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ประสิทธิภาพของ Ion Generator ในการยับยั้งเชื้อแบคทีเรียและเชื้อรา

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Efficacy of Ion Generator Against Bacteria and Fungi

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หลักการและวัตถุประสงค์: ปัจจุบันมีวิธีต่างๆ หลายวิธีที่นำมาใช้ในการกรองอากาศเพื่อควบคุมและป้องกันการแพร่กระจายของจุลชีพ Ion generator เป็นเทคโนโลยีใหม่ที่ทำให้อนุภาคของน้ำในอากาศแตกตัวเป็นประจุบวก (H^+) และประจุลบ (O_2^-) ในการศึกษาครั้งนี้ได้ทำการทดสอบประสิทธิภาพของเครื่อง Ion Generator (IG) ในการยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียและเชื้อราอย่างละ 5 ชนิด ได้แก่ *Streptococcus pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Burkholderia pseudomallei* *Candida albicans*, *Aspergillus fumigatus*, *Rhizopus* sp, *Pennicillium* sp และ *Microsporum* sp.

วิธีการศึกษา: ได้ทำการทดลองในตู้พลาสติกขนาด 100x50x50 ซม. โดยการวาง plates เปิดฝา ที่มีเชื้อ แบคทีเรีย และเชื้อราจำนวนรวมกัน 10 ชนิดๆละ 10^2 CFU หรือการพ่นเชื้อแบคทีเรียจำนวนตั้งแต่ 10^4-10^8 CFU/mL จำนวน 5 มิลลิลิตร หรือเชื้อราจำนวนประมาณ 10^3-10^4 CFU/mL ให้เป็นละอองฝอยภายในกล่องเพื่อทดสอบประสิทธิภาพของ IG เมื่อเปิดเครื่องเป็นเวลา 30, 60 และ 120 นาที นอกจากนี้ยังได้ทำการทดสอบในห้องปฏิบัติการการจุลชีววิทยาจำนวน 2 ห้องขนาด 22-24 $ม^2$ สูง 3 ม.

ผลการศึกษา: พบว่าเครื่อง IG สามารถทำให้ปริมาณเชื้อแบคทีเรียลดลงได้ตั้งแต่ร้อยละ 73.1 - 99.3 แล้วแต่ชนิดของเชื้อ โดยเครื่อง PCI มีประสิทธิภาพสูงสุดในการยับยั้งเชื้อร้อยละ 99.3, 91.3, 90.1, 87.96 และ 83.1 สำหรับเชื้อ *E. coli*, *P. aeruginosa*, *B. pseudomallei*, *B. subtilis* และ

Backgroud and Objective: Many methods were used for air purification to prevent the spreading or to control microorganisms. Ion generator is a new technology to split the airborne molecules of water into positively (H^+) and negatively (O_2^-) charges. In this study, the bacterial and fungal killing efficacy of ion generator (IG) was evaluated against 5 bacteria and 5 fungi including *Streptococcus pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomanas aeruginosa*, *Burkholderia pseudomallei, Candida albicans*, *Aspergillus fumigatus*, *Rhizopus* sp, *Pennicillium* sp and *Microsporum* sp.

Methods: The experiments were done in a plexi glass chamber (size of 100x50x50 cm). Plates containing approximately 10^2 CFU of each bacteria or fungal strains were left opened in the chamber or five milliliters of 10^4-10^8 CFU/ml of the bacteria or 10^3-10^4 CFU/ml of fungi, one at a time, were sprayed into the chamber and evaluated the killing efficiency of IG after 30, 60 and 120 min operation time. The efficacy of IG was also evaluated in the atmosphere of two microbiology rooms with the size of 22-24 m^2 high 3 m.

Results: The percentages of bacterial killing were varied from 73.1 to 99.3. The killing efficacy was demonstrated to be varied from one type of microorganisms to the others. The maximum killing efficacy of IG were 99.3%, 91.3%, 90.1%, 87.96%, and 83.1% for *E. coli*, *P. aeruginosa*, *B. pseudomallei*, *B. subtilis* and *S. pneumoniae,* respectively.

