

THE DEVELOPMENT OF PIGMENTED BACTERIA IN WATER SAMPLES AND THEIR BEHAVIOUR TOWARD THE ACTION OF SYNTHETIC ANTIMICROBIALS

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Abstract

Pigmented bacteria as *Chryseobacterium* spp., *Serratia marcescens*, and *Chromobacterium violaceum* are an important group of microorganisms distributed in water, liquid products, soil and other environments. An experimental work was conducted related with the isolation and identification of some pigmented bacteria from water samples and the monitoring of their development under the action of synthetic antimicrobials. The aim of the study was not focused only in the reduction of the growth of those microorganisms but in evidencing also the changes in some of their morphological characteristics under the action of the pharmaceutical product Vibramycin (generic name: doxycycline). This is a known product for its powerful antimicrobial activity. (The research work was based also in the former results achieved observing the action of other antimicrobials in the growth of non-pigmented bacteria).

Microorganisms were isolated from samples of water taken from water supply network. Isolated, purified and identified bacteria, were treated with Vibramycin solutions in different concentrations in order to observe all the changes of macro colonies and their characteristics. The treatment was performed using disk diffusion antibiotic sensitivity testing (KB-testing). The discoloration of light absorbing compounds, the reduction of the size of colonies and sporulation tendencies were evaluated.

An interesting conclusion was the fact that in pigmented bacteria the cell wall integrity was affected as well as evident changes in the specific chemical characteristics and reproduction process. This ultimately resulted in some cases of creation of mutant forms.

Key words: *Chryseobacterium* sp., *Serratia marcescens*, *Chromobacterium violaceum*, Disk diffusion method, Light absorbing compounds, Cell wall, Mutant forms.

1. Introduction

Pigmented bacteria as *Chryseobacterium* spp., *Serratia marcescens*, and *Chromobacterium violaceum* are an important group of microorganisms distributed in water, liquid products, soil, etc. During various experimental works a great number of such pigmented bacteria were developed and it is safe to say that they have an important role in taxonomic studies [1, 3, 4, 6, and 8].

These bacteria have been identified in several liquid samples, water and liquid foods and they represent even more interest due to the chemical compounds they contain and their ability to absorb light [3, 4, and 9].

Therefore it was deemed of great interest to expand further the knowledge on these microorganisms by conducting an experimental work to isolate and identify some pigmented bacteria from water samples and monitor their development under the action of a powerful synthetic antimicrobial, Vibramycin (generic name: doxycyclin).

Changes of some of the morphological characteristics under the effect of Vibramycin were a very important objective of this study and some interesting conclusions that will be described further in the paper are: effects on the microorganisms cell wall integrity, evident changes in the specific chemical characteristics and reproduction processes as well as some cases of creation of mutant forms [10, 11].

2. Materials and Methods

After isolating bacterial charges from water obtained from the regular supply network, all the colonies pigmented in red, yellow and those of a white opalescent color were selected to create a group of microorganisms that would be tested with inhibition tests using the synthetic inhibitor, Vibramycin as well as natural antibacterials extracted with CO₂.

This paper only describes the use of the synthetic antimicrobial selected which is an antimicrobial substance with a wide spectrum - Vibramycin (active ingredient is doxycycline, an antibiotic belonging to the class of tetracycline's that are known for their effective use against different types of infections).

Colonies were developed in Petri dishes in Plate Count Agar (PCA) medium and afterwards in Malt Agar (MA). Some of them underwent complete taxonomic tests with the aim to identify them until class and species level together with other discovered microorganisms [2, 5, 7, 10, and 11].

Chryseobacterium sp., *Serratia marcescens*, *Chromobacterium violaceum* were identified in such way as well as some other non-pigmented bacillus colonies.

For the colonies in liquid medium their characteristics were evaluated for a three day incubation period taking into consideration that for the Kirby Bower testing the colonies needed to be tested in their exponential growth phase, after 24 - 48 hours of incubation.

Depending on the pigments produced they could be developed as pigmented macro-colonies. The effect of the chemical compounds present in the selective substrates used might have influenced in the pigmentation of the cell wall.

3. Results and Discussion

The results as an evidence of microbial growth in the liquid medium were shown as turbidity, precipitates, ring forming, membrane forming and gas release.

During the evaluation some tests of developing in liquid medium were performed and afterwards counting of the colonies developed in 10 fields on the microscope in order to assess the degree of growth for each isolated bacterial culture.

The development of the colonies was fairly good, therefore the period of incubation as recommended in the Kirby Bower method was followed.

