Adaptive Laboratory Evolution of *Kitasatospora* sp. SeTe27 Enhances Selenite Tolerance and Alters Cellular Morphology and Redox Stability

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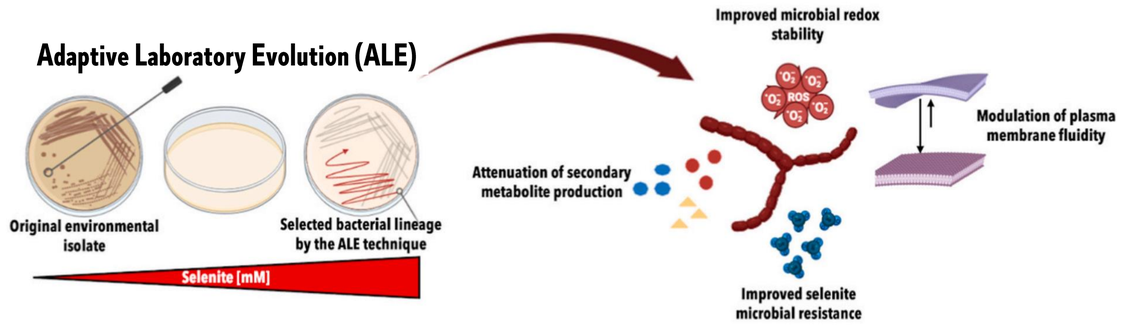
1. INTRODUCTION

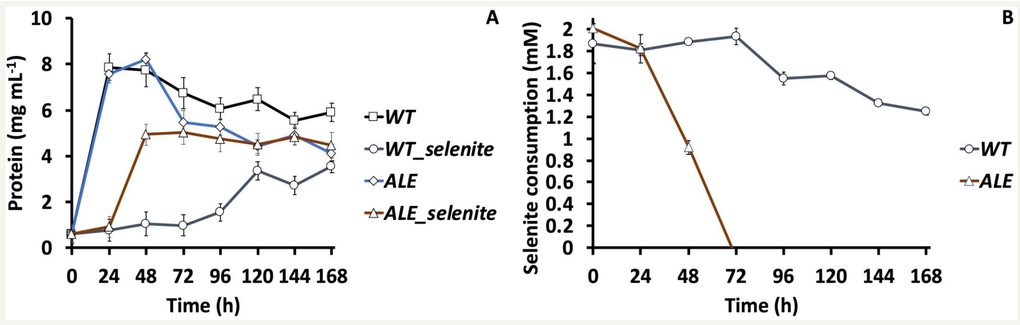
Selenium (Se) is a vital micronutrient for living organisms. It plays a crucial role in various biological functions through selenoproteins like glutathione peroxidases and thioredoxin reductases[1,2]. However, in high concentrations, selenium can be toxic, especially in its soluble oxyanion forms, **selenite (SeO_3^{2-})** and **selenate (SeO_4^{2-})**. **Selenite toxicity** arises from its ability to induce oxidative stress and disrupt protein functions[3]. However, certain microorganisms can mitigate selenite toxicity through their unique biochemical strategies. These strategies include reduction to elemental selenium, assimilation into seleno-amino acids, and volatilization. The potential of these microbial processes is vast, offering opportunities for **eco-friendly bioremediation and biotechnological applications**, such as producing selenium nanoparticles. Actinobacteria are promising due to their genetic diversity and metabolic capabilities[4].

This study investigates the ***Kitasatospora* sp. SeTe27 strain**, originating from uncontaminated agricultural soil in Sicily, to understand its tolerance and adaptive mechanisms to selenite stress. The study aims to uncover genetic and physiological adaptations enhancing the strain's resilience to selenite. These findings could aid in developing **biotechnological strategies for selenite pollution management and advancing microbial biotechnology**.



