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Boric Acid and EDTA Combination Inhibits Growth of *Candida albicans* and *Gardnerella vaginalis*

Tyler Beach, Benjamin Hart, Thomas Piotrowski, Bryan Larsen, PhD

Abstract

Candida albicans and *Gardnerella vaginalis* are two common infectious organisms of the female urogenital tract. Single agent boric acid (B) topical preparations have been a treatment for resistant gynecologic *Candida* and *Gardnerella* infections, but it is worth exploring combinations of inhibitors to determine if a more effective treatment may be developed. Our study aims to determine the efficacy of BA alone in comparison to the combination of BA and EDTA (C) by measuring organism growth, size, and morphology. Three concentrations of boric acid are B1=5%, B2=0.5% and B3=0.05%; BA:EDTA ratio=10:1.

Methods

- Overnight cultures of *C. albicans* grown in Sabourad's broth, varying concentrations of B, or varying concentrations of C media provided dose-response data
- Forty-eight hour cultures of *G. vaginalis* grown in modified Brain-Heart-Infusion (BHI) broth, varying concentrations of B, or varying concentrations of C media provided dose-response data
- C. albicans* was imaged using light microscopy
- G. vaginalis* was imaged using scanning electron microscopy (SEM)
- Forward scatter and cell count data measured by flow cytometry
- Fluorescence data based on flow cytometry fluorescence channel 1 (FL1-H)
- Protease data collected using protease assay kit and fluorescence plate reader

