

Testing Plant and Fungal Extracts for Anti-Quorum Sensing Activity in *Chromobacterium violaceum*

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Introduction

- Development of antibiotic resistant microbial species are reducing efficacy of many antibiotics the more frequently these treatment types are employed (Ventola, 2015). Since the first implementation of antibiotic treatments, a continuous occurrence of resistance phenotype has been recorded among selected species. (Fig. 1).
- The gram negative species, *Chromobacterium violaceum* is a biofilm forming bacteria and relies on Quorum Sensing (QS) for film development. (Fig 2) Previous disk diffusion assays provided evidence of potential anti-quorum sensing (anti-QS) activity when treated with essential oils of Peppermint (*Mentha piperita*) and Reishi (*Ganoderma lucidum*) (Fig 3).
- Many plant species (ex: legumes) produce secondary compounds to control the growth of associated microbial species (Contreras et al. 2006). These secondary compounds may mimic homoserine lactones: a crucial autoinducer in gram negative bacteria (Poli et al. 2018)
- The objective of this study was to provide evidence of anti-QS activity of Eucalyptus (*Eucalyptus tubulus*) and Reishi (*Ganoderma lucidum*) extracts.

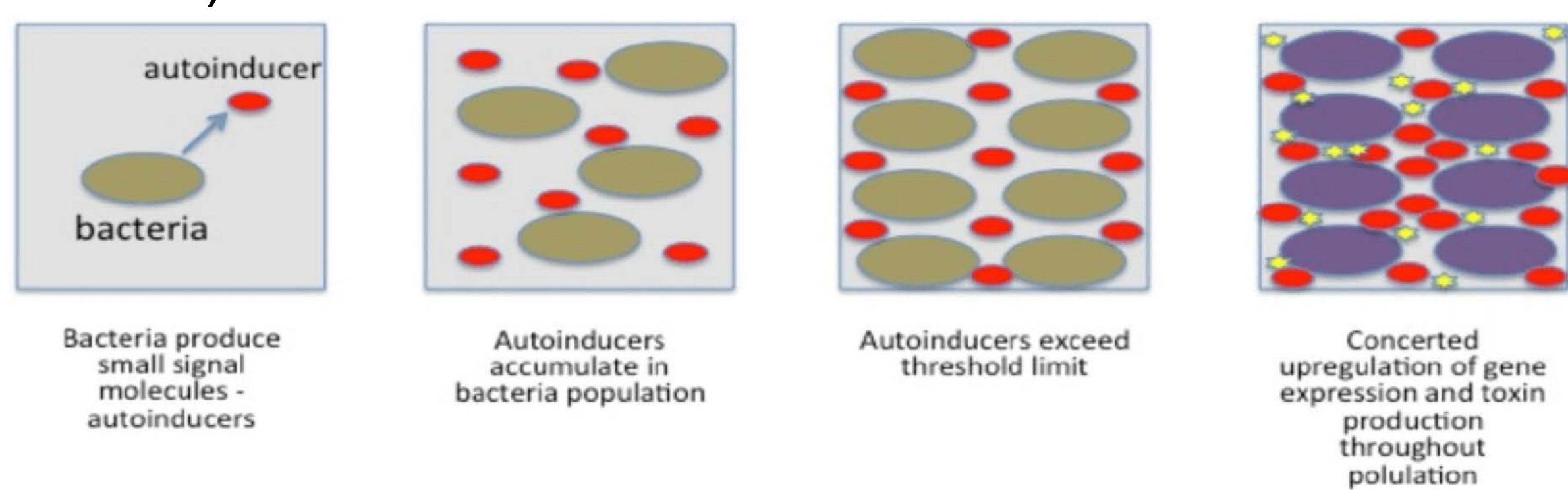


Figure 2. Diagram of biofilm composition and QS pathway. Images from <https://www.slideshare.net/hannahgrazia/biofilms-33285351>