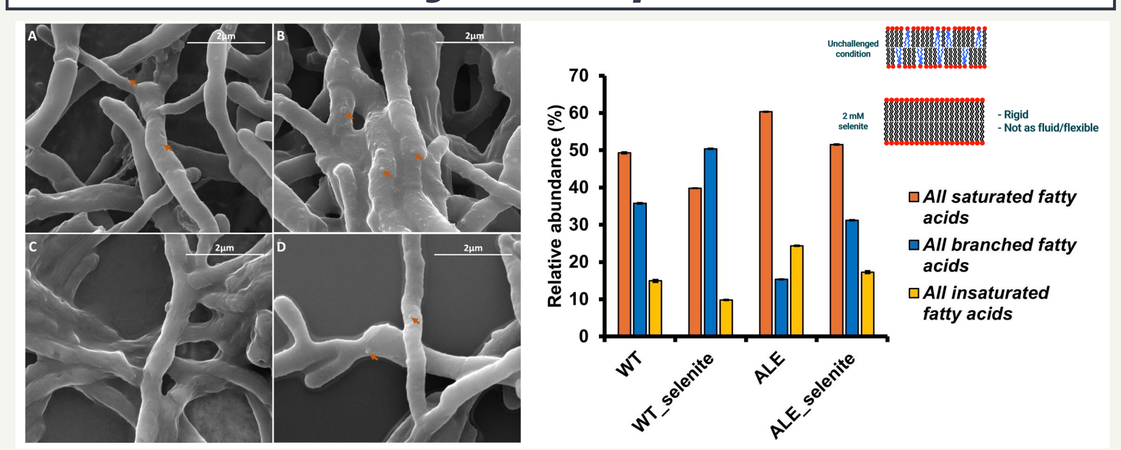
2. RESULTS

Growth profiles and selenite consumption capacities of *Kitasatospora* sp. SeTe27 WT and ALE strains



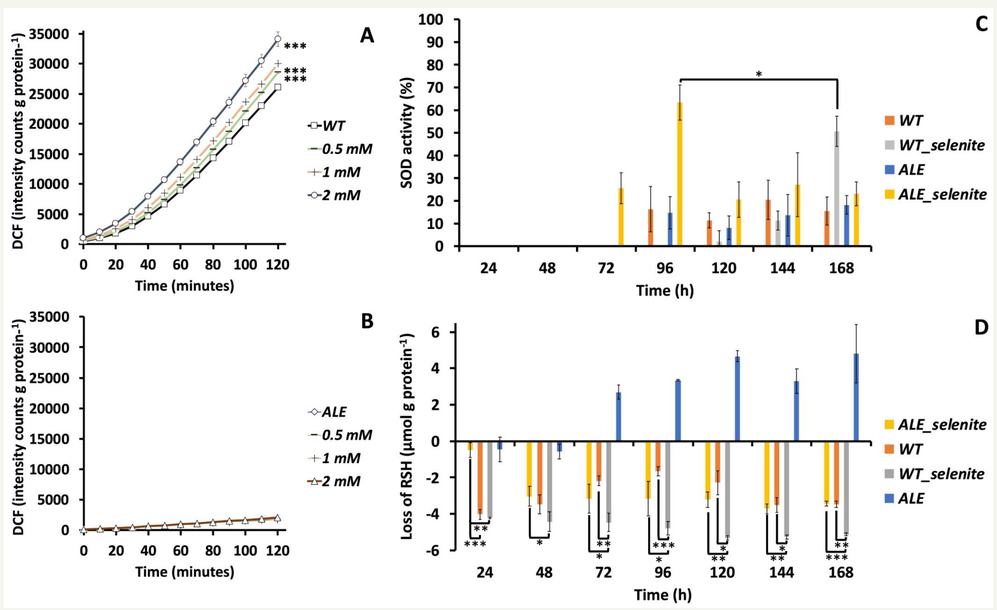
Kitasatospora sp. SeTe27 WT and ALE strain cultures and residual selenite content determination: growth profiles in the absence or the presence of 2 mM selenite (A) and selenite consumption kinetic (B).

Cell membrane adaptation and morphological changes in selenite-challenged *Kitasatospora* WT and ALE cells



1. *Kitasatospora* sp. SeTe27 hyphae's morphological features: unchallenged WT (A) and ALE (B) mycelium, while (C) and (D) depict those grown in the presence of 2 mM selenite. Arrows indicate surface membrane blebs.
2. Fatty acids profile of *Kitasatospora* sp. SeTe27 WT and ALE cells grew up to the late exponential growth phase in the absence or presence of selenite (2 mM).

