

# Weissella confusa enhances the defense mechanism against vulvovaginal candidiasis in vaginal epithelial cell

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## PURPOSE

To investigate whether *Weissella confusa* isolated from dandelion-kimchi inhibits the vulvovaginal candidiasis (VVC) by modulating cytokine and blocking adherence.

## METHOD

To investigate the adhesion ability of *W. confusa*, competition test was carried out. For the competition test, the vaginal epithelial cells (VK2/E6E7) were seeded at  $1 \times 10^5$  cell/ml, and  $1 \times 10^7$  CFU/ml of beneficial bacteria (BB; *W. confusa* and *L. reuteri*, *L. rhamnosus*, the mixture of *L. reuteri* and *L. rhamnosus* with 1:1 ratio) were co-cultured with *C. albicans*, respectively. After gram-staining of VK2/E6E7 cells, BB-attached cell were counted under the 400X microscopy.

The concentration of IL-6 and IL-8 were measured by ELISA to determine immune response induced by BB.  $1 \times 10^5$  cell/ml of VK2/E6E7 cells were inoculated with *C. albicans* ( $1 \times 10^7$  CFU/ml) for 12 hours. After washing, *W. confusa* and *lactobacillus* spp. were added with  $1 \times 10^7$  CFU/ml for an additional 24 hours, respectively. This test was carried out using four groups; defined as ① Only VK2/E6E7 as negative control (group1), ② VK2/E6E7 added with BB as positive control (group2), ③ VK2/E6E7 and *C. albicans* (group3), and ④ VK2/E6E7 with *C. albicans* and BB (group4). The supernatants of each group were detected by IL-6 and IL-8 using ELISA kit. *P*-value < 0.05 was considered as statistically significant.

Table 1. Cell line and strain information.

Cell line
Vaginal Epithelial cell (VK2/E6E7)
Pathogen
<i>Candida albicans</i>
Experiment strain
<i>Weissella confusa</i>
Positive control
<i>Lactobacillus reuteri</i>
<i>Lactobacillus rhamnosus</i>
Mixture_1:1 of reuteri and rhamnosus

*Candida albicans* were used as a pathogen yeast. To test the ability of *W. confusa*, *L. reuteri*, *L. rhamnosus* and, mixture of these were used as a positive control.