Results

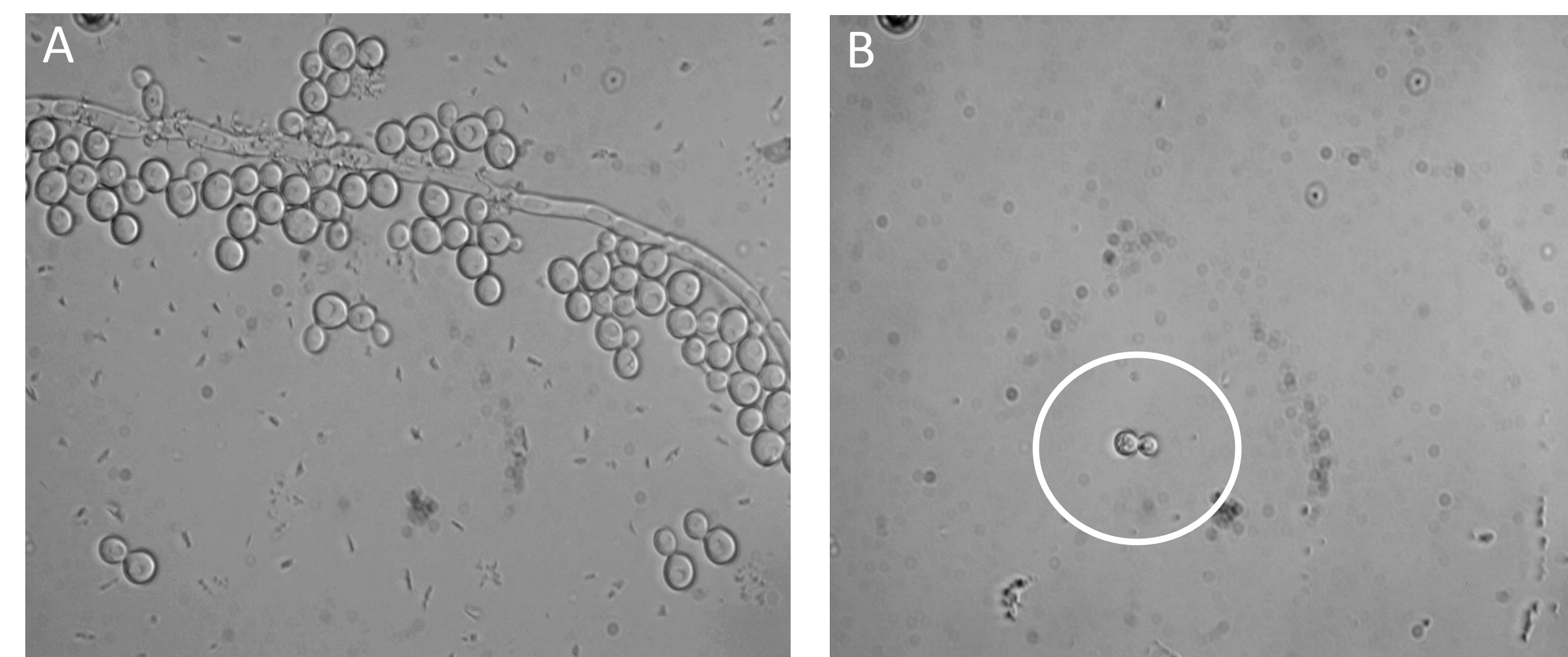


Figure 1. Light micrographs of *C. albicans* (strain 6). **A** Growth control grown overnight in Sabourad's broth. Notice the hyphal growth and size of cells. **B** Cells grown overnight in 5% boric acid. Notice the lack of hyphal growth and the small cell size compared to control.

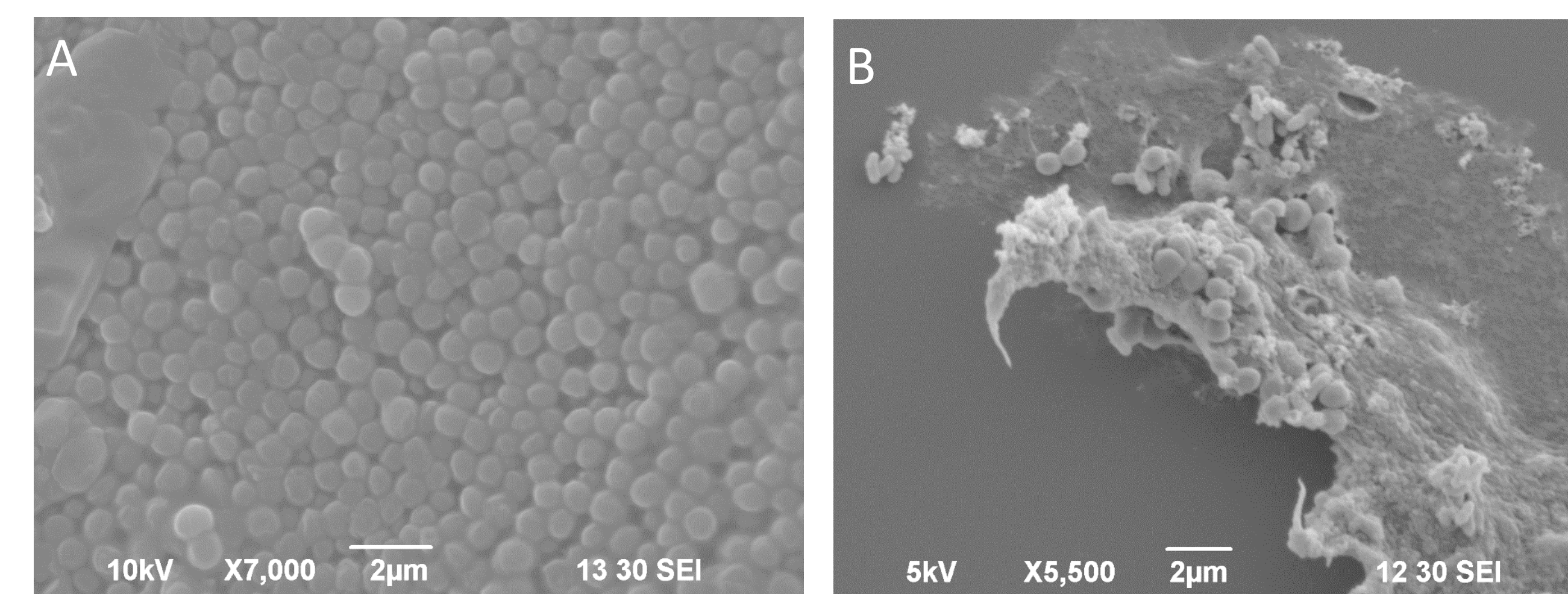


Figure 2. Scanning electron micrographs of *G. vaginalis* (BEI strain 19). **A** Growth control (48 hours in modified BHI broth). Note the uniformity and abundance of the cells. **B** Cells grown in 5% boric acid solution. Notice the aberrant bacterial forms having appearance of rod-shaped organisms and generally smaller size.