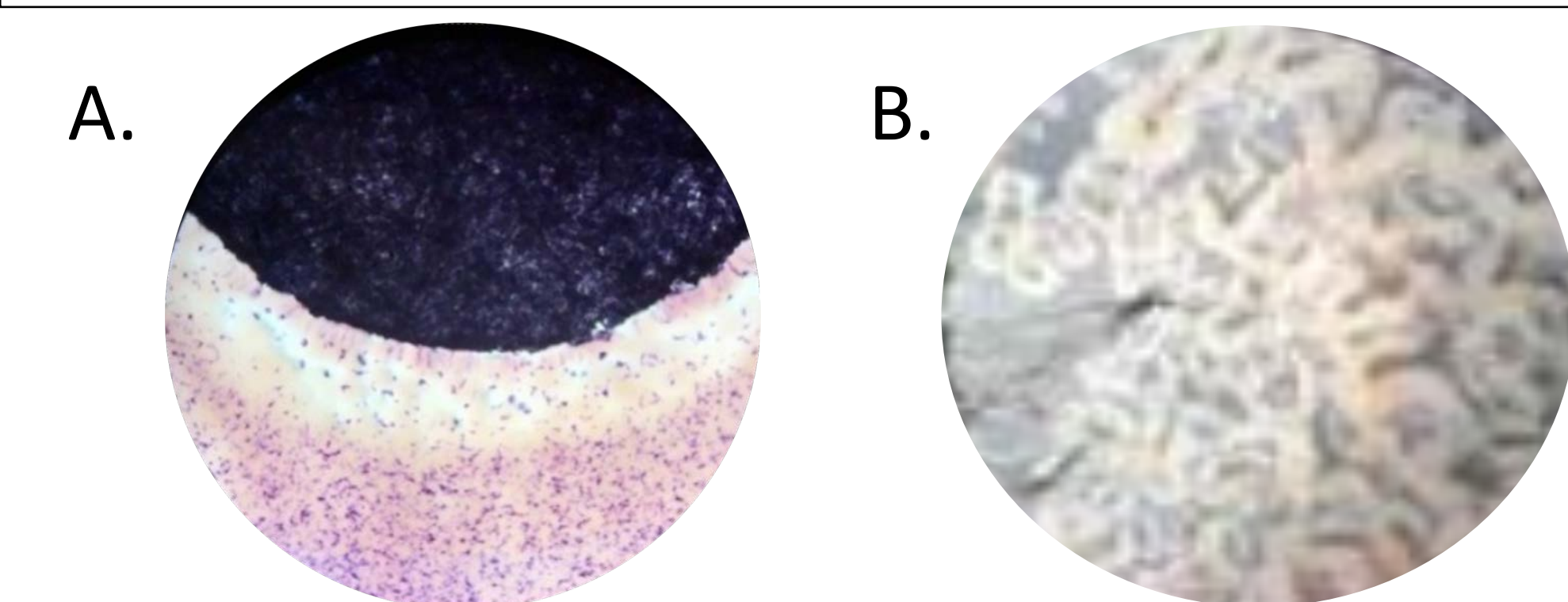


Figure 3. Evidence of potential anti-Quorum sensing activity in the presence of Peppermint essential oil from disk diffusion experiment. A. Edge of disk from diffusion assay. B. Biofilm morphology.

Materials and Methods

- A Spot Plate Assay was employed to measure the efficacy of treatment types in control of microbial growth followed by a spectrophotometric analysis of liquid cultures for violacein production. An initial tenfold serial dilution of essential oils and tinctures was developed using ethyl alcohol as a diluent for both tinctures and essential oils providing a general minimal inhibitory concentration: MIC (Poli et al., 2018). Refined dilution trials were developed upon determination of MIC and tested against *C. violaceum* (CV1).
- Colonies were then grown in liquid, varying in concentrations of treatment. Treatment types consisted of Reishi tincture and Eucalyptus extract (*Eucalyptus tubulus*). A spectrophotometric analysis and determination of colony forming units (CFUs) were used to quantify anti-quorum sensing.
- Agents that show inhibition of violacein production and/or biofilm "fragmentation" were the main interest in this study. Biofilm fragmentation was recorded as changes in uniform growth of bacterial colonies without evidence of biocidal effects.

Results

- Spot plate treatment of *C. violaceum* with Eucalyptus showed evidence of potential anti-quorum sensing at levels of 10% and 15%.
- CFUs and spectrophotometric analysis was inconclusive.

