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Estradiol effect on vaginal *Lactobacillus crispatus*

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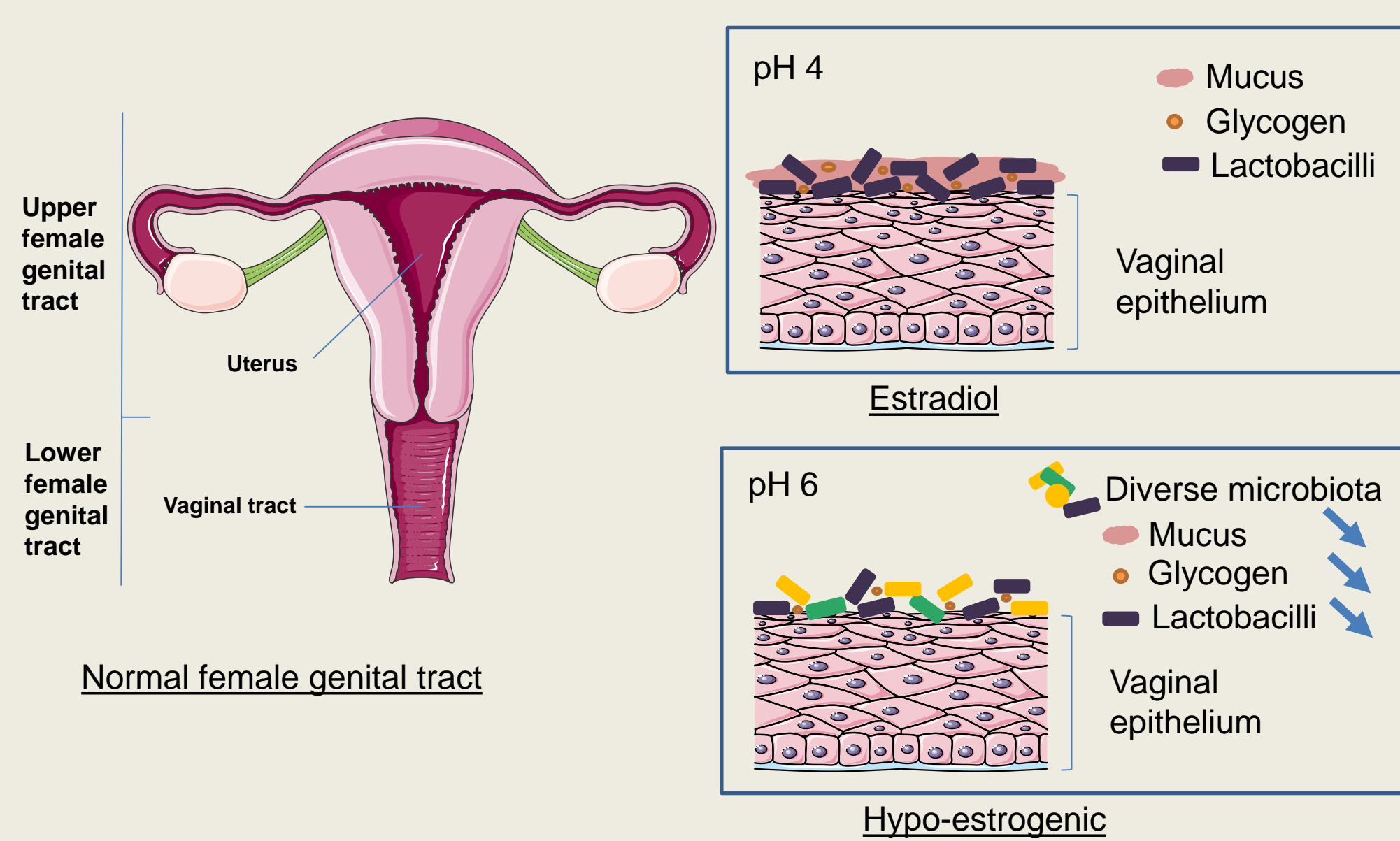
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Introduction

Lactobacilli are the major phylum of the normal human vaginal microbial community. Several studies have revealed that vaginal disorders and dysbiosis are associated to an important decrease of the Lactobacilli population and the emergence of pathogens. Many strategies have been proposed to protect the vaginal epithelium and re-equilibrate the microbiota to inhibit proliferation of undesired bacteria. Their real impact remains insufficient presumably because of the complex interactions between the microbiota and the host. Indeed, events such as menopause or child-birth are associated to both hormonal and microbiota variations. Moreover, a direct effect of estrogens on the vaginal microbiota has never been investigated. We choose to focus the present study on the potential effects of estradiol on *Lactobacillus crispatus*, one of the dominant species in the healthy vagina. *L. crispatus* strains CIP104459 (Institut Pasteur) and V4 from vaginal swab origin were chosen to investigate the effects of estradiol on their growth, biofilm formation, auto-aggregation and adhesion.