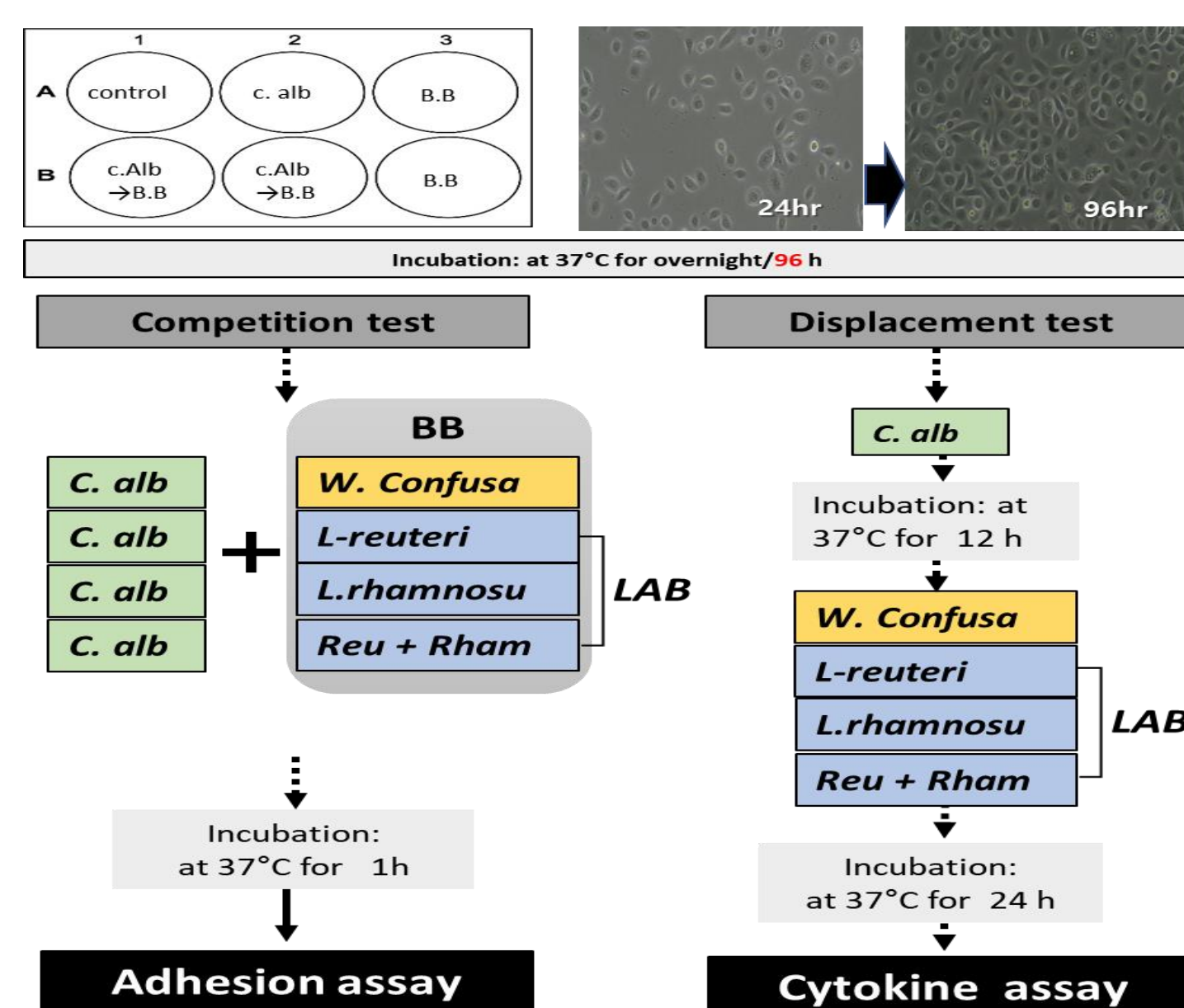


Figure 1. Protocol of competition test and Cytokine assay. Every strain were cultured respectively after the vaginal epithelial cell were confluent. LAB: lactic acid bacteria.

## RESULT

### *W. confusa* can block adherence of *C. albicans* on vaginal epithelial cell.

Biofilm formation of *candida* spp. is an essential step because it enhances its pathogenicity and colonizes after settling down. The VK2/E6E7 is surrounded by BB whereas without *C. albicans* were counted to estimate how BB can inhibit the adherence of *C. albicans*. The results are displayed in table 2. There was no significant difference between *W. confusa* and other LAB. All bacteria inhibited the attachment of *C. albicans* to VK2/E6E7.

Table 2. Cell count

Strains	Total Cell number	Attached Cell	Average (%)
<i>W. confusa</i>	45.89 ± 3.99	43.33 ± 4.18	93.78 ± 2.84
<i>L. reuteri</i>	45.9 ± 3.06	44.5 ± 2.65	97.48 ± 1.54
<i>L. rhamnosus</i>	46.5 ± 4.26	42.75 ± 4.61	91.04 ± 3.94
MTX	48.4 ± 4.23	47.56 ± 4.84	95.23 ± 1.2

MTX: Mixture of *L. reuteri* and *L. rhamnosus* with 1:1 ratio. Results are expressed by mean of cell number ± Standard distribution (SD).

