohexidine's mechanism of action. We were not able to determine an exact MIC for chlorohexidine on *S. epidermidis*. This may either be due to that the MIC lies outside our measured range of concentrations, or that an error has been made in the dilution or inoculation of the samples.

S. epidermidis displayed a somewhat higher MLC than *E. coli*. This was unexpected given chlorohexidine's mechanism. However the granularity of the measurement means that these results are not necessarily significant. One source of uncertainty in the measure of MLC for *E. coli* was that a gradient of decreased growth across 3-4 segments of the platter was observed instead of binary growth/no-growth segments. Segment 4 did display some bacterial colonies along the path of inoculation however it was decided to count this as no-growth given the relative number of colonies.

It is not anticipated that not shaking the bacterial solutions prior to inoculation significantly affected the results given the high viscosity and uniform turbidity of the bacterial solutions.

Laboratory excersice 5 - Susceptibility of microorganisms to antibiotics and other organic substances

Introduction

The aim of this lab is to investigate the susceptibility of a Gram-positive (*Staphylococcus epidermidis*) and a Gram-negative (*Escherichia coli*) bacteria and a yeast (*Saccharomyces cerevisae*) to 5 antibiotic/antifungal compounds (penicillin G, erythromycin, polymyxin B, tetracycline and cycloheximide) and one sample with unknown antimicrobial properties (horseradish).

Method

The horseradish solution was made by extracting the juice from finely grated horseradish by squeezing. The practical steps were carried out as per the laboratory instructions.

Results

Results of studied microorganisms degrees of resistance can be found in Table 3. Measures of resistance S, I and R taken from table "Tolkningsschema" included in lab instructions.

Table 3: Diameter of inhibited growth by substance and corresponding diameter classifications in mm. Suscebtible (S), Intermediate (I), Resistant (R).
*-Regrowth of resistant bacteria present
†-Indeterminate.

	S. epidermidis		*E. coli*		*S. cerevicae*		([?]) S	([?]) R
Penicillin G	16	I	0	R	0	R	23	15
Erythromycin	29.5	S	11.5*	R	0	R	27	16
Tetracycline	31.5	S	29	S	22	I	28	21
Polymyxin B	13	R	15	†	15*	†	15	15
Cyclohexamide	0	N/A	0	N/A	0	N/A	N/A	N/A
Horseradish	0	N/A	0	N/A	0	N/A	N/A	N/A

Discussion

Penicillin G (Benzylpenicillin)

Penicillin G inhibits cell wall synthesis in Gram-positive bacteria, causing the wall-structure to be incomplete. The cell lysis that follows is bactericidal [2]. This correlates with our results, as the only organism affected by the antibiotic was the Gram-positive *S. epidermidis*, which showed intermediate resistance.

Penicillin G was I for *S. epidermidis* but R for the other samples. This was expected because Penicillin affects Gram-positive cell walls and *S. epidermidis* is Gram-positive.

Erythromycin

Erythromycin is a bacterial protein synthesis inhibitor that binds to the P-site of 50S ribosomal subunit in the cytoplasm, disabling the binding of tRNA to the donor site. It can be bactericidal or bacteristatic, depending on concentration and species [3]. Erythromycin is active against both Gram-negative and Gram-positive bacteria [4].

The 50S ribosomal subunit is only a part of the prokaryotic ribosome and as such *S. cervicae* resistance is inherent, expected and confirmed by the results. The strain of *E. coli* studied seem to have gained resistance.

Polymyxin B

Polymyxin B destabilizes the outer cell membrane of Gram-negative bacteria by altering membrane permeability. All Gram-positive bacteria and fungi are, according to previous research, resistant to Polymyxin B [5]. This did not correlate with our results as all three microorganism were affected by the antibiotic.

Tetracycline

Tetracycline interferes with protein synthesis by binding to the 30S ribosomal unit and preventing binding to the mRNA. The binding is reversible and tetracycline is considered bacteriostatic [6].

The 30S ribosomal subunit is only a part of the prokaryotic ribosome, this is confirmed by the results regarding *E. coli* and *S. epidermidis*. Results regarding *S. cerevicae* are unexpected as it should be unaffected by the primary mechanism. The discrepancy is likely explained by statistical uncertanty in the test lookup-table data. This could be verified by repeating the test.

