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In vitro activity of farnesol against *vaginal Lactobacillus* spp.

Fengjuan Wang, Zhaohui Liu*, Dai Zhang, Xiaoxi Niu

Department of Obstetrics and Gynecology, Peking University First Hospital, Beijing 100034, China

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ABSTRACT

Objective: Farnesol, a quorum-sensing molecule in *Candida albicans*, can affect the growth of certain microorganisms. The objective of this study was to evaluate the *in vitro* activity of farnesol against vaginal *Lactobacillus* spp., which play a crucial role in the maintenance of vaginal health.
Methods: Growth and metabolic viability of vaginal *Lactobacillus* spp. incubated with different concentrations of farnesol were determined by measuring the optical density of the cultures and with the MTT assay. Morphology of the farnesol-treated cells was evaluated using a scanning electron microscope. *In vitro* adherence of vaginal *Lactobacillus* cells treated with farnesol was determined by co-incubating with vaginal epithelial cells (VECs).
Results: The minimum inhibitory concentration (MIC) of farnesol for vaginal *Lactobacillus* spp. was 1500 μM. No morphological changes were observed when the farnesol-treated *Lactobacillus* cells were compared with farnesol-free cells, and 100 μM farnesol would reduce the adherence of vaginal *Lactobacillus* to VECs.
Conclusion: Farnesol acted as a potential antimicrobial agent, had little impact on the growth, metabolism, and cytomorphology of the vaginal *Lactobacillus* spp.; however, it affected their adhering capacity to VECs. The safety of farnesol as an adjuvant for antimicrobial agents during the treatment of vaginitis needs to be studied further.

Introduction

Lactobacillus species play an important role in decreasing the prevalence of vulvovaginal candidosis (VVC), gonorrhea, bacterial vaginosis (BV), and human immunodeficiency virus (HIV) infection by colonizing the vagina in women [1]. Studies have demonstrated an association between vaginal microflora dominated by *Lactobacillus* species and prevention of vaginal infectious diseases [2,3]. Colonization of the vaginal epithelium by *Lactobacillus* strains is a natural barrier against infection, and agents used to treat vaginal infections must have little impact on this important and natural barrier.

Farnesol, a sesquiterpene alcohol generated by the dephosphorylation of farnesyl pyrophosphate during the mevalonate biosynthetic pathway in yeast cells, has been reported as a quorum-sensing molecule in *Candida albicans* [4]. Farnesol generally has a wide variety of biological activities, for example, it can inhibit the growth of many microorganisms, block biofilm formation, suppress the transition of some dimorphic fungi from the yeast to filamentous stage, and increase microbial susceptibility to antibiotics [4,5]. It had also exhibited an important role in the resistance to oxidative stress [6] and has a cytotoxic effect on *C. albicans* as well as other microorganisms by inducing apoptosis at certain concentrations under specific environmental conditions [7].

Several studies have confirmed the inhibitory effect of farnesol on the growth of some bacteria [8–10]. The objective of this study was to evaluate the *in vitro* activity of farnesol against clinical strains of vaginal *Lactobacillus* strains.

Materials and methods

Organisms and culture conditions

Vaginal *lactobacillus* strains were provided by the Department of Obstetrics and Gynecology, Peking University First Hospital, Beijing, China. This study was a microbial basic study, no human right was involved, in other words, the strains were originally collected in an anonymized and de-identified form from regular clinical examination without damaging the patients' interest. So there's no Ethics Committee(s) formal approval documents. We received the strains from our Hospital Repository Department to conduct this microbial basic research after reviewed by Peking

* Corresponding author at: Peking University First Hospital, No 1, Xianmen Street, Xicheng District, Beijing 100034, China
E-mail address: 23662161@qq.com (Z. Liu).

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University First Hospital Ethics Committee. Freshly grown *lactobacillus* cells from de Man, Rogosa, Sharpe (MRS, BD, USA) agar plates were propagated in MRS broth and sequentially incubated at 37 °C and 5% CO_2.

Vaginal epithelial cells (VECs) were purchased from the American type culture collection (ATCC). Defined Keratinocyte-SFM (1×) (K-SFM) containing growth supplements and 1% Penicillin-Streptomycin (Gibco) was generally used to culture VECs in 75-cm^2 flasks to a confluent monolayer at 37 °C and 5% CO_2.

Vaginal Lactobacillus *growth and cell viability assay after farnesol treatment*

For this assay, a mixture of stereoisomers of farnesol (assay ≥ 96%; GS, sum of isomers; Sigma-Aldrich) was diluted in 100% methanol. Vaginal *Lactobacillus* strains (1×10^3 to 5×10^3 CFU/ml) were inoculated in a complex medium, MRS broth, supplemented with farnesol at different final working concentrations to a final volume of 5 ml in glass tubes. Farnesol-free controls were supplemented with 3% methanol. The cultures were allowed to grow for 48 h at 37 °C and 5% CO_2.

For the *in vitro* growth assay, the number of cells at each specific time interval was determined by using 200-μl culture mixtures to measure the optical density at 600 nm. The minimum inhibitory concentration (MIC) for farnesol was defined as the lowest concentration that resulted in 50% inhibition of cell growth when compared with the farnesol-free control culture mixtures.