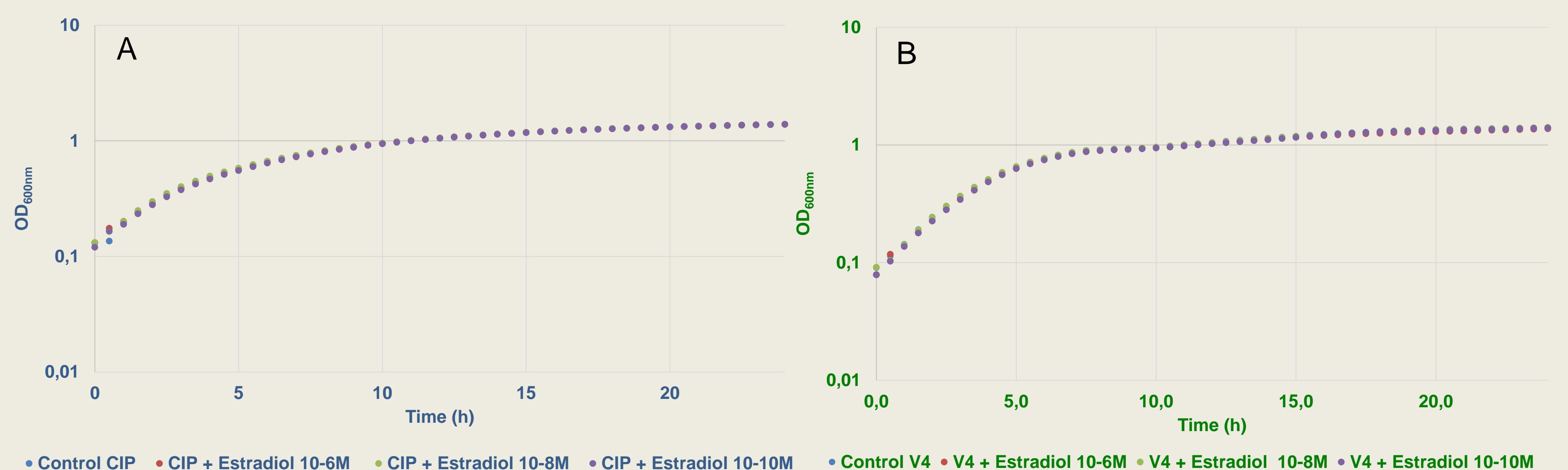
1

Vaginal environment



2

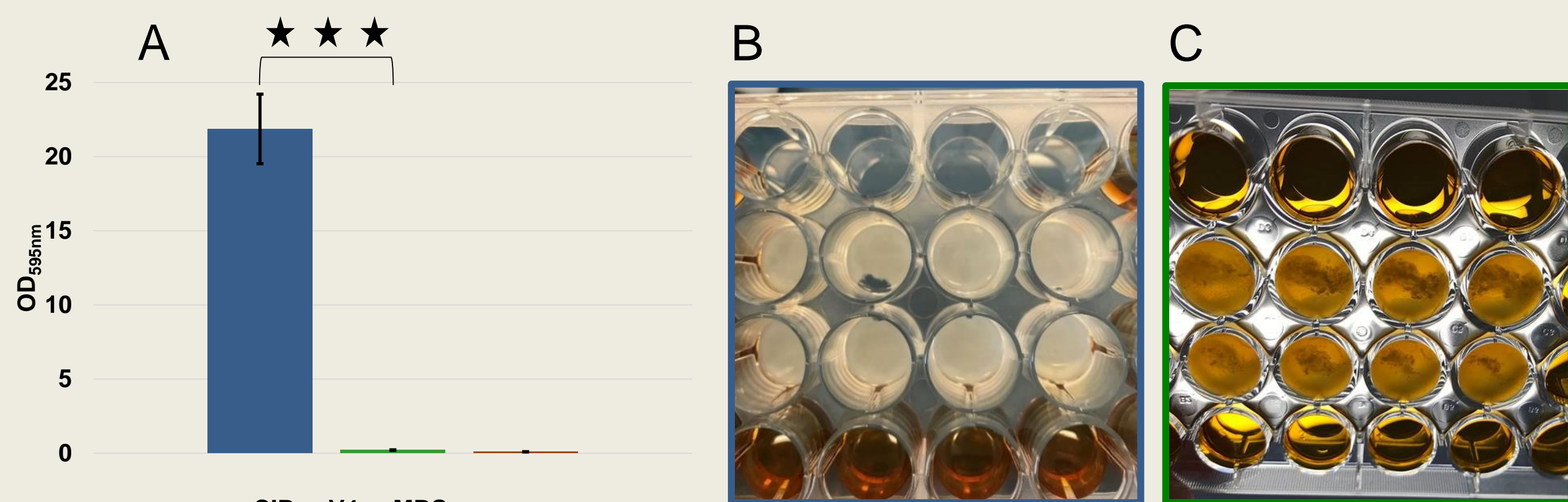
Effect of 17 β -estradiol on CIP and V4 strains growth