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S. pneumoniae ตามลำดับ สำหรับเชื้อรา ประสิทธิภาพในการฆ่าเชื้อจะอยู่ที่ร้อยละ 42.6 - 93.9 เชื้อ *Rhizopus* sp. เป็นเชื้อที่ไวต่อการถูกทำลายมากที่สุด (ร้อยละ 91.9 - 93.9) ในขณะที่เชื้อ *A. fumigatus* มีความทนต่อ IG มากที่สุด (ร้อยละ 42.6 - 53.9) ในทางตรงข้ามเมื่อนำเครื่อง IG มาเปิดเป็นเวลา 1 ชั่วโมงในห้องปฏิบัติการจุลชีววิทยาทั้งสองห้อง พบว่าสามารถลดเชื้อได้ประมาณร้อยละ 10-14

สรุป: เครื่อง IG จะไม่มีผลต่อการฆ่าเชื้อแบคทีเรียและเชื้อราที่เจริญอยู่บนอาหารเลี้ยงเชื้อแต่มีประสิทธิภาพในการฆ่าเชื้อแบคทีเรียและเชื้อราที่อยู่ในอากาศภายในเวลา 30 นาที โดยที่เชื้อราจะมีความไวต่อการถูกทำลายน้อยกว่า

คำสำคัญ: เครื่อง Ion generator, การฆ่าเชื้อแบคทีเรีย, การฆ่าเชื้อรา, พลาสม่าไอออน

For fungi, the rate of killing was ranged from 42.6 to 93.9%. *Rhizopus* sp. was found to be the most susceptible (91.9 to 93.9%) whereas *A. fumigatus* was the most tolerance (42.6 to 53.9%). In contrast, the operation of IG for 1 hour in 2 microbiology laboratories could reduce the microorganisms in the air at around 10-14%.

Conclusion: The IG was found to have no effect on bacteria or fungi that grown on agar plates but it can be used efficiently to kill bacteria and fungi in the air within 30 min of operation but the fungi were found to be less susceptible.

Keywords: Ion generator, bacterial killing, fungal killing, Plasmacluster Ion.

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Introduction

The quality of air surround us is important to our health[1]. It is not only pollutants in the air such as volatile organic compounds (VOCs) but also microorganisms distributed in it that should be concerned. Several pathogens that cause infectious diseases such as influenza, tuberculosis, melioidosis, bacterial meningitis, and anthrax can infect people through inhalation of contaminated droplets or particles in the air into human body[2, 3]. The incidence of emerging infectious diseases was also increased due to the climate change[4]. Moreover, indoor airborne microflora has been shown to be implicated as the etiology of hypersensitivity pneumonitis, allergic rhinitis and some type of asthma[5, 6, 7]. Therefore, the indoor air quality due to the present of several microorganisms is recently become highly concerned[8, 9]. Many methods were used for indoor air purification to prevent the spreading or to control these microorganisms such as HEPA air filtration[10], short ultraviolet wavelength (UV-C) light[11], photocatalytic oxidation (PCO)[12] plus UV air purifiers with positively and negatively charges cluster ion generator[13, 14]. Each method has some limitations such as HEPA air filtration that could not trap virus particles or mycoplasma due to their small size. UV-C does not efficiently kill the microorganisms if they are not directly exposed to the UV light. Moreover, the light could cause some health hazard to human in that it could not be operated during work. Ion generator (IG) is a new technology that splits the airborne molecules of water into positively charged hydrogen (H^+) and negatively charged oxygen (O_2^-). The generated ions have a property of forming clusters around microscopic particles and microorganisms and create groups of highly reactive OH^- radicals that can kill the germs in the air. In developing countries, with limited research budget for set up the high quality of Biosafety level 2 or 3 as required for microbiology work, therefore, the efficacy of the IG was evaluated whether it would be able to control microbial contaminants in laboratories or rooms that need clean air.