For the analysis of cell metabolic viability, 100 μl of the culture mixtures was transferred to 96-well tissue culture plates after inoculation and growth for 24 h, and then a solution containing 5 mg of MTT per ml of 0.15 M phosphate-buffered saline (PBS) was added to each well to obtain a final concentration of 0.5 mg/ml. After incubation for 4 h at 37 °C, 100 μl of the reaction mixture (10 g SDS, 5 ml isobutanol, 0.1 ml of 10 mol/l HCl, and 100 ml ddH_2O) was added to solubilize the MTT formazan product. After incubation for 12 h at 37 °C, MTT formazan formation was measured at 578 nm by using a spectrophotometer (VersaFluor Fluorometer, Bio-Rad). Control wells contained medium plus MTT and farnesol to determine background formazan values. All assays were performed at least in triplicate. The minimal lethal concentration (MLC) for farnesol was defined as the lowest concentration that resulted in 50% cell death when compared with the farnesol-free control cells.

Effect of farnesol on Lactobacillus *cytomorphology*

The cytomorphology assay was performed using 4-cm glass culture plates, each containing a sterile coverslip. To each plate, 2 ml of the adjusted vaginal *Lactobacillus* cultures with different farnesol concentrations was added and incubated at 37 °C and 5% CO_2 for 24 h. The farnesol-free controls were supplemented with 1% methanol. After incubation for 24 h, 1 ml of 1.5% glutaraldehyde was added for fixing overnight at 4 °C. Then, cell morphology was assessed using a scanning electron microscope after dehydration, desiccation, and gold plating.

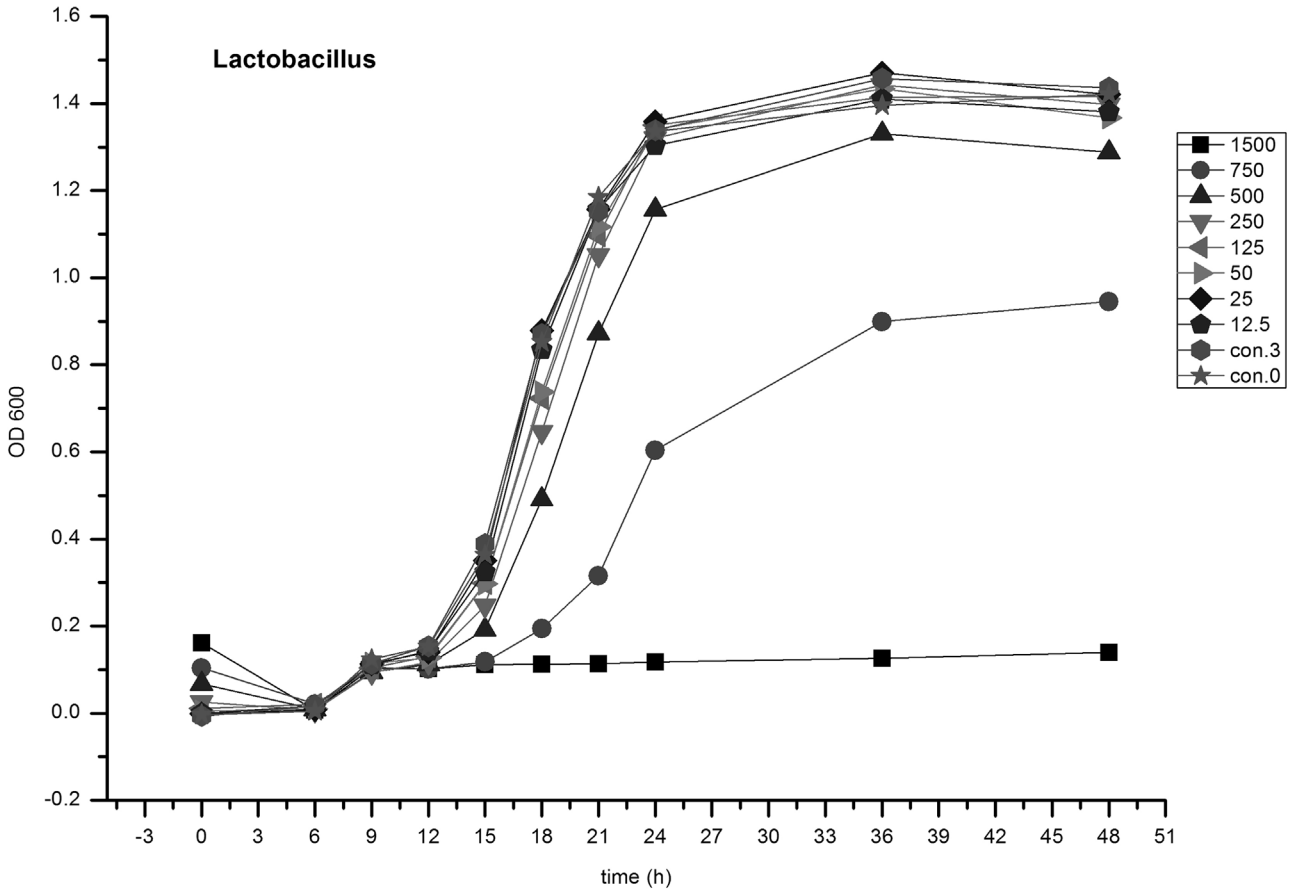

Fig. 1. Effect of farnesol on the growth of Vaginal Lactobacillus.
Growth curves of Lactobacillu cells incubated in the presence of different concentrations of farnesol. The number of cells at each specific time point was assessed by measuring the OD absorption at 600 nm, after stationary cultures. The growth curve of culture was prepared by plotting the values of OD600 *vs.* incubation time.