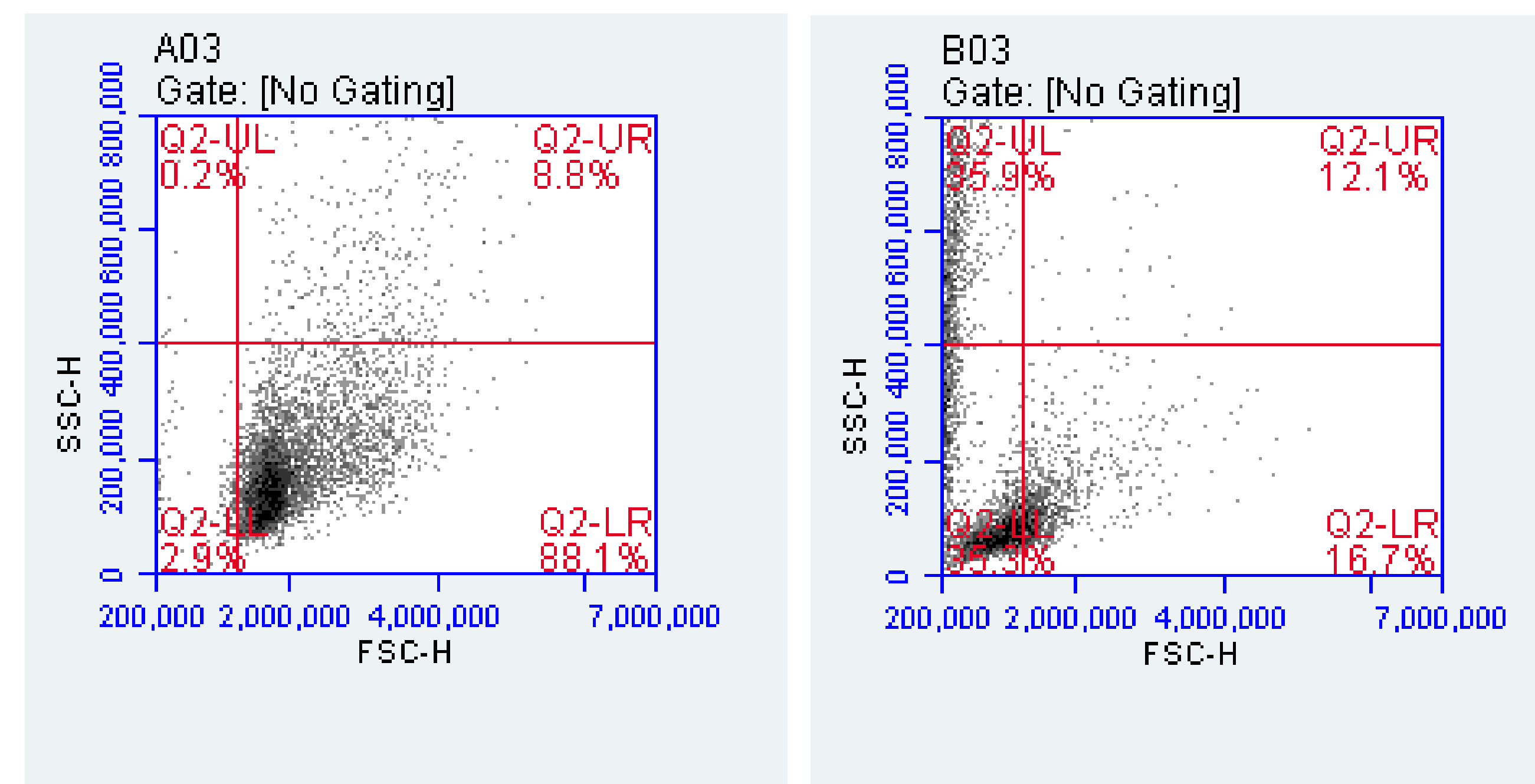


Figure 3. Flow cytometry plot of *C. albicans* forward scatter (strain 3). Note that the control on the left shows greater cell size (higher FSC) than cells grown in boric acid. Also notice that two distinct populations of smaller size are evident in cells grown in high concentration of inhibitor. Similar results were seen in cells grown in combination media.