Materials and Methods

Microorganisms

Bacteria and fungi strains used in this study were obtained from the stock culture in Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. The bacteria used were *Burkholderia pseudomallei, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Streptococcus pneumoniae* and fungi were *Aspergillus fumigatus, Rhizopus* sp, *Pennicillium* sp, *Microsporum* sp. *and Candida albicans.* All bacterial strains were cultured in Brain Heart Infusion

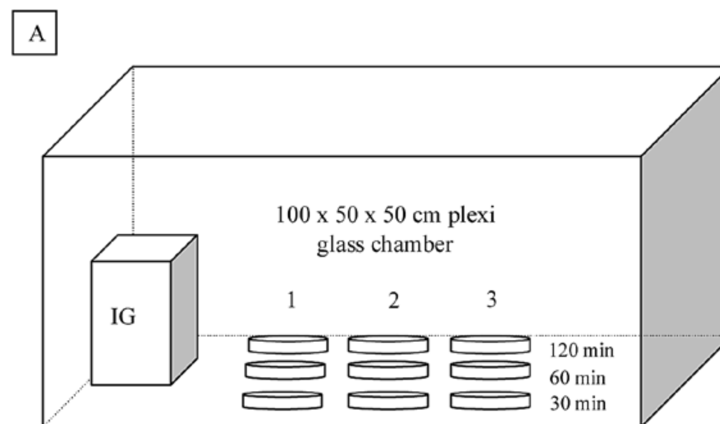
(BHI) broth and nutrient agar (NA) except *S. pneumoniae* that used blood agar whereas the fungi were cultured in potato dextrose agar (PDA).

Ion Generator

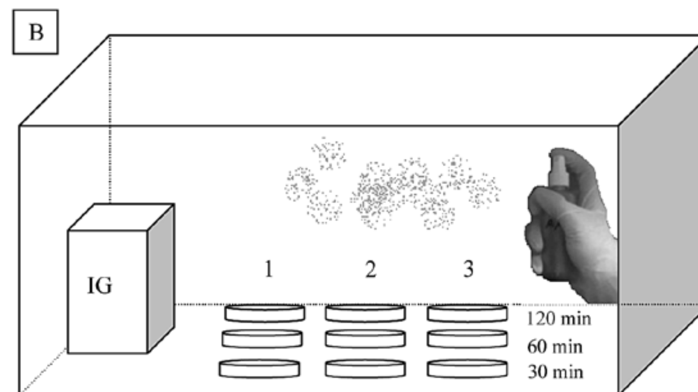
Two ion generators (PCI model IG-A10TA), provided by Sharp Thai Co. Ltd, were used throughout this study. The model was evaluated for their capacity for the area of 10 m^2 according to the manufacturer instruction.

Research Design

The studies were done in 3 separated conditions (Figure 1).The number of bacterial or fungi colony (CFU) was determined before and after being exposed to the ion generated from Plasmacluster Ion® generator machine at the duration of 30, 60 or 120 min.



Condition I: The tested microorganism was cultured on agar plates.



Condition II: The tested microorganism was sprayed into the chamber.

Figure 1 Illustration of the chamber and design for condition I and II. The size of the chamber is 100 x 50 x 50 cm. In condition I, 9 NA plates (3 sets) containing approximately 10^2 CFU of each bacteria or fungal strains were left opened in the chamber. After IG operation, 3 plates at a time were removed simultaneously after 30, 60 and 120 min (A). In condition II, liquid culture containing 10^4-10^8 CFU/ml (5 ml) of bacteria or 10^3-10^4 spores/ml (5 ml) of fungal strains were sprayed into the chamber. Nine sterile NA plates were put into the chamber and the IG was operated. Three plates were removed after 30, 60 or 120 min of the operation time (B).

Condition I: The efficacy of ion generator machine to reduce the microorganisms in culture plates. The experiment was performed in a plexi glass chamber (size of 100 x 50 x 50 cm). Nine NA plates were freshly spread with either 10^2 CFU of each bacteria or each fungal strain. The plates were left opened in the chamber and then the IG was operated. After 30 min of exposure, 3 plates were removed from the chamber and did so after 60 and 120 min of exposure. The plates were incubated for an appropriated period of time and temperature depending on the microorganisms before being evaluated for the number of colonies in comparison with those exposured in the chamber without ion generator.