Kinetics of growth of *Lactobacillus crispatus* (A) CIP and (B) V4 strains with 17 β -estradiol at three concentrations under anaerobic conditions at 37°C for 24h in MRS broth. The optical density was measured each 15 minutes at 600nm. All data are the mean of four independent experiments.

3

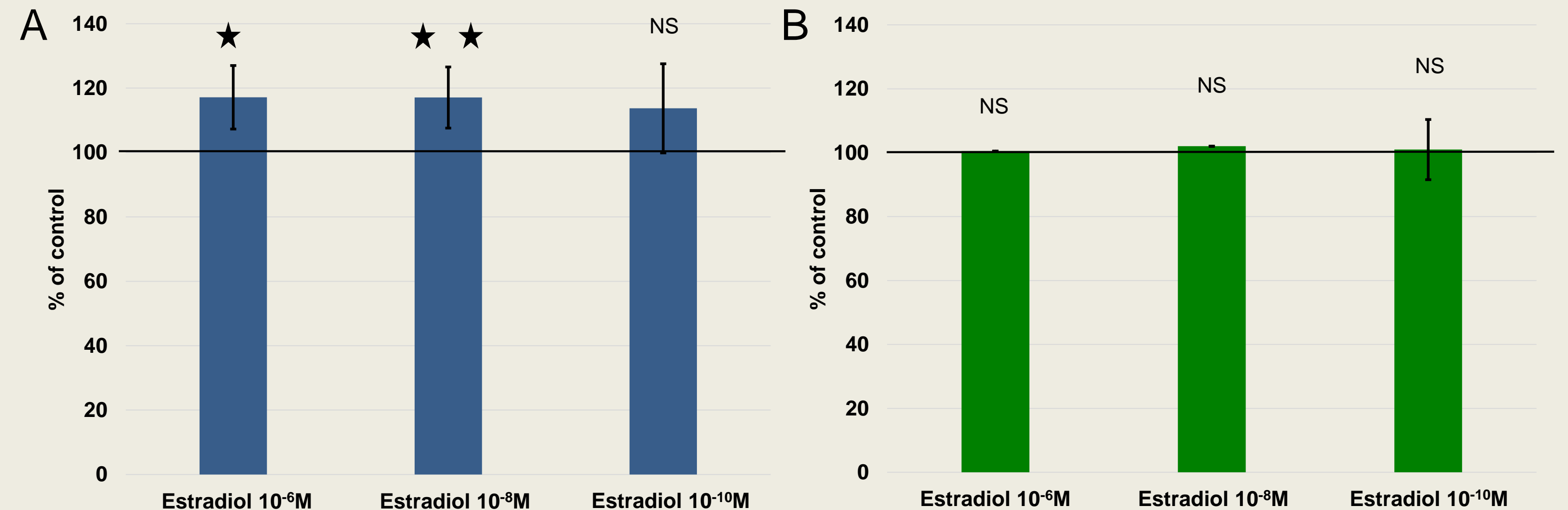
L. crispatus biofilm formation differences