In Figure 1 it is shown the development of the pigmented bacteria in liquid medium (types of bacteria are codified in the chart from K1 to B10) after 24, 48 and 72 hours.

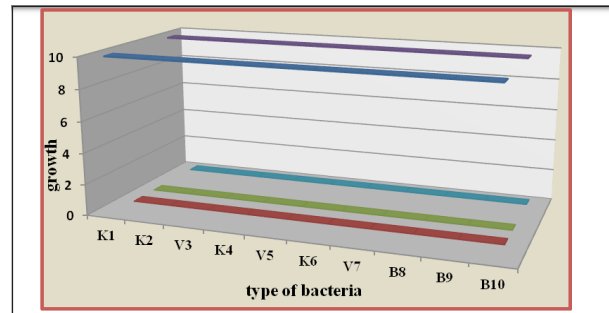


Figure 1. Growth in liquid medium of the pigmented bacteria colonies (After 24 hours there is no change in the growing of the bacteria)

The characteristics related with the bacterial development (based on the color coding above) in liquid selected medium are as follows:

- Red - no precipitation (negative).
- Green- no ring forming (negative).
- Light Blue - no membrane forming (negative).
- Dark Blue - a presence of turbidity (positive).
- Purple - a presence of fermentation observed by CO₂ production (positive).

The inhibition tests with Vibramycin were performed for all 10 cultures.

The strains were inoculated and spread through cotton tampons in Petri dishes in order to study the stains of inhibition. The colonies were developed first in solid media and incubated for 24 - 48 hours and afterwards cultivated in PCA in the center of the plate.

Table 1. Inhibition of the pigmented macro-colonies with the synthetic inhibitor Vibramycin.

Type	Type of inhibition	Colony Diameter - Dc/mm	Spot Diameter - Ds/mm	Ratio Ds/Dc Inhibition in %
K-1	Complete inhibition + sporulation	3	40	13.3
K-2	Complete inhibition + sporulation	5	40	8
V-3	Complete inhibition + sporulation	3	40	13.3
K-4	Complete inhibition + sporulation	4	40	10
V-5	Complete inhibition	5	40	8
K-6	Complete inhibition	5	40	8
V-7	Visible inhibition	6	28	4.7
B-8	Visible inhibition	9	25	2.7
B-9	Visible inhibition	6	26	4.3
B-10	Visible inhibition	6	22	3.7

Dc - Colony Diameter

Ds - Spot Diameter

Ds/Dc ratio - the ratio between the Colony Diameter and Spot Diameter

The results are summarized in the Table below (Table 1) and in the respective Figure (Figure 2).

As it is evident from the table and chart above Vibramycin achieved a visible and clear bacterial inhibition for these bacterial colonies that are capable of producing pigments and maintaining their percentage and specific color without being attacked by other synthetic or natural inhibitors of their growth.

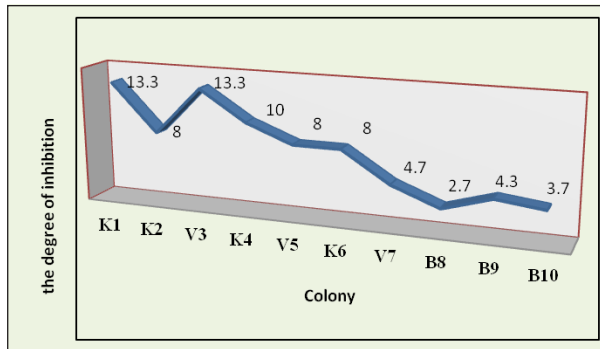


Figure 2. Degree of inhibition according to the ratio Ds/Dc

4. Conclusions

- A more complete inhibition with the greatest value of the ratio belonged to K-1 and V-3, the first being a characteristic red colored bacteria that for its characteristics is believed to belong to the *Serratia* class (it was also compared to a selected culture of *Serratia marcescens* isolated during a research work in a bilateral project with University of Ioannina); this is currently being kept in storage for further identifications until species level.

V-3 is a typical bacterial colony of a yellow color that after the study of the macro colony is believed to belong to the flavobacteria group.

- All other cases of pigmented bacteria showed a greater grade of inhibition compared to the identified bacilli and to the white bacterial colonies identified. To fully confirm this statement it remains to perform new tests and comparisons between pigmented bacteria tests and non-pigmented ones.

- A typical change of the pigments was seen as time passed. The colonies treated with Vibramycin at first lost their red or yellow color and afterwards if the incubations period was prolonged they hardly re-gained their original color - meaning that the chromophore pigment was evidently affected and the formation of mutant forms was a possibility that could be excluded.

- An evident loss of color was especially seen in the red colored pigment.

5. References

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