Cycloheximide

Cycloheximide interferes with protein synthesis in eukaryotic organisms, while the exact mechanism is unknown it disrupts the translocation step in elongation. It is believed to achieve this by binding to the 60S ribosomal subunit. As subunit 60S is only present in eukaryotic ribosomes, effects onbacteria is unexpected[7].

Cycloheximide did not have any effect on the growth of the three microoorganisms studied. This is unexpected in the case of *S. cerevicae* as cycloheximide has in the past been used as an agricultural fungicide.

Horseradish

Horseradish forms Allyl isothiocyanate (AITC) when grated. This is an electrophilic compound and part of strong sensation that comes with eating horseradish is from he activation of the TRPA1 reponse system. Horseradish was choosen to see if it's reactive, electrophilic qualities would proove antibacterial.

Our results indicated no antibacterial or antifungal activity. However AITC is known to be highly antibotic with a mechanism similar to Polymyxin B and to be most effective on Gram-negative bactgeria [8]. AITC is highly volitie and our negative results could be explained by evaporation eg. in the drying step.

Antiboitic choice

Based on the results from this test the authors would choose Tetracycline or Erythromycine agains the Gram-positive *S. epidermidis* and Tetracycline against the Gram-negative *E. coli*. The authors would further use cycloheximide against *S. cerivicae* in a labaratory settings, despite the results of this test, as it is generally accepted to be an effective antifungal agent. Difference in effect of some antiboitics on differnt Gram-types is explained by antibiotics having diffent modes of action with some specifically targeting Gram-negative cell walls and others not.

Laboratory exercise 6 - the Ames test

Introduction

The aim of this lab is to investigate eventual mutagenic effects of grated horseradish using an Ames test. Horseradish contains a compound known as allyl isothiocyanate which exhibits attributes of a cancer chemopreventative agent, mutagenic properties of horseradish are therefore not expexted.

Method

The method was carried out as per lab instructions. The horseradish solution was made by extracting the juice from finely grated horseradish by squeezing.

Results

The results of the aims test for the positive control (4-nitro-o-phenylenediamine or NOP4), negative control (dH_2O) and sample (horseradish solution) can be seen in Table 4.

Table 4: Number of *Salmonella typhimurium* TA98 and contaminant colonies on the horseradish, positive control (4-nitro-o-phenylenediamine or NOP4) and negative control (dH_2O) tests after incubation for just under 24 hours.

	Number of TA98 colonies	**Number of contaminant colonies**
Negative control (dH_2O)	407	48
Sample (Horseradish)	235	20
Positive control (4NOP)	380 / 370	unknown

Discussion

The Ames test is used to investigate mutagenic properties of chemical compounds. Microorganisms that are sensitive to reverse mutation are cultured on a medium with the chemical compound being investigated. Auxotrophic bacteria - bacteria who are unable to synthesize one or more compounds nessesary for their growth - who's auxotrophy is induced by a a frame-shift mutation can be cultured on a medium lacking the nutrient they can't syntesise. Only colinies who have mutated in such a way as to revert the frame-shift mutation will grown and the relative rate of mutation can be measured by counting the number of such colonies.

In this experiment we used a His^- strain of *S. typhimurium* called TA98 cultured on a low-histadine plate. A small ammount of histadine was included in the growth medium to allow for a few generation cycles and thus a higher chance of mutation. A mutagenic sample would be expected to result in more colonies than the negative control.

One limitation of the Ames test is that if the compound under study has antibacterial properties it will supress colony formation and may give a false negative result. Another limitation is that an extremely mutagenic compound may cause such widespread and severe mutations that it has a static effect, also supressing colony formation and giving a false negative result.

A positive and negative control were used to establish baseline mutation values for a known non-mutagenic and mutagenic compound under test conditions. It would be expected that the negative controll would have fewer colonies than the positive, however this was not the case for this test. The resason is unknown but may be due to antibacterial contamination in the positive test or that the mutagenic compound was sufficiently mutagenic to have a significant static effect.

Our results showed significantly lower colony count on the horseradish sample than the negative control. This indicates that either the horseradish extract contains an antibacterial compound or is sufficiently mutagenic to have a significant static effect. As grated horseradish is know to contain Allyl isothiocyanate (AITC) which is a known antibacterial this is taken to be the more likely explination. Given the antibacterial effect the mutagenicity of horseradish can not be concluded from this test.

References

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