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光觸媒二氧化鈦薄膜對茭白細菌性基腐病原菌（*Enterobacter cloacae* SM1）之抑菌作用

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摘　要

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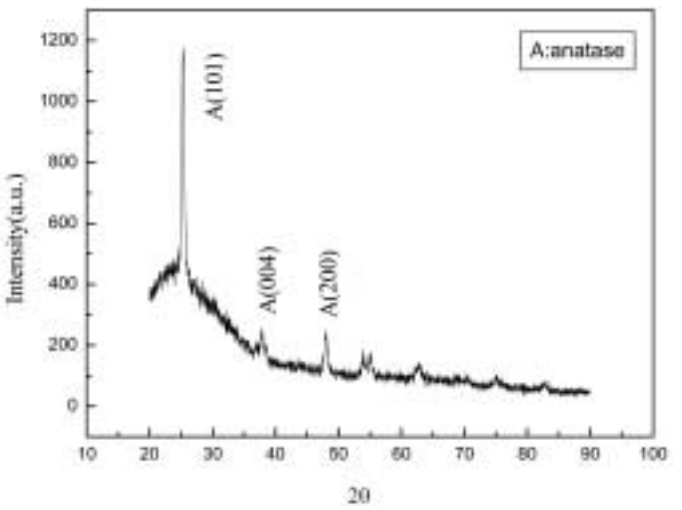
爲解決化學農藥所帶來的殘毒及環境污染問題，尋求新的替代作物病害防治方法，已成爲防範植物病害重要的課題。近年來，利用紫外光 A(UV-A) 與二氧化鈦 (TiO_2) 奈米粒子之光觸媒殺菌 (photocatalytic inactivation) 技術能有效的殺滅微生物且不會形成有害物質，應是一種可以嘗試的替代防治方法。*Enterobacter cloacae* SM1 主要引起茭白細菌性基腐病，且造成茭白作物栽培之損失。本文利用溶膠凝膠法 (sