

Superoxide Dismutase Activity is Important for Oxidative Survival, Biofilm Formation and Mucoviscosity of *Klebsiella pneumoniae*



Klebsiella pneumoniae

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Abstract

Klebsiella pneumoniae is the causative agent of several nosocomial and community acquired infections (1, 2). Some of its virulence determinants have been identified but it is not known how it copes with reactive oxygen species. Superoxide dismutase (SOD) is responsible for removal of toxic superoxide radicals (3), and from NCBI database *K. pneumoniae* genome has three superoxide dismutase genes coding for Mn-, Fe- and CuZn- cofactored SODs. This work is designed to evaluate the importance of each SOD in oxidative survival, capsular polysaccharide production and biofilm formation of *K. pneumoniae*.

Phenotypic characterization of single, double and triple isogenic SOD mutants indicated that *sodB* alone or in combination with *sodA*, have a major impact on total SOD activity. It was also found that lack of one SOD is compensated through induction of remaining SODs. Finally, overexpression of *sodA* in single Δ *sodB*, Δ *sodC* and double Δ *sodBC*, has significantly increased capsular polysaccharide production and biofilm formation, which is linked to *K. pneumoniae* virulence.

Objectives

1. To demonstrate SOD activity in *K. pneumoniae*.
2. To assess SOD's role in *K. pneumoniae* growth in oxidative conditions.
3. To evaluate the importance of SOD in capsular polysaccharide production and biofilm formation of *K. pneumoniae*.

Methods

1. SOD activity and gene expression have been determined by WST kit and RT-PCR, respectively.
2. Growth assays have been done in LB and MB-media under aerobic condition and without or with addition of 60 μ M redox active compound methyl viologen dichloride hydrate (paraquat).
3. After overnight growth, capsular polysaccharide was extracted using hot-phenol *water* (4) and measured by the phenol-sulfuric acid method (5).
4. Biofilm formation was assessed by crystal violet staining (6), and structure by Scanning Electron Microscope (SEM) (7).

Results

- The major SOD activity of *K. pneumoniae* is encoded by *sodB* as the mutation of this gene alone or in combination with *sodA* or *sodC* would significantly reduce total SOD activity (Figure 1).
- The lack of one SOD is compensated through induction of remaining SODs (Figure 2).

- Mutation of *sodA* alone or in combination with *sodB* and *sodC*, reduces *K. pneumoniae* growth, capsular polysaccharide production and biofilm formation (Figure 3, 4, 5, respectively).

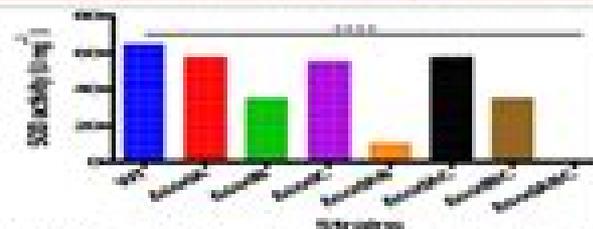


Figure 1. Quantitative SOD activity assay. Significant differences were observed between wild type (WT) and the mutants (***) $p < 0.001$, n=3.



Figure 2. *sod* gene expression (*sodA*, *sodB* and *sodC*) in *K. pneumoniae* in response to oxygenated environment. Relative gene expression was determined in LB cultures in oxygenated environment. Difference of ± 2 fold, up or down, was considered to be significant (n=3).

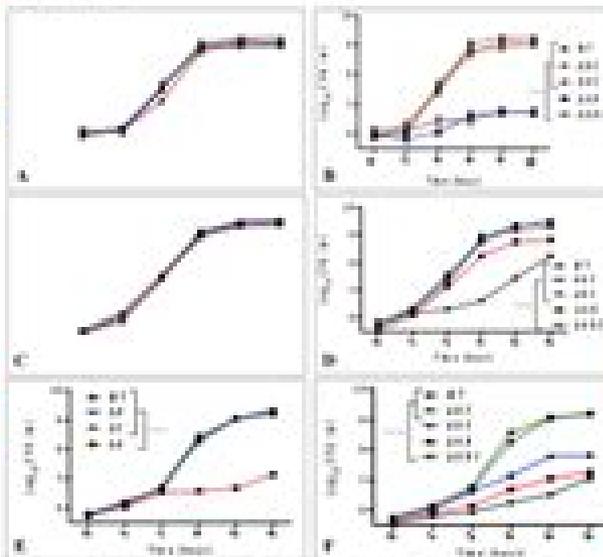


Figure 3. Growth curves of wild type and mutants under aerobic condition in MB medium (A and B), and in LB medium without (C, and D) and with 60 μ M oxidizing agent paraquat (E and F) (n=3).

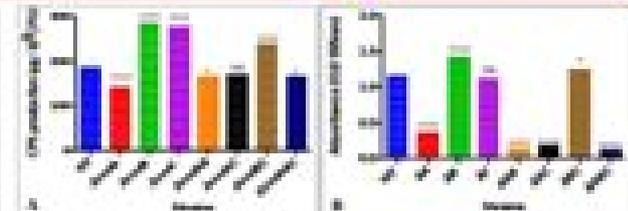


Figure 4. (A) The capsular polysaccharide production of WT and mutant strains grown in LB broth. The capsular polysaccharide was extracted and determined by sulfate and assay. Data shown are mean of one experiment in triplicate.

(B) Biofilm formation of WT and mutants using LB broth medium in microplate wells after overnight growth in aerobic condition. Data shown are the mean value from eight independent experiments each done in triplicate. (***) $p < 0.001$.

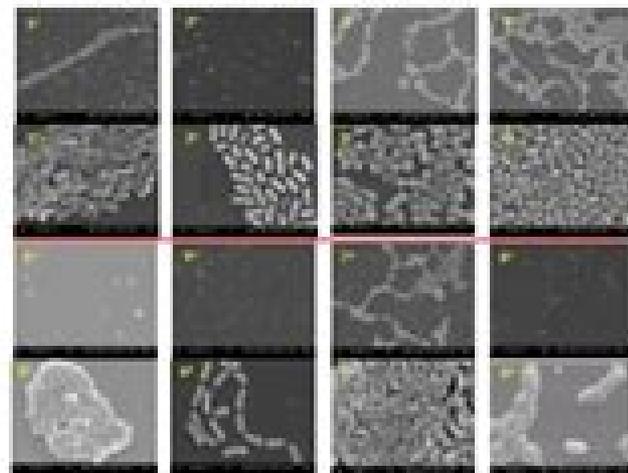


Figure 5. Biofilm structures of *K. pneumoniae* strains grown in LB medium under microaerobic conditions. Images were taken by scanning electron microscope. (A) WT, Δ *sodA*, Δ *sodB*, Δ *sodC*, Δ *sodA* Δ *sodB*, Δ *sodA* Δ *sodC*, Δ *sodB* Δ *sodC*. (B) High magnification indicates the presence of extracellular polysaccharide materials on the surface of bacterial cells. Scale bar, 5 μ m.

Conclusions

1. SOD activity is required for aerobic growth of *K. pneumoniae*.
2. *sodB* was found to be responsible for the major SOD activity in *K. pneumoniae*, and *sodA* genes are inducible.
3. Over expression of *sodA* significantly enhanced capsular polysaccharide production, biofilm formation and changed the biofilm structure.

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