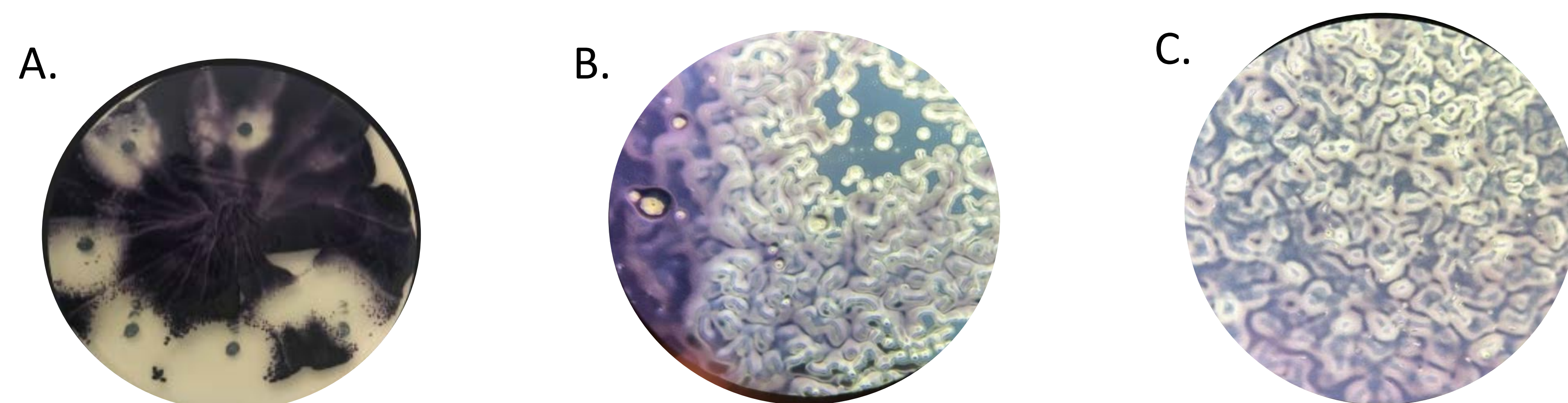


Figure 4. A. Spot plate assay to determine the effect on quorum sensing by serially diluted Eucalyptus oil. At a concentration at 10%, violacein production is inhibited. C. At 15% further inhibition of violacein.