(A) Comparison of capacities of biofilm formation by *Lactobacillus crispatus* CIP and V4. The two strain were compared under the same culture conditions in MRS broth. Plates were incubated anaerobically at 37°C for 48 hours. (B) Biofilm formed by the CIP strain at the bottom of the well. (C) Biofilm formed by the V4 strain: aggregative form, not attached at the well's bottom. The relative propensities of *L. crispatus* to form biofilms were measured by absorbance spectrophotometry after staining with crystal violet 0,1%. All data are the mean of four independent experiments. ★ ★ ★ P<0,001

4

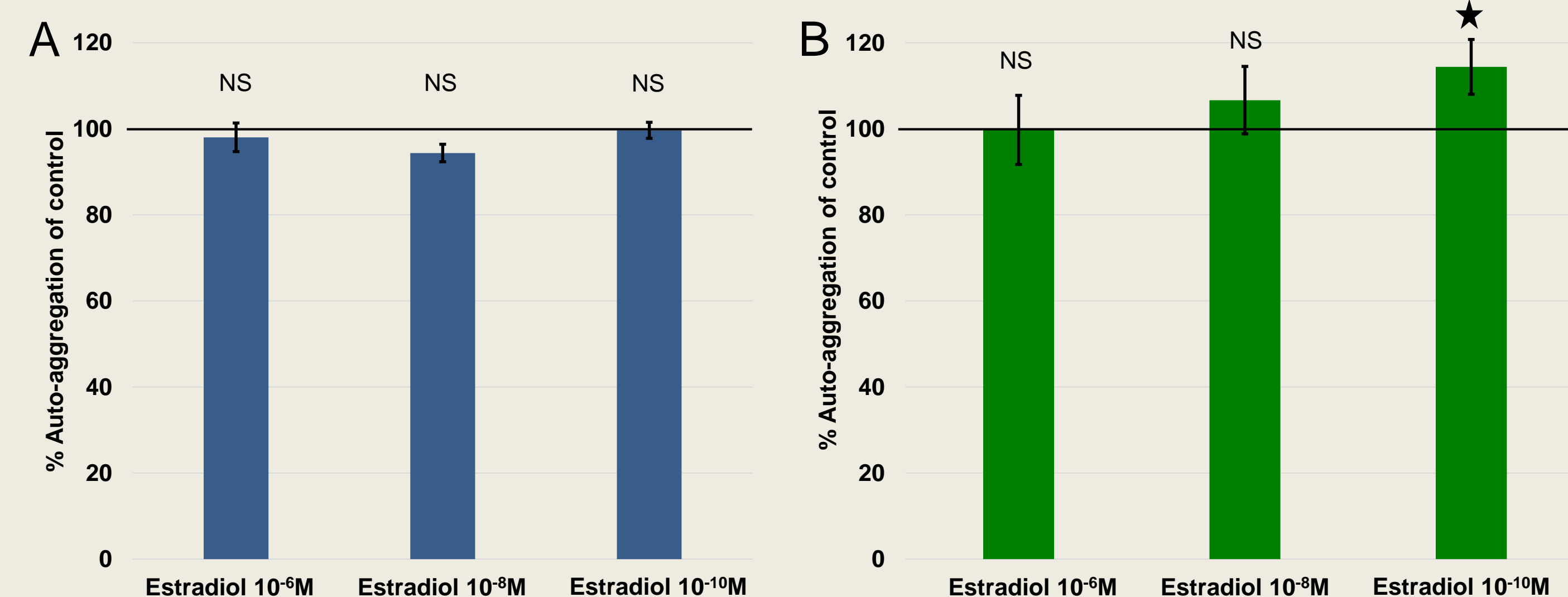
Effect of 17 β -estradiol on *L. crispatus* biofilm formation



Biofilm formation by *Lactobacillus crispatus* (A) CIP and (B) V4 treated with 17 β -estradiol. The two strains were grown under the same culture conditions in MRS broth with three estradiol concentrations. Plates were incubated anaerobically at 37°C for 48 hours. The relative propensities of *L. crispatus* to form biofilms were measured by absorbance spectrophotometry after staining with crystal violet 0,1%. And the percentage was compared to control 100% (black line). All data are the mean of four independent experiments. ★ P<0,05; ★ ★ P<0,01; NS: No significantly different.

