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Biofilm exudates reactivate viable but non-culturable bacteria and fungi

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In response to stresses, microbes become dormant and show a low level of metabolic activity, which is known as a viable but non-culturable (VBNC) state. The metabolic activity of VBNC microbes has been hypothesized to be triggered by biofilm exudates (BFEx), which contain biochemicals exuded from developed fungal-bacterial biofilms. The present study was designed to evaluate this property in mineral-associated microbes. Serpentine, feldspar, and Eppawala rock phosphate (ERP) mineral samples were collected from Ussangoda, Kaikawala, and Eppawala, respectively. Mineral samples were crushed, sieved, and suspended in solutions, followed by mixing and serial dilution. The diluted mineral suspensions were inoculated onto Nutrient Agar and Potato Dextrose Agar plates using two treatments: one with the addition of sterile BFEx (10 µL) and one without BFEx, each with three replicates. The enumeration was performed to assess the total bacteria and fungi in each treatment. The difference between the counts with and without BFEx was used as a proxy for the VBNC count. The data were analyzed using a one-way Analysis of Variance test, and the means were separated using Tukey's honestly significant difference test. The results showed that the total number of VBNC bacteria was greater than the total number of VBNC fungi for each mineral type. On average, the serpentine, ERP, and feldspar minerals consisted of significant amounts of VBNC bacteria with, 72%, 54%, and 45%, and for VBNC fungi with 43%, 64%, and 25%, respectively. The serpentine had significantly higher VBNC bacterial counts ($p < 0.05$) compared to the other two minerals. However, VBNC fungal counts demonstrated no significant differences across tested mineral types. In conclusion, the findings suggest that BFEx more effectively reactivates mineral-associated VBNC bacteria and fungi. Further research is necessary, using more advanced methods, to reveal the underlying mechanisms of VBNC in mineral-associated microbes.

Keywords: *Biochemicals, biofilms, enumeration, minerals, VBNC*