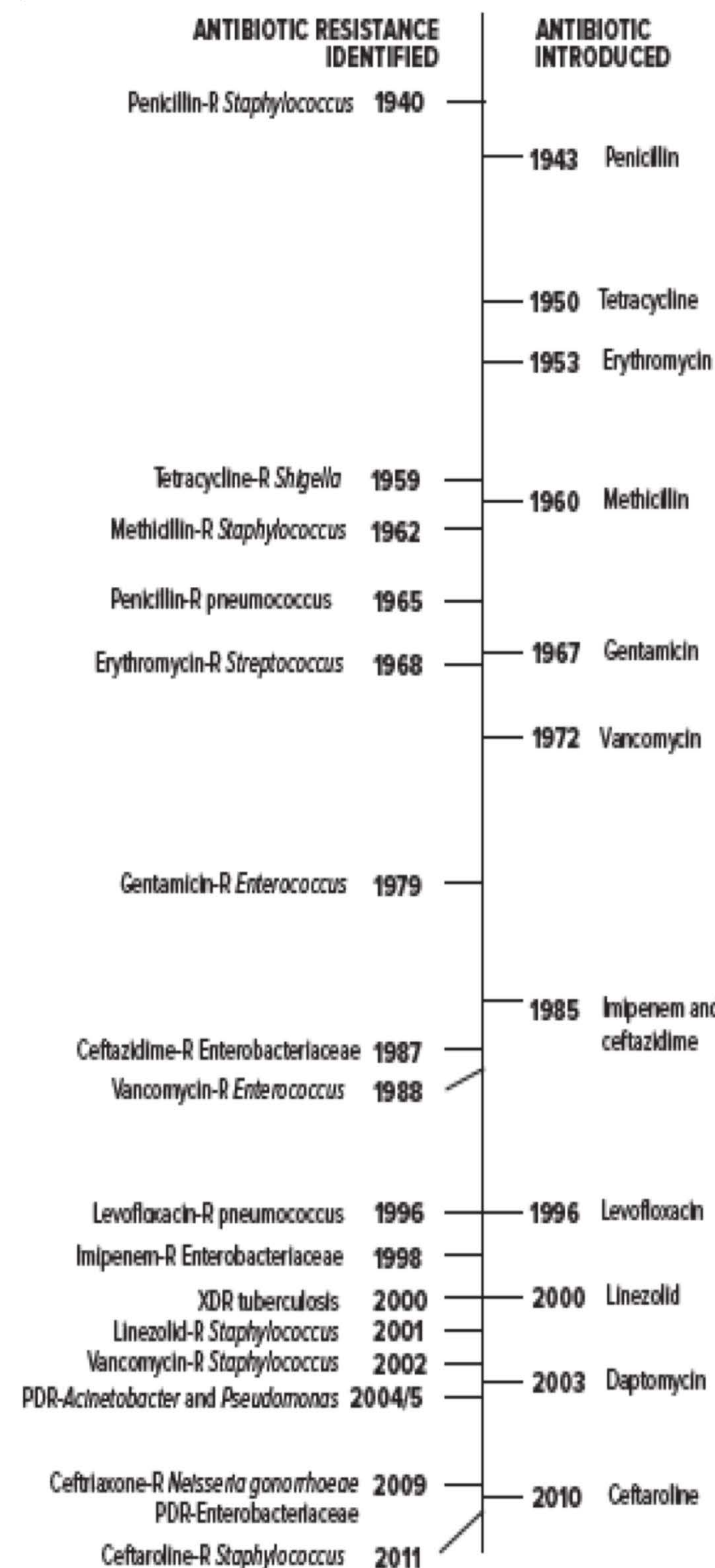


Figure 1. development of the resistance phenotype upon antibiotic implementation. (Ventola. 2015).

Results (continued)

- Spot plate treatment of Reishi elicited changes in colony morphology and an apparent loss in violacein production. Biofilm morphology appeared fragmented, as reported in previous studies (Fig 5).
- Analysis of Reishi treated inoculates showed a decrease in colony forming units as treatments levels increased.
- Violacein absorbance remained similar between the control, 5%, and 10% and significantly decreased when treated with 20% Reishi.