5

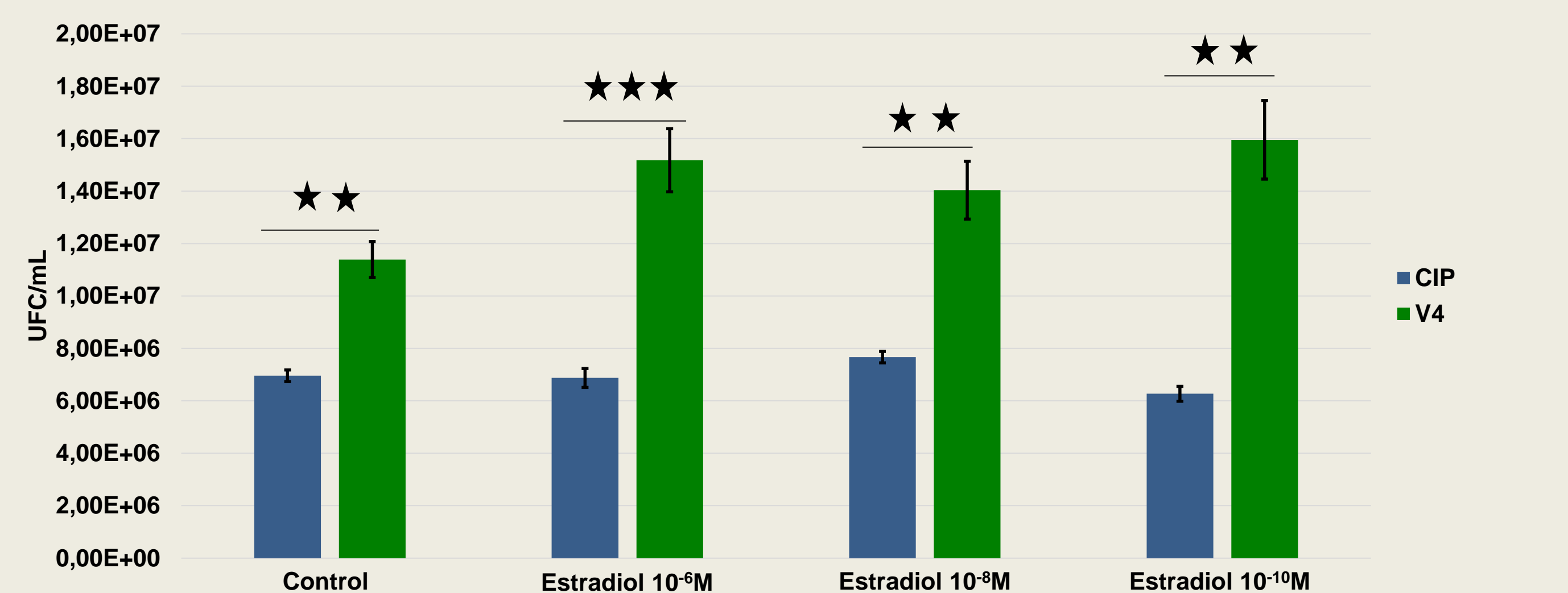
Effect of 17 β -estradiol on *L. crispatus* auto-aggregation ability



Comparison of the autoaggregation ability of *Lactobacillus crispatus* (A) CIP and (B) V4 strain with 17 β -estradiol at three concentrations to compare with the control (black line). Under static conditions 18 hours of incubation at 37°C in anaerobic atmosphere. The auto-aggregation was measured after 30 minutes resuspended in MRS culture supernatant fluids. Error bars represent standard deviations of the mean values of results from four independent experiments. ★ P<0,05; NS: No significantly different.

6

Effect of 17 β -estradiol on *L. crispatus* adhesion on vaginal cells



Adherence of *L. crispatus* CIP and V4 to vaginal epithelial cells VK2/E6E7 (ATCC® CRL-2616™) with 17 β -estradiol. The strains was incubated with 17 β -estradiol 18h in MRS media before adhesion test. Epithelial cells were incubated with bacterial inocula of MOI:100 and incubated for 2 hour. The number of viable CIP and V4 adhering to the cell monolayer surface was determined by plating onto MRS agar. The data are averages of three independent experiments performed in triplicate. Error bars indicate standard deviations of the mean values of results from three independent experiments. ★ ★ P<0,01; ★ ★ ★ P<0,001

Conclusion

This study revealed that CIP and V4 strains have different behaviors in terms of biofilm forming potential and auto-aggregation abilities, and that estradiol can modulate these activities. In addition, *in silico* studies suggest that the effects of estradiol on *L. crispatus* could be explained by the expression of ortholog(s) of the human estradiol receptor allowing bacteria to adapt their physiology in response to estradiol.