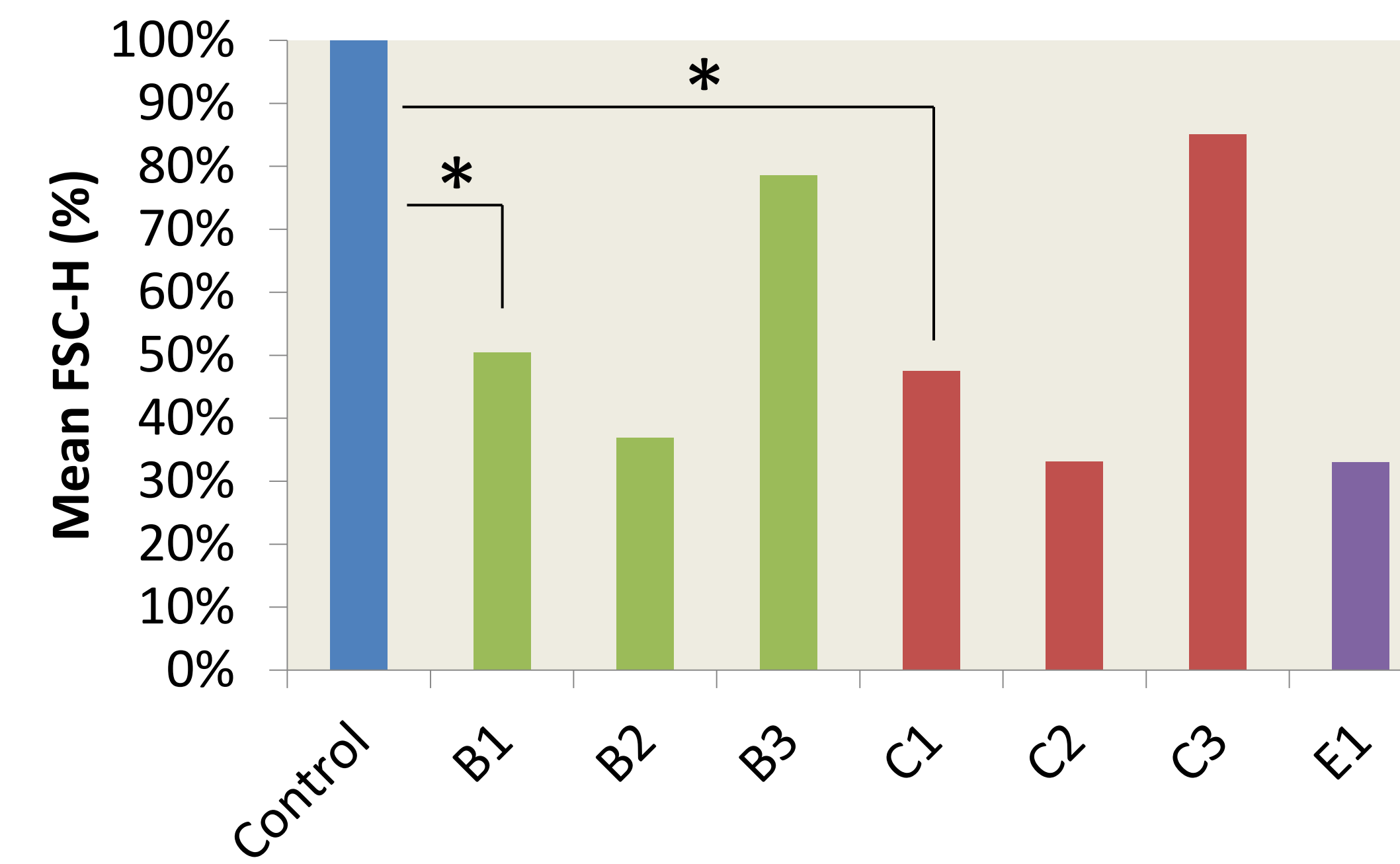


Figure 4. Average forward scatter data collected from 2 sets of 11 strains, 3 concentrations of 2 inhibitors (154 observations) of *C. albicans* grown overnight. Cells grown in B1 media showed a 50% decrease in cell size relative to growth control. Also notice cells grown in C1 media exhibited a 52% decrease in average cell size. * = $p < 0.001$