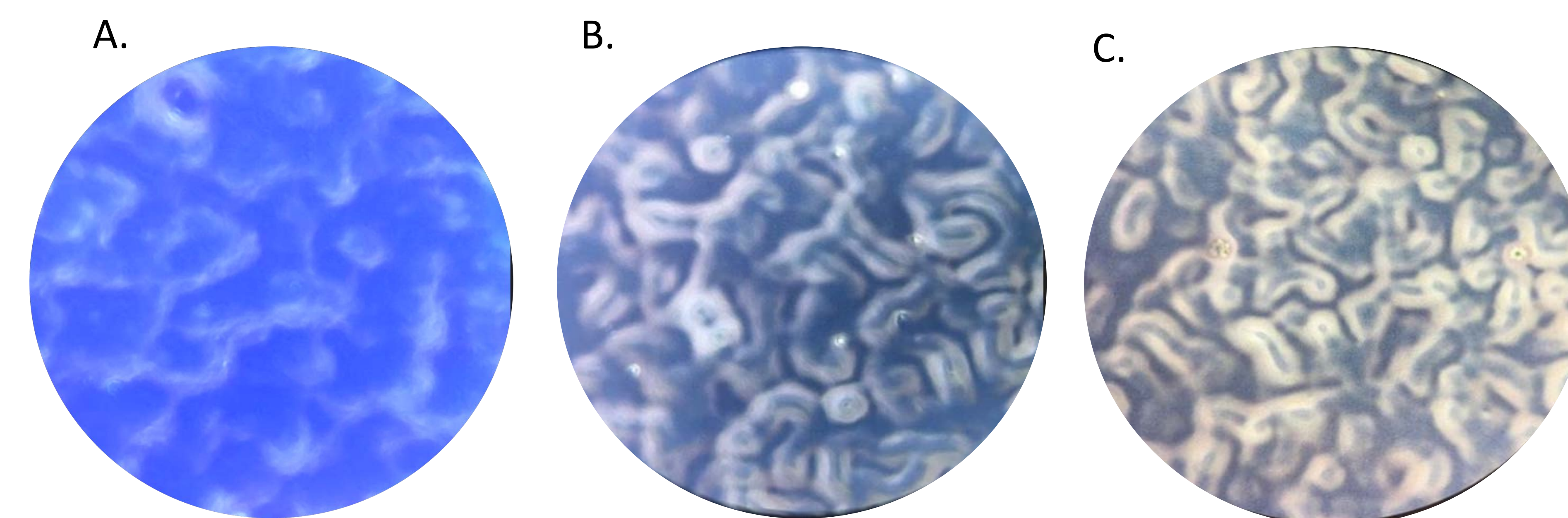


Figure 5. A. *C. violaceum* growth in the presence of no Reishi effects on CV1 shows distinct changes in colony growth. B. *C. violaceum* growth in the presence of 5% Reishi with an apparent loss in pigmentation C. *C. violaceum* grown in media of 20% Reishi.