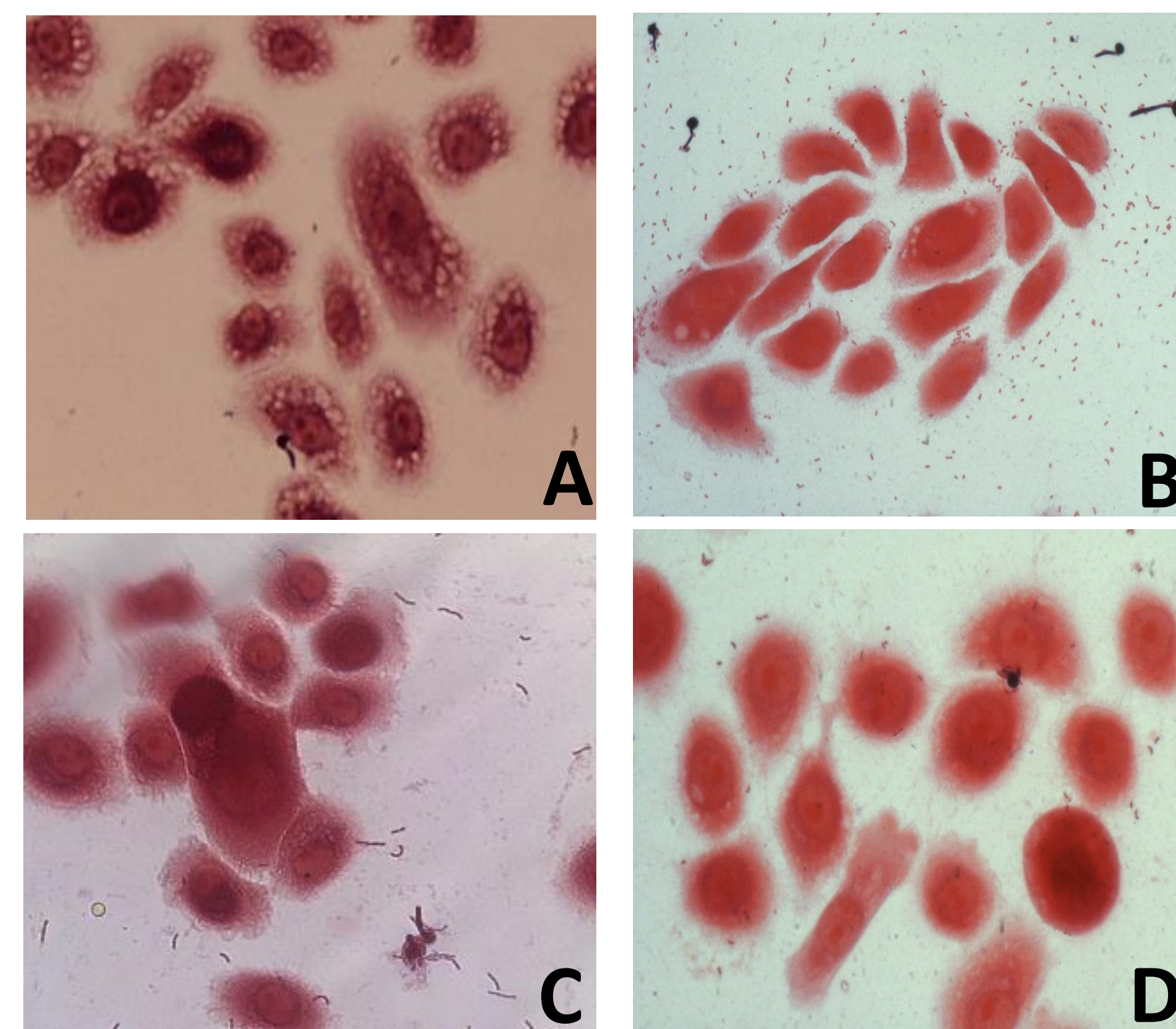


Figure 2. Morphological changes of VK2/E6E7 induced by *C. albicans* and BB.

The VK2/E6E7 were observed after gram-staining under 400X microscope. *Candida albicans* were dyed with bluish purple, in contrast VEC and bacteria were stained with red. (A) *Weissella confusa*(x400), (B) *C. albicans* and *L. reuteri*(x400), (C) *L. rhamnosus*(x400) and (D) MTX with *C. albicans*.

### *W. Confusa* can modulate the immune response.

This cytokine assay was performed to investigate whether BB can modulate cytokine secreting by using four groups, defined in table 3.

Table 3. VK2/E6E7 with different stimulation.

GROUP	Stimulation
Group 1	VK2/E6E7
Group 2	VK2/E6E7 + BB
Group 3	VK2/E6E7 + <i>C. albicans</i>
Group 4	VK2/E6E7 + <i>C. albicans</i> > BB

The results showed that group 4 of *W. confusa* produced a significantly higher concentration of IL-6 than group 3 (*P* < 0.05), and *L. rhamnosus* showed same pattern but not significant. The concentration of IL-8 showed the opposite result. Regardless of bacteria strains, group 3 as an infection status by *C. albicans* elevated than VK2/E6E7 medium only (group1). Sequentially, the supernatant of group 4 reduced IL-8 level and only *W. confusa* has a significant difference (*P* < 0.05). Interestingly, the supernatant of group 4 with *L. rhamnosus* were significantly higher than group 3 (*P* < 0.05). This result support that VK2/E6E7 can be modulated against inflammatory response using IL-8 and IL-6 spontaneously by probiotics.