Condition II: The efficacy of ion generator machine to kill the microorganisms in the air. The evaluation was performed in the same chamber. The broth containing 10^4-10^8 CFU/ml (5 ml) of bacteria or 10^3-10^4 spores/ml (5 ml) of fungi, one at a time, were sprayed into the chamber that contained 9 sterile NA plates that just left opened. After operated for 30 min, 3 plates were removed and did so after 60 and 120 min of exposure. The plates were incubated and the number of colony was counted in comparison with that in the chamber without ion generator.

Condition III: The efficacy of ion generator machine to reduce the microorganisms present in the atmosphere. The experiment was done in 2 microbiology laboratory rooms (size of 6 x 4 m^2 and 7.35 x 3.13 m^2). The number of microorganisms in each room was detected before and after the ion generator machine was operated. Four NA plates were placed at one meter from each corner of the room for 1 hour to detect the number of microorganisms present in the room and then removed. After that, another 4 plates were placed at the same positions and the machine was operated for 1 hour. All plates were incubated and the number of colonies was counted.

All experimental designs were approved by human ethical committee of Khon Kaen University, Khon Kaen, Thailand.

Results and discussion

The number of bacterial count on solid media (Figure 2) is displayed in Figure 3. The results demonstrated that the IG did not have any significant effect on the tested organisms when they were grown on solid media. This same observation was obtained when fungi were used to evaluate (data not shown). The generated ions may not be able to kill the microorganisms on the surface of the agar plates due to the instability of the ions on moisture surface. However, some reports indicated the negative ion is more effective than the positive one in reducing the gram-negative and gram-positive bacteria by detecting the microorganisms growing on agar plates[15, 16]. From our design experiment, the ions were designed to generate very close to the plate's surface. The possible mechanisms responsible for bactericidal process proven by other experiments was ozone that plays a major role and electroporation as a secondary role and not directly by the negative ions itself[16]. However, the ozone was known to have toxic effect to human health[17]. As IG works by emitting positive and negative ions into the air which change into OH^- radicals when adhered or surround to the surface of airborne pathogens and destroy them[14]. Moreover, the IG was reported to generate less than 10 ppb (≤0.01 ppm) of ozone.

When IG efficacy was tested by using microorganisms in a spray condition, it could kill all of the tested bacteria and most of fungi within 30 min (Figure 4). The killing efficacy at 30 min to tested bacteria was ranged from 73.1-99.1%. However, when the operation time was extended to 60 or 120 min, the killing capacity was not much different except for *P. aeruginosa* that was increased from 73.1% to 91.30%. *E.coli* was found to be the most susceptible whereas *P. aeruginosa* was the least susceptible. The maximum killing efficacy of IG on *E. coli*, *P. aeruginosa*, *B. pseudomallei*, *B. subtilis* and *S. pneumoniae* were 99.3%, 91.3%, 90.1%, 87.96%, and 83.1%, respectively. *B. pseudomallei*, which was classified in category B of possibly being used as a bio-weapon by CDC, USA, was very sensitive to IG killing. In fungal experiment, the percentage of killing

tested fungi ranged from 42.6 to 93.9%. *Microsporum* sp. and *Rhizopus* sp. were found to be the most susceptible in all time course (92.0 to 95.3% and 91.9 to 93.9%, respectively) whereas *A. fumigatus* was quite resistant (42.6 to 53.9%). At 30 min of IG exposure, the percentage of killing of *A. fumigatus, C. albican, Rhizopus* sp and *Microsporum* sp were 44.4%, 76.1%, 91.9% and 95.3% respectively. When the exposure time was extended for 60 or 120 min, the killing efficacy was not significantly different. This result suggested that fungus spores are more resist to IG. This finding is not unexpected as fungus spores have a rigid structure.