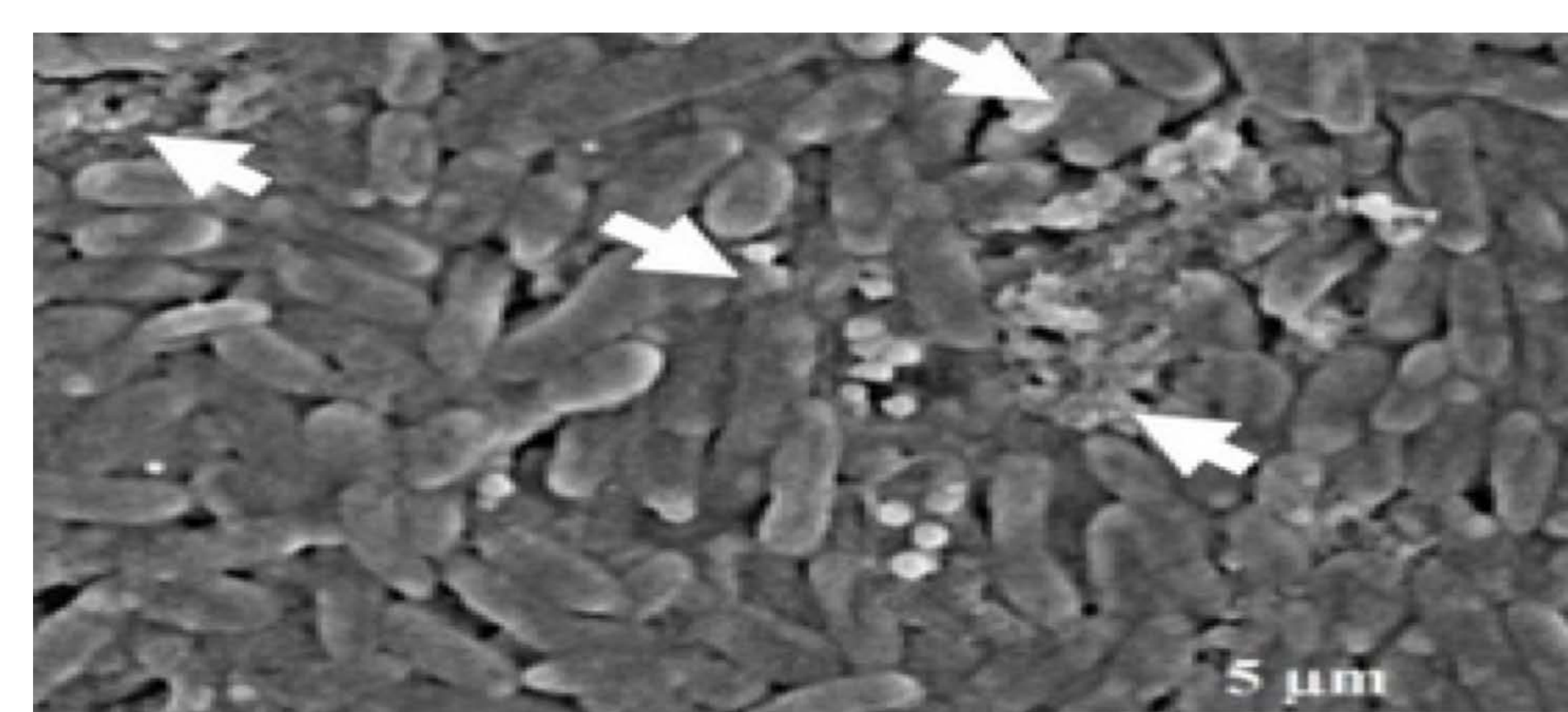


Figure 7. scanning electron microscopy of *C. violaceum* with apparent "fragmentation" indicated by arrows. Fragmentation within biofilm subsequently leads to loss in pigmentation. (Poli et al, 2018)

Discussion

- Based on the results of the spot plate study, *Reishi* derived extracts show potential evidence of anti-qS activity against *C. violaceum*.
- Decreases in pigmentation, along with changes in colony morphology and apparent biofilm fragmentation among *C. violaceum* colonies showed potential evidence of anti-QS activity (Fig 7)(Givskov and Rasmussen 2006).
- Implementing essential oils via spot plate assay showed incomplete absorbance into agar plates. Oils were prone during spread plate inoculation. A potentially more effective treatment delivery system is illustrated in Figure 6.
- Spectrophotometric analysis showed little changes in violacein production at the lowest concentrations. Since this was a pilot experiment with no replication, more spectrophotometric analysis is required to determine anti-quorum sensing versus biocidal activity of treatment.
- Eucalyptus in concentrations of 10-15% appeared to show anti-QS activity, however, further spectrophotometric analysis and determination of CFUs is required.
- Our results may be due to mimicry of N-Hexanoyl-L-homoserine lactone by other compounds found within Eucalyptus and Reishi.

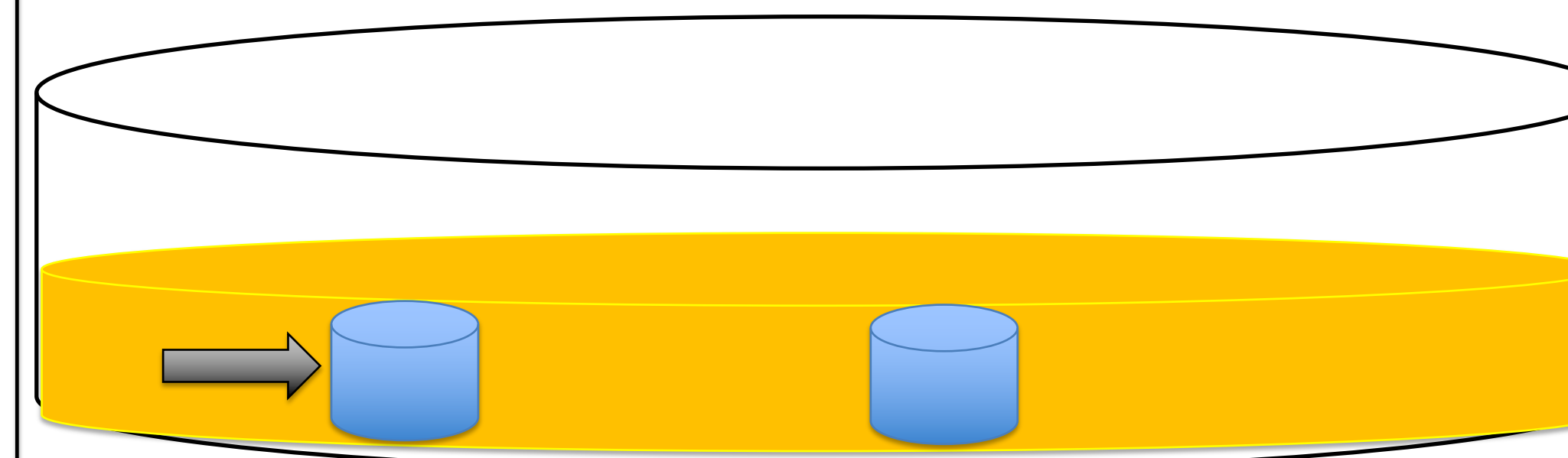


Figure 6. Essential oils could be implemented into agar via open holes in the agar. *C. violaceum* strains will be grown in top agar.