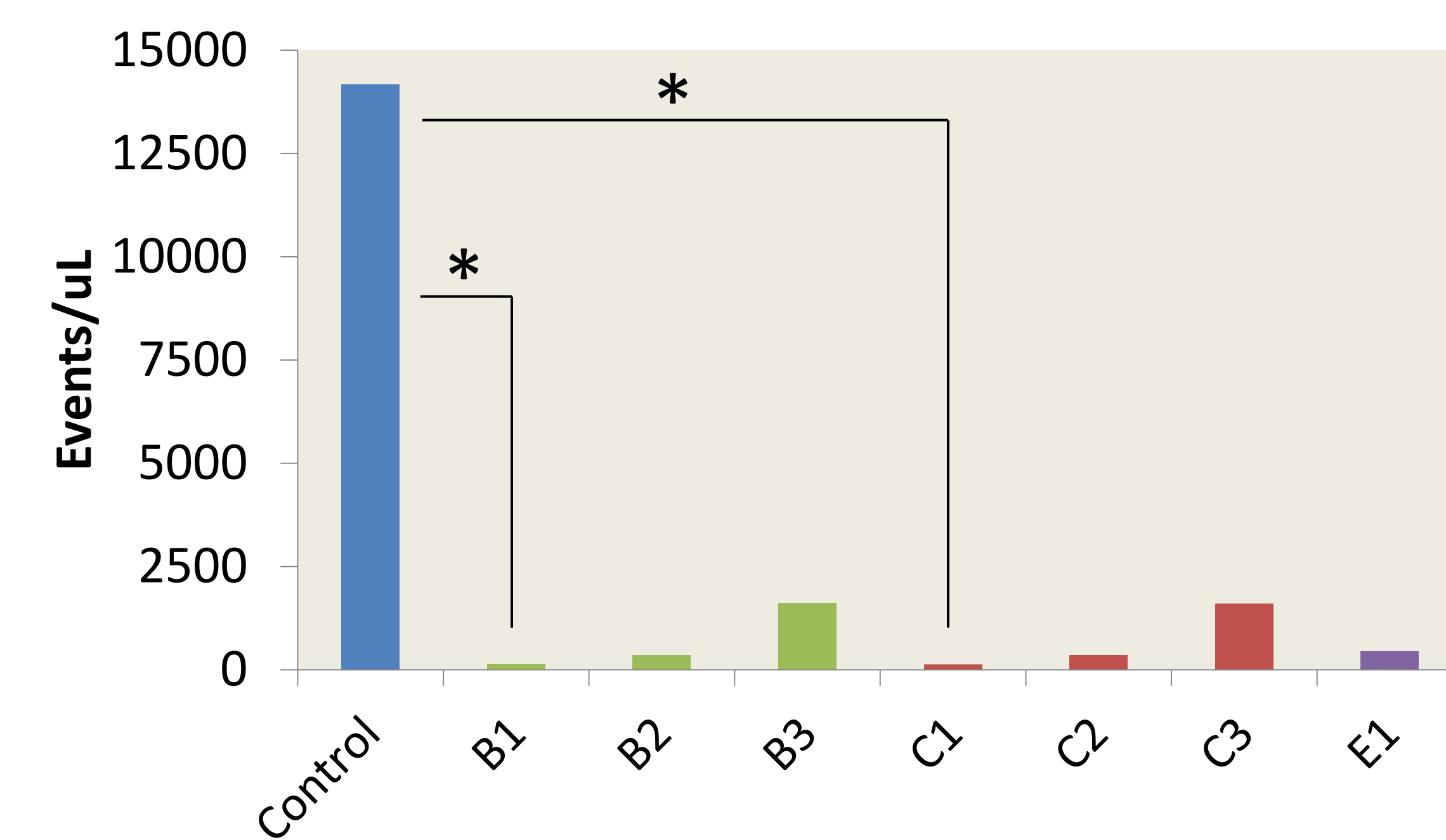


Figure 5. Events/ μ L collected from 2 sets of 11 strains of *C. albicans* grown overnight in three concentrations of boric acid and combination media. Notice that cells grown in high concentrations of inhibitors B1 and C1 are significantly reduced in number compared to control (* = $p < 0.001$).