Cellular stress and oxidative responses of *Kitasatospora* sp. SeTe27 WT and ALE strains under selenite pressure



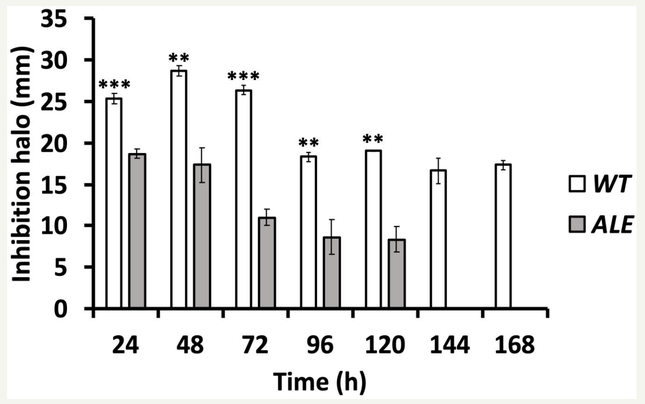
ROS, SOD, and RSH content determination: kinetic of ROS production by the *Kitasatospora* sp. SeTe27 WT (A) and ALE (B) strains as a function of selenite concentration, SOD induction (C), and loss of the intracellular RSH pool (D) in the WT and ALE strains upon 2 mM selenite challenge over time (*p-value <0.05, **p-value <0.01, ***p-value <0.001).

Genomic insights into the selenite resistance trait of the *Kitasatospora* sp. SeTe27 ALE strain

Missense mutations identified in the *Kitasatospora* sp. SeTe27 ALE strain genome.

Locus tag	Effect	Gene Product	Predicted function	Affected functional domain
001777	Ala6549Val	non-ribosomal peptide synthase/ polyketide synthase	Biosynthesis of secondary metabolites	13th condensation domain
004491	Asp731Ala	amino acid adenylation domain-containing protein		–
005291	Arg23Gly	response regulator transcription factor (DraR)		alpha-helix of the response regulator receiver domain
003756	Arg310Pro	DegT/DnrJ/ EryC1/StrS family aminotransferase	Unknown	–
004585	Val206Ile	pyridoxal-dependent decarboxylase	Metabolism of aromatic amino acids	Major domain
003444	Cys104Tyr	dipeptide ABC transporter ATP-binding protein	OppF, dipeptide uptake	ATP-binding domain
004215	Pro188Ala	TetR/AcrR family transcriptional regulator	LiuX, Isoleucine degradation	Repressor domain
004256	Leu204His	CbtA family protein	Cobalt uptake	5th TM alpha-helix
004455	Glu26Val	infB, translation initiation factor IF-2	protein synthesis	–
006314	Ala198Gly	SulP family inorganic anion transporter	anion:anion exchange transporter	7th TM alpha-helix
005760	Thr365Asn	ROK family transcriptional regulator	Unknown	–
006364	Arg17Trp	Ig-like domain-containing protein	Unknown	Signal peptide
007127	Glu9Ala	serine/threonine-protein kinase	Unknown	phosphorylase kinase domain 1
006013	Ser112Leu	hypothetical protein	Unknown	–

"-" indicates that the substitution does not fall in any of the functional/structural domains predicted via InterProScan.



1. Missense mutations identified in the *Kitasatospora* sp. SeTe27 ALE strain genome.
2. Comparative antimicrobial efficacy of *Kitasatospora* WT and ALE strains against *Staphylococcus aureus* ATCC 25923: zone of inhibition halos (mm) estimated consequently to *S. aureus* cells growth in the presence of cell-free spent media collected, over the incubation, from the WT and ALE strain cultures (**p-value <0.01, ****p-value <0.001).

3. CONCLUSION

This study provides a comprehensive analysis comparing the ALE-evolved strain to the wild-type (WT) strain. The key findings of this research are:

- **Enhanced Selenite Removal:** The ALE technique significantly improved the SeTe27 strain's ability to remove selenite.
- **Improved Redox Stability and Bioprocessing:** The ALE variant exhibited better redox stability and selenite bioprocessing capabilities than the WT strain.
- **Morphological Adaptations:** The SeTe27 strain showed adaptive responses such as changes in fatty acid composition, hyphae aggregation, and the formation of membrane-like vesicles to mitigate selenite stress on the cell membrane.
- **Genetic Mutations:** The ALE variant identified specific missense mutations in genes related to primary and secondary metabolism, suggesting a trade-off between energy-intensive processes and selenite bioprocessing, reflecting a survival strategy under selenite stress.

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