

## Effect of bioavailable copper on *Oleidesulfovibrio alaskensis* G20 biofilm formation

*Tuesday, May 14, 2024 5:00 PM (20 minutes)*

Copper is known to have inhibitory effects on bacterial growth. However, sulfate-reducing bacteria (SRB) have demonstrated the ability to grow in the presence of toxic levels of metal ions. This study aimed to evaluate the influence of Cu(II) ions on biofilm formation by the SRB strain *Oleidesulfovibrio alaskensis* G20 (OA-G20) in a lactate-based medium supplemented with varying concentrations of Cu(II). When exposed to media containing high levels of Cu(II) (30 $\mu$ M), OA-G20 exhibited inhibited growth in its planktonic (free-floating) state. Conversely, under the same experimental conditions with elevated Cu(II) concentrations (e.g., 30-100 $\mu$ M??), OA-G20 displayed enhanced biofilm formation on glass surfaces. Microscopic observations revealed that the Cu-induced biofilms exhibited changes in cellular morphology and increased accumulation of carbohydrates and proteins compared to Cu(II)-free biofilms. Consistent with these findings, gene expression analysis using qPCR showed a significant upregulation of genes involved in sulfur and energy metabolism, EPS production, and stress response in the copper-induced biofilms. In contrast, genes related to cellular division (*ftsZ*, *ftsA*, *ftsQ*) were negatively regulated compared to the control. These results suggest that the presence of Cu(II) ions triggers alterations in the cellular morphology and gene expression levels of OA-G20, impacting its ability to adhere to surfaces and produce EPS. This adaptation, characterized by enhanced biofilm formation, represents a crucial strategy employed by OA-G20 to resist and cope with metal ion stress.

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**Session Classification:** Biology - Parallel

**Track Classification:** Biology