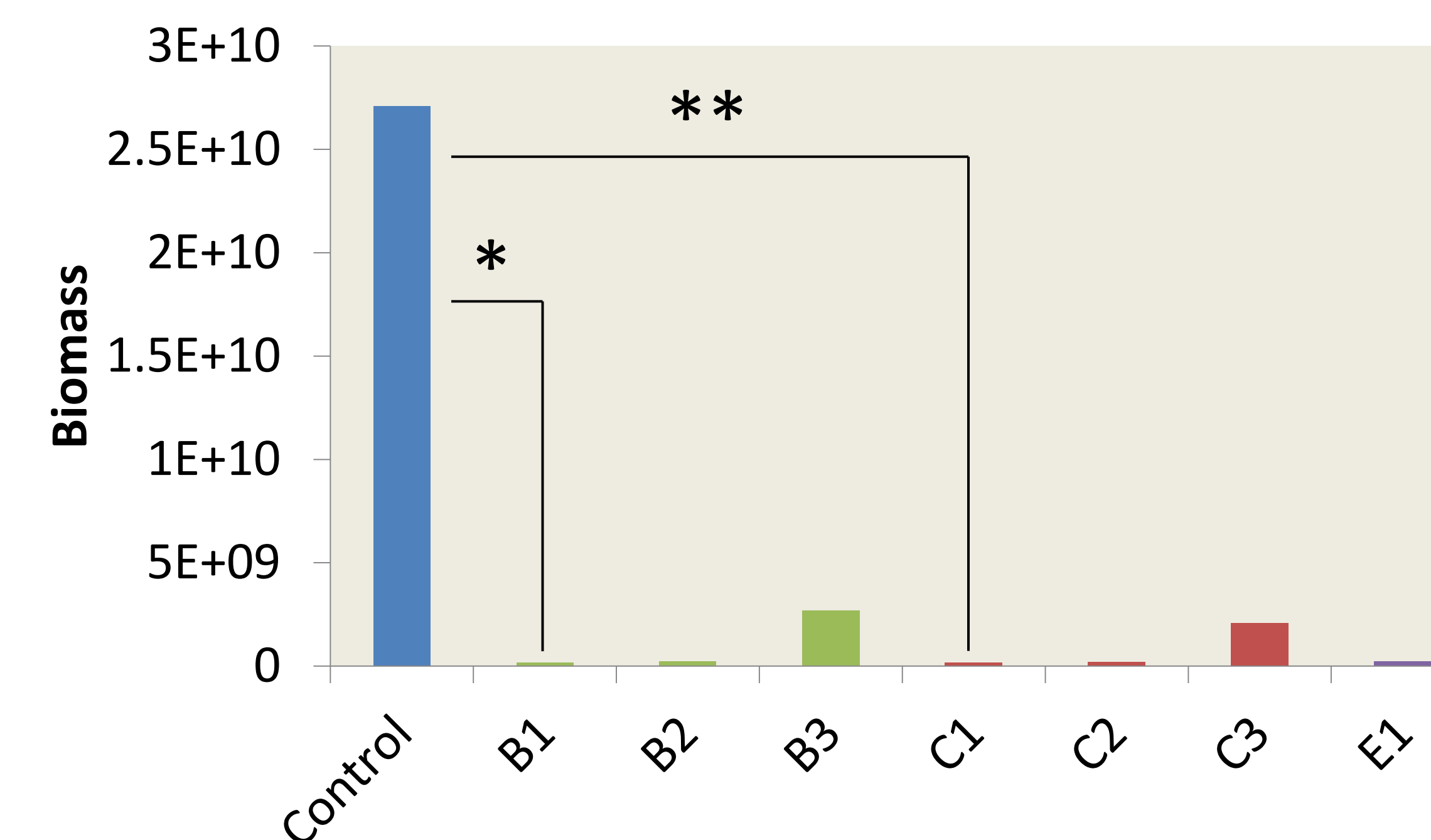


Figure 6. Mean biomass of 2 sets of 11 strains of *C. albicans* was calculated as a product of FSC-A and events/ μ L. Biomass of cells grown in high concentration of inhibitors was significantly reduced. * = $p < 0.01$, ** = $p < 0.001$