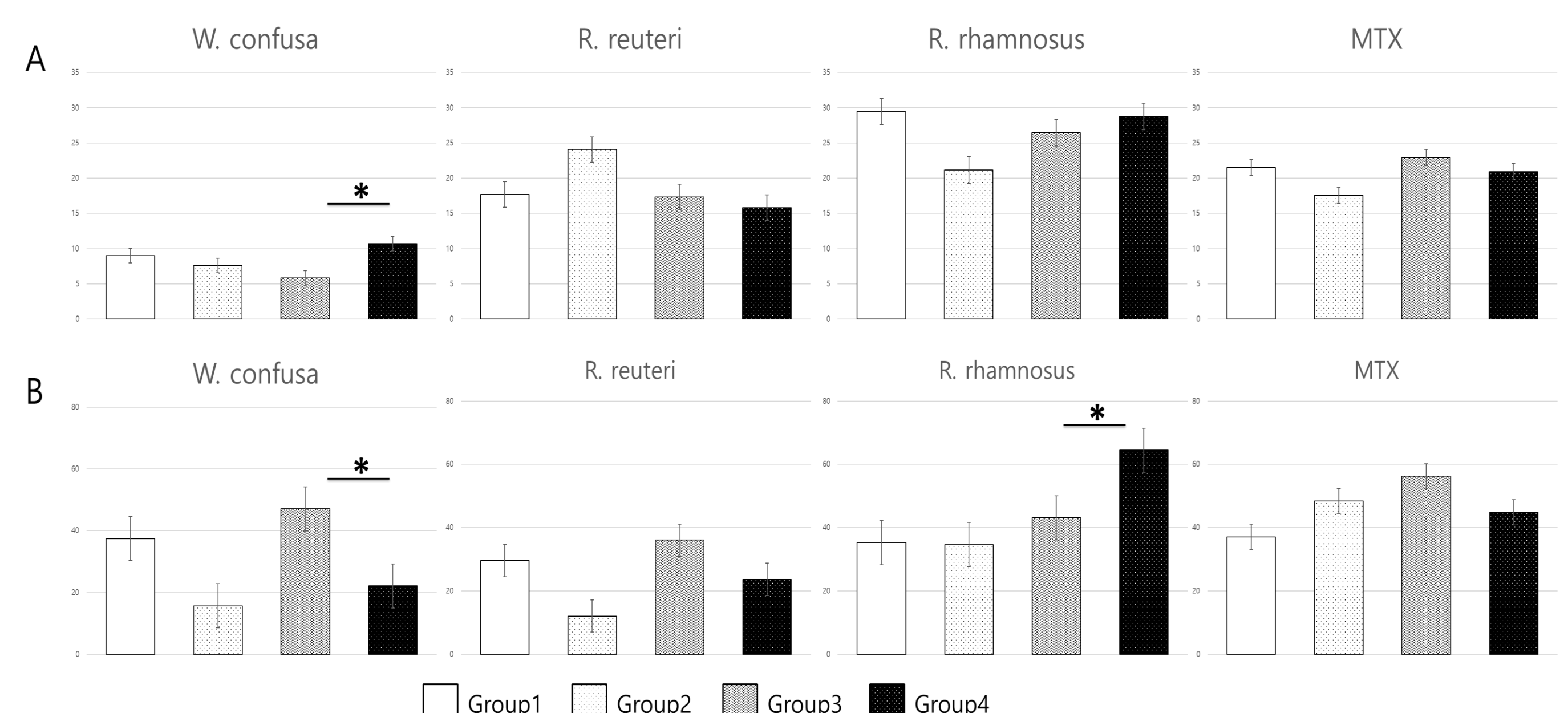


Figure 3. Cytokine level of VK2/E6E7 with respective bacteria strains.

(A) Concentration of IL-6 and (B) Concentration of IL-8. MTX: Mixture of *L. reuteri* and *L. rhamnosus* with 1:1 ratio. Graphs are represented Mean ± Standard distribution (SD). \*: *P*-value < 0.05

## CONCLUSION

Our findings suggest that the *W. confusa* isolated from a kimchi can maintain vaginal ecosystem and inhibit VVC infection as much as *L. reuteri* and *L. rhamnosus*. Moreover, *Weissella confusa* can be used as component to support women's vaginal health by modulating of immune response.