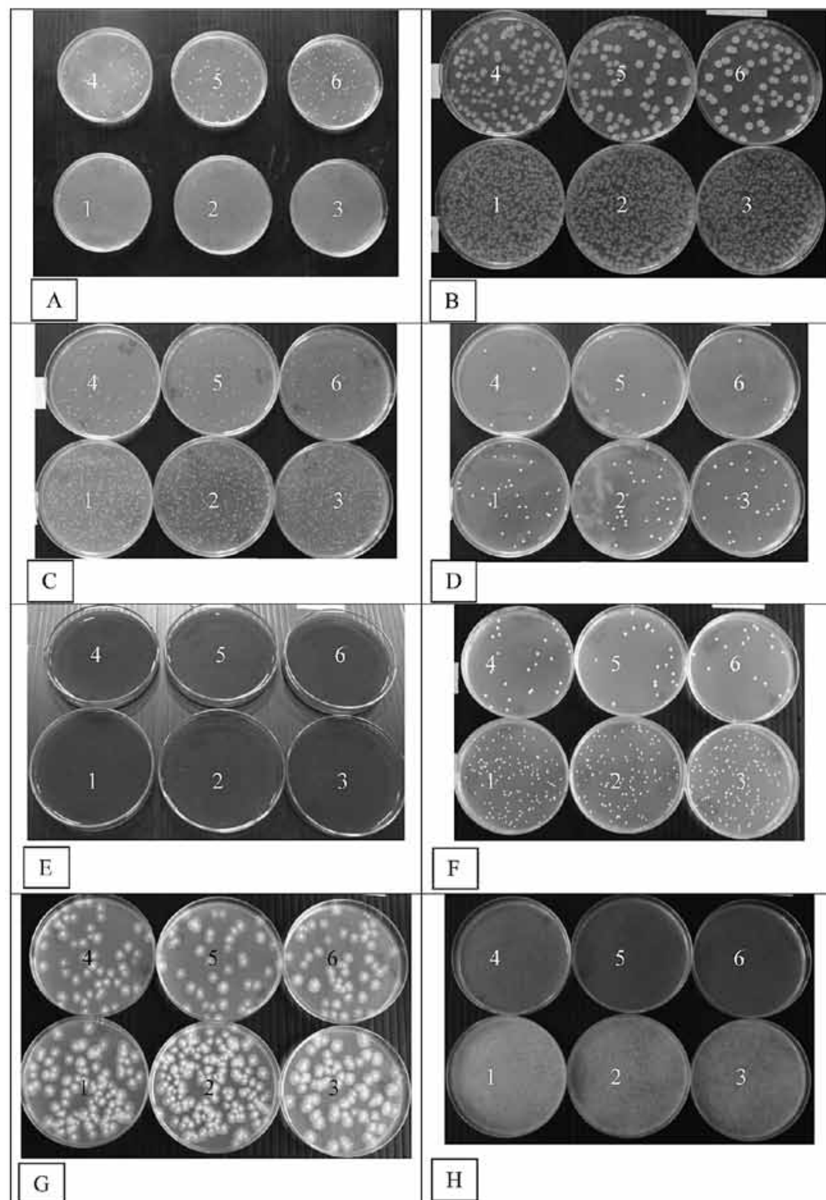


Figure 2 The appearance of colonies on solid agar plate as a result after each microorganism in sprayed form was exposed to IG in comparison with without exposure. The appearance of colony without IG operation (1, 2 and 3) and with IG operation (4, 5 and 6) for 30 (1 and 4), 60 (2 and 5) and 120 (3 and 6) min. of *Escherichia coli* (A), *Bacillus subtilis* (B), *Burkholderia pseudomallei* (C), *Pseudomonas aeruginosa* (D), *Streptococcus pneumoniae* (E), *Candida albicans* (F), *Aspergillus fumigatus* (G) *and Rhizopus* sp (H).

