# Cerium Nitrate: Anti-*Candida albicans* activity in Planktonic cells and Biofilms

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## Introduction

***C. albicans*** **ability to form biofilms has important clinical repercussions since it results in a reservoir of cells with promoted antifungal resistance to antifungal agents. Biofilms are commonly related with medical indwelling devices such as intravascular or urinary catheters. Cerium is a member of the lanthanides or rare earth elements. Several medical applications have been addressed to cerium compounds, based upon its antiemetic, antineoplastic, antiseptic and immunomodulatory properties. All these applications are still controversial, with the exception of its topical use for the treatment of burn wounds. Nevertheless studies addressing its antimicrobial effect produced contradictory results.**

**Aim: To clarify cerium nitrate anti-*Candida* activity, its ability to impair biofilm formation and its potential use in *C. albicans* biofilm treatment.**

## Material and Methods

**Clinical isolates of *C. albicans* (n=6), were tested accordingly to the classical microdilution susceptibility protocol M27-A3. Serial concentrations of Cerium nitrate ranging from 1 to 0.244 M were used and the minimal inhibitory (MIC) and minimal lethal (MLC) concentrations were determined.**

**To investigate cerium nitrate antimicrobial activity by Flow Cytometry *C. albicans* ATCC 90028 was incubated with different concentrations of cerium (0.03, 8 and 50mM) for 0.5, 1.5, 3, 6 and 24h, at 35 ° C, with agitation. At each time point yeast cells were collected, stained with FUN-1 0.5µM (a metabolic marker) or PI 1µg/ml (a membrane injury marker) and analyzed in a flow cytometer (FACSCalibur BD Biosciences, Sydney). Cytometric readings were performed in the FL2 fluorescence channel (BP 585/42 nm) for FUN-1 staining and FL3 fluorescence channel (LP 670 nm) for PI. Results are represented as a staining index (SI) for FUN-1, representing the reason between treated and untreated cells; and as the percentage of dead cells for PI. From each suspension, yeast cells were plated on Sabouraud dextrose agar and incubated for 24h for assessment cell viability.**

**Biofilms were grown in polystyrene plates, in the presence of three cerium concentrations (0.03, 8 and 50 mM) for 24 hours. The biofilm metabolic activity and total biomass were quantified colorimetrically with XTT and crystal violet assays, respectively. Biofilm susceptibility was also assessed (tested cerium concentrations ranging from 0.5 mM to 1 M); MIC end point for biofilms (SMIC) was determined based on the lowest drug concentration producing 50% reduction of the metabolic activity relative to the metabolic activity of control (measured by the XTT reduction assay).**

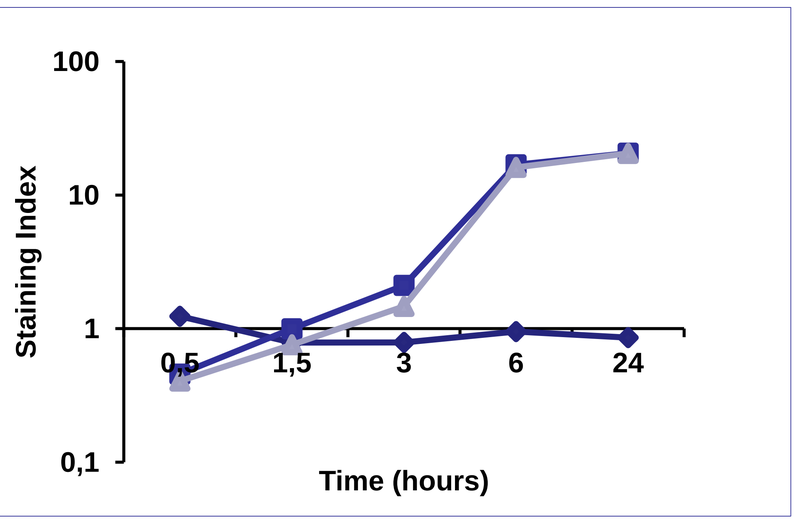
## Results

| Planktonic cells | | Biofilm cells (24h) | |
|---|---|---|---|
| $MIC_{50}$ (mM) | MLC(mM) | $sMIC_{50}$ (mM) | $sMIC_{90}$ (mM) |
| 0.03 | 8 | 500 | 1000 |

