2015 Wood Chemistry – 10th Week Antifungal Effects of Essential Oil (Ⅱ)

TA: Seong-Min Cho

Minimum Inhibitory Concentration (MIC) and Checkerboard Method

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. It is used by diagnostic laboratories mainly to confirm resistance of antibiotics.

Checkerboard method has been one of the traditional methods for the measurement of antibiotic synergism. Particularly, synergistic effect is generally defined as requiting a 4-folds reduction in MIC of both antibiotics in combination, compared with each used alone. In addition to it, there are different type of interaction between two antibiotics, addictive effect and antagonistic effect.

In our experiments today, we assess the MIC of several terpenoid compounds and test the synergism of their combinations.

1. Materials

① Terpenoid

Group 1: Nerolidol + Citral

Group 2: Farnesol + Nerolidol

Group 3: Citral + Farnesol

② Fungi (Dermatophytes): *Epidermophyton floccosum*, *Trichophyton rubrum*

③ Culture Media: SDB (Sabouraud Dextrose Broth) media

④ Equipment: Autoclave, Stationary-incubator, Clean bench, GC/MS

2. Methods

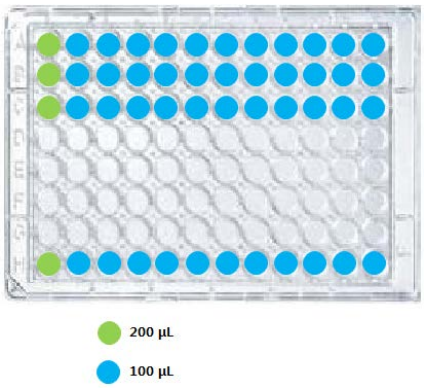
§ MIC

① Prepare sterilized 100 mL of SDB media (Auto-clave at 121℃, 15 min) and cool it down at RT.

② Prepare the spore suspension of pre-cultured strain by doing dilution with sterile water.
(McFarland Turbidity Standard No. 0.5, OD_{600}=0.132, 1.5×10^8 CFU/mL)

③ Put SDB media into 96 well microplate at clean bench as follows.

(The last line is control)



④ Put 5 μL of terpenoid sample into well of first column (green spot) with sufficient pipetting.

⑤ Take 100 μL of sample-media mixture and mix the next (blue spot) with sufficient pipetting.

⑥ Repeat step ⑤ along the line in sequence (serial dilution).

⑦ Put 10 μL of prepared spore suspension into all well.

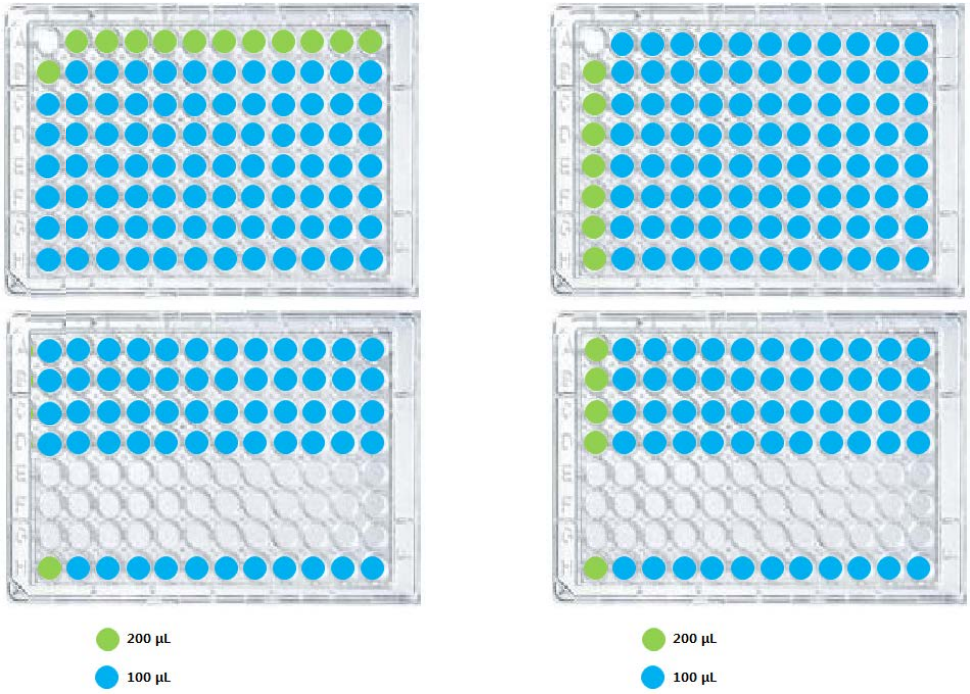
⑧ Cultivate at 28℃ for 1 days and check the turbidity of each well with the naked eyes.

§ Checker board method

① Prepare sterilized 100 mL of SDB media (Auto-clave at 121℃, 15 min) and cool it down at RT.

② Prepare the spore suspension of pre-cultured strain by doing dilution with sterile water.
(McFarland Turbidity Standard No. 0.5, OD_{600}=0.132, 1.5×10^8 CFU/mL)

③ Put SDB media into 96 well microplate at clean bench as follows (left-side of below figures).
(The last line is control)



④ Put 10 μL of terpenoid Ⓐ into well of first column and B1 (green spot) with sufficient pipetting.

⑤ Take 100 μL of sample-media mixture and mix the next (blue spot) with sufficient pipetting.

⑥ Repeat step ⑤ along the line with downward in sequence (serial dilution).

⑦ Put 100 μL of SDB media into well of first law (right-side of above figures).

⑧ Put 5 μL of terpenoid Ⓑ into well of first law (green spot) with sufficient pipetting.

⑨ Take 100 μL of sample-media mixture and mix the next (blue spot) with sufficient pipetting.

⑩ Repeat step ⑤ along the line with rightward in sequence (serial dilution).

⑪ Put 10 μL of prepared spore suspension into all wells.

⑫ Cultivate at 28℃ for 1 days and check the turbidity of each well with the naked eyes.

⑬ Calculate fractional inhibitory concentration (FIC) index and determine what kind of mutual effect (synergistic, antagonistic or additive) relation between terpenoid Ⓐ and Ⓑ has.

$$\textbf{FIC index} = \frac{\textbf{MIC of A with combination}}{\textbf{MIC of A alone}} + \frac{\textbf{MIC of B with combination}}{\textbf{MIC of B alone}}$$

$FIC \leq 0.5$ Synergistic effect
$0.5 < FIC < 2$ Additive effect
$FIC \geq 2$ Antagonistic effect

§ Report

※ Describe data of 9th week and 10th week together, and should include results of other groups.

※ Report should be written by MS words (10 points, line spacing 1) or Hancom office (10 points, line spacing 120)

※ Report must be taken in the following order (in Korean or English): 1. Introduction, 2. Materials and Methods, 3. Results and Discussion, 4. Conclusions, 5. References

※ Please explain Dermatophytes which is fungus used in our experiment.

※ Please illustrate, in your own words, how three different effects (synergistic, additive, antagonistic) can occur.

※ Assignment should be appended to report. (If you copy and paste, you can not get a grade)

※ Inquires: ① Wood Chemistry Lab (6203) Seong-Min Cho, ② csmin93@snu.ac.kr ③ 010-6623-5449