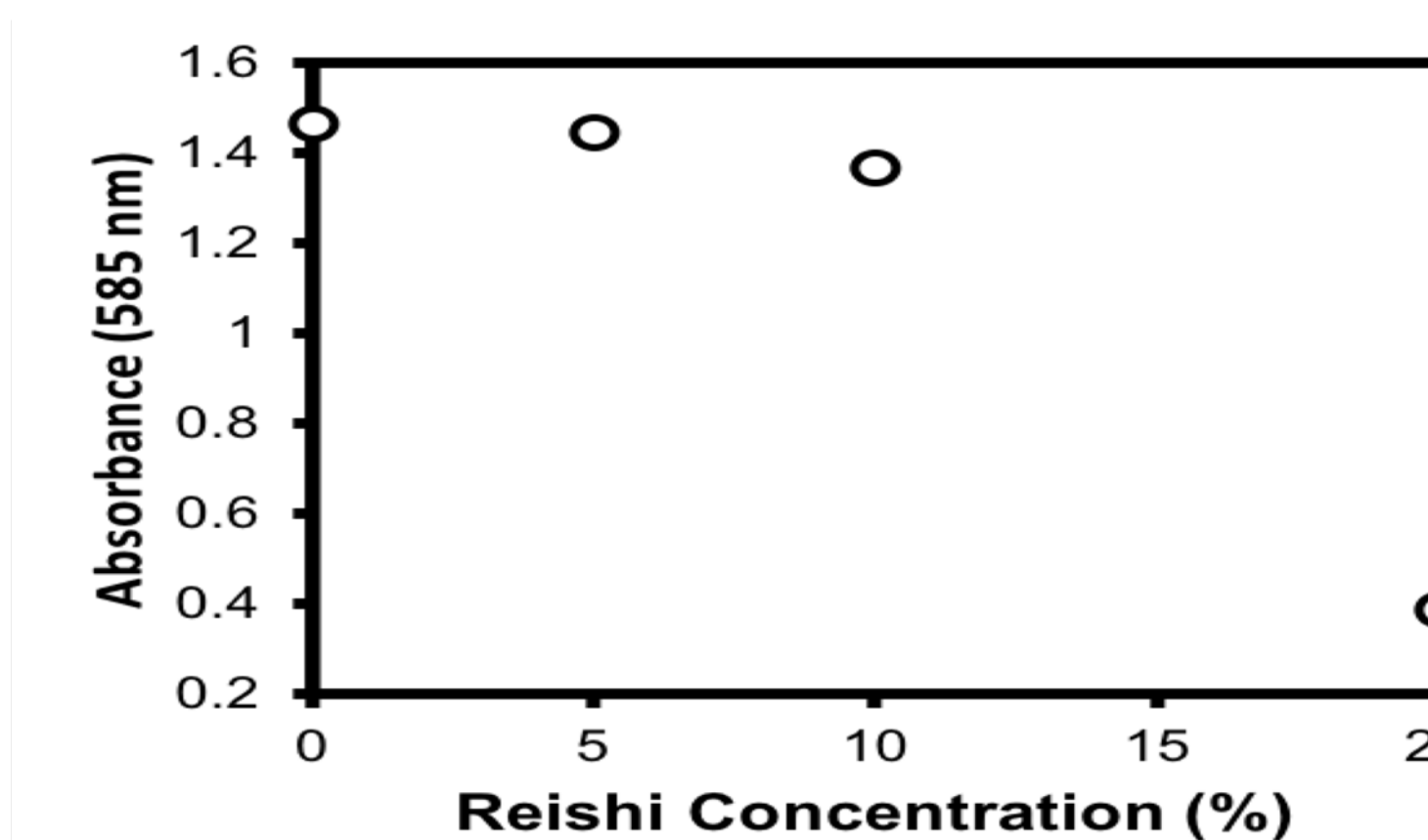


Figure 7. Spectrophotometric analysis showed pigmentation loss in *C. violaceum* colonies as treatment increased.

Literature Cited

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