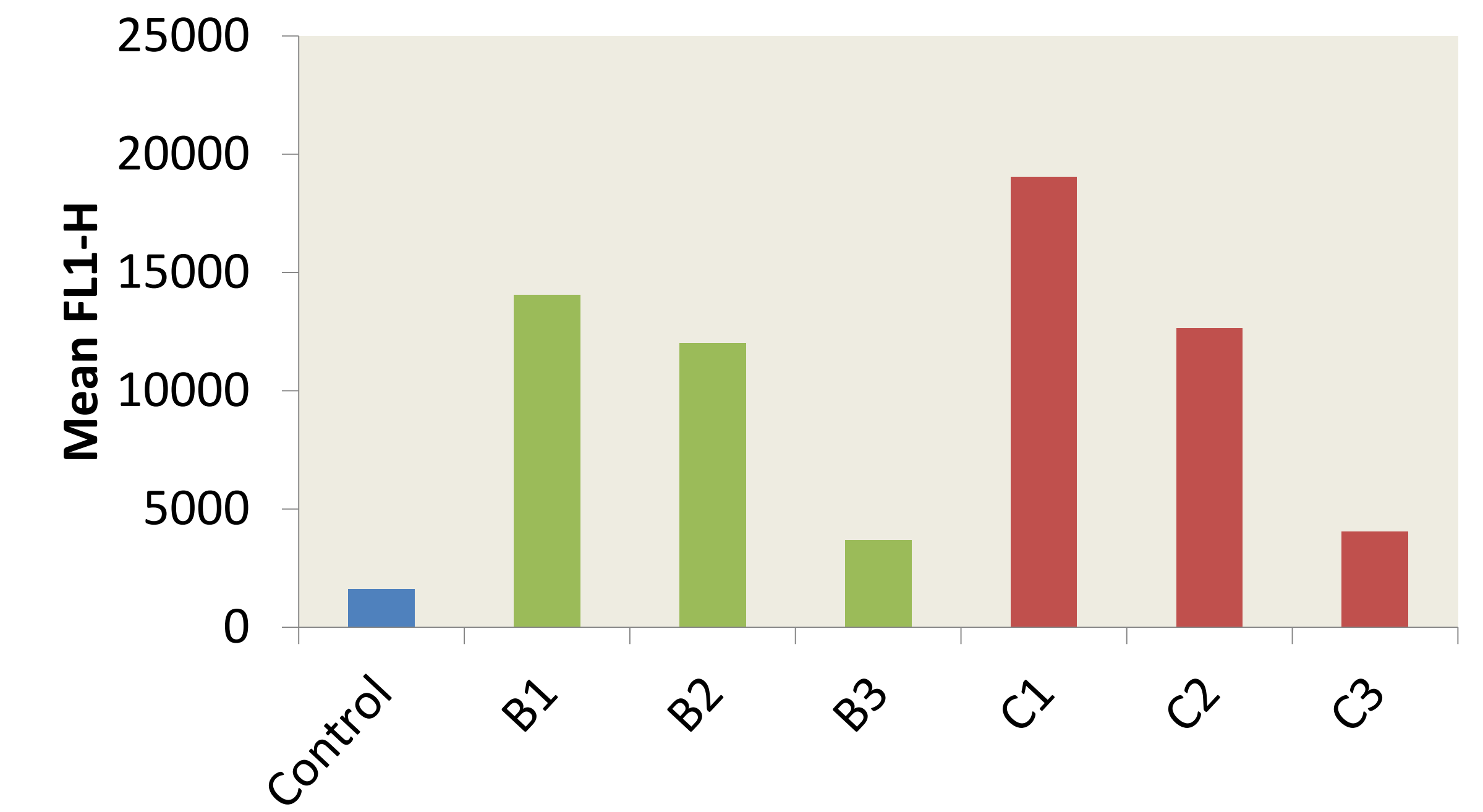


Figure 7. Autofluorescence of 11 strains of *C. albicans*. Cells grown in inhibitors showed higher incidental fluorescence in channel 1, potentially indicating a stress response to boric acid and EDTA. Cells grown in high concentration combination media exhibited the greatest autofluorescence.