**Cerium nitrate demonstrated antimicrobial activity against *C. albicans* planktonic and sessile cells, although biofilm cells needed a much higher concentration to obtain the same percentage of inhibition.**

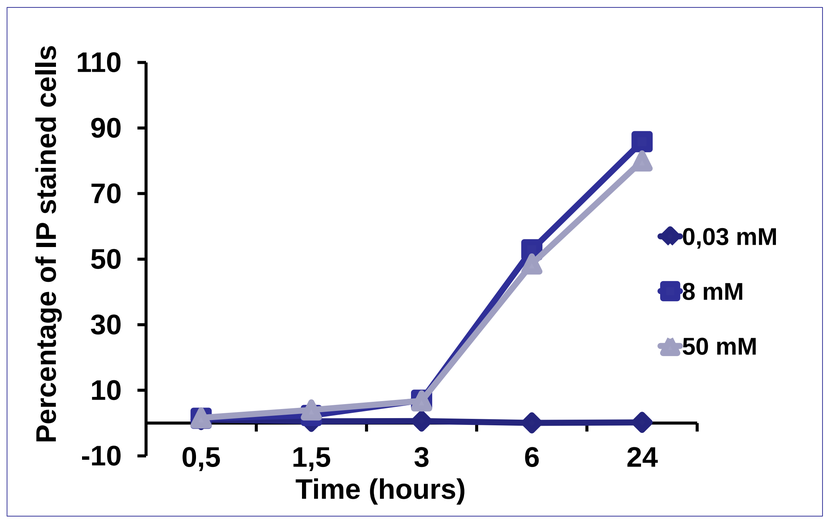
**Treatment of pre-formed biofilms with cerium resulted in 90% reduction in the biofilm metabolic activity with 500 mM, and 92% of total biomass disaggregation with 16 mM.**

**Cerium Nitrate effect in cell metabolic activity**



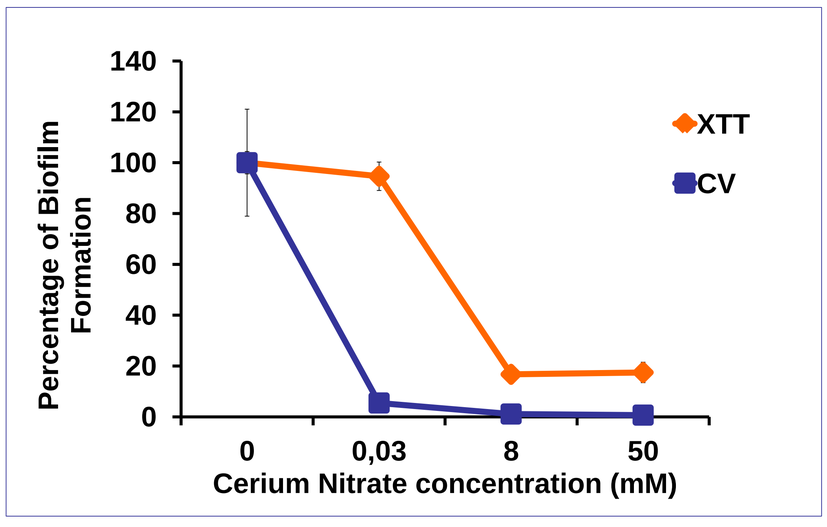

**Cerium Nitrate impairs *C. albicans* metabolic activity, as seen by the raise in FUN-1 fluorescence. This effect starts at 3h for the 8 and 50mM concentrations, increasing with the incubation time**

**Percentage of cells with membrane injury caused by Cerium Nitrate**




**The capacity to cause membrane damage is only significantly achieved at 6h time point for the 8 and 50mM concentrations reaching approximately 80% at 24h**

**Cerium Nitrate impact in biofilm formation**




**Cerium nitrate was able to reduce biofilm metabolic activity up to 83% at MLC concentration**

**The biomass formation was efficiently impaired even in the MIC concentration**

## Conclusion

**Cerium nitrate exhibits not only antimicrobial but also anti-biofilm activity against *C. albicans,* being a promising agent for the prevention or treatment of candidosis associated with medical indwelling devices.**

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