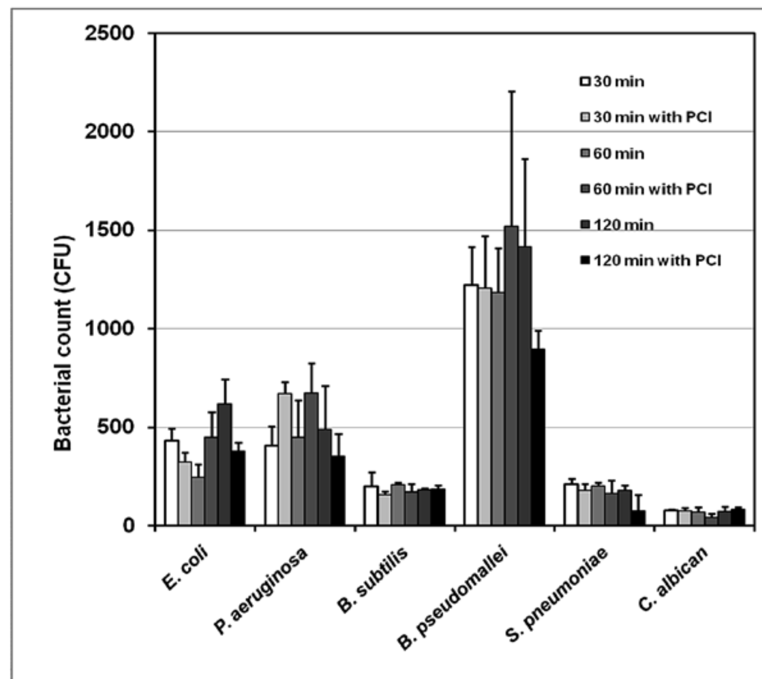


Figure 3 The efficacy of IG against bacteria and yeast on agar plates. The average colony counts of *E. coli, P. aeruginosa, B. subtilis, B. pseudomallei, S. pneumoniae and C. albicans* after 30, 60 and 120 min with or without exposure to IG were shown. Each bar represents mean ± SD.

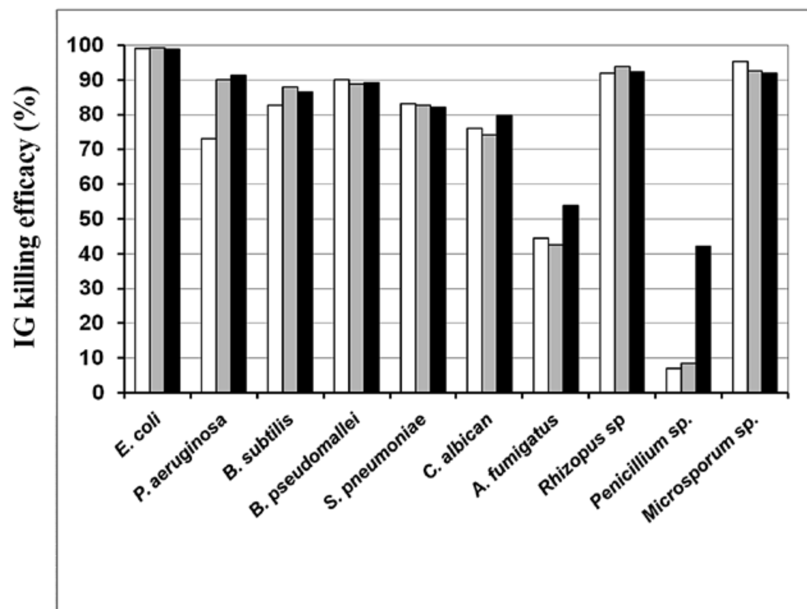


Figure 4 The efficacy of IG against bacteria and fungi in sprayed condition. The percent reduction (mean) in colony counts after exposure to IG at 30 (□), 60 (▦) and 120 (■) min of *E. coli*, *P. aeruginosa, B. subtilis*, *B. pseudomallei*, *S. pneumoniae*, *C. albicans*, *A. fumigatus, Rhizopus* sp., *Penicillium* sp. and *Microsporum* sp.

The operation of IG in the two microbiology laboratories could reduce microorganisms in the air at the mean of 10-14% in 1 hour. This result was less efficient than expected. This might be due to the size of the room (22-24 m^2) that were larger than that (10 m^2) given in the specification of the machine model IG-A10TA (10 m^2). The low number of microorganisms (10-14 colonies/m^2) in the control group (no IG operated) found in the environment of the room tested might be another reason.

Conclusion

The IG could efficiently kill bacteria in the air if being used as specified but not on the agar plates. This condition is suitable for using in routine microbiology laboratory where bacteria are generally stored on solid media. As fungal spores are more resist to the ions, the IG model with HEPA filter should be more suitable and the suitable size of the room with a correct IG model should be concerned to obtain an efficient mechanism for prevention of the airborne pathogens.

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