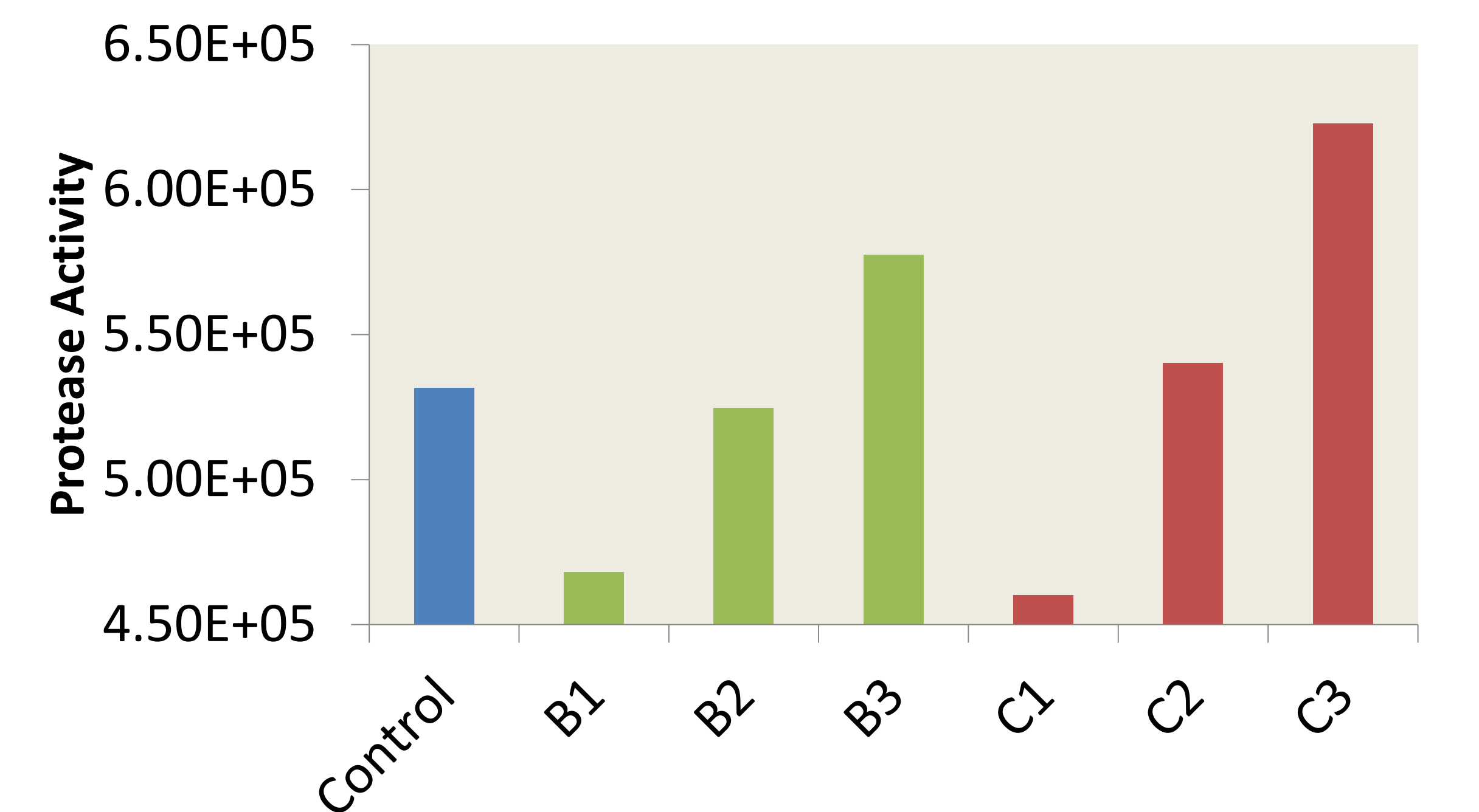


Figure 8. Inhibition of virulence-associated protease activity in 11 strains of *C. albicans*. Protease activity was decreased in cells grown in high concentration of inhibitors. Cells grown in high concentration combination media show less protease activity than those inhibited by high concentration boric acid. Also notice that low concentrations of inhibitor appear to increase protease activity.

Conclusion

These data provide evidence supporting the potential use of a combination therapy in *Candida* and *Gardnerella* infections and prompt additional investigation into combination therapies with EDTA. Physical evidence of cell involution in boric acid suggests the mechanism of inhibition may involve apoptosis. The finding of increased autofluorescence may be a novel finding in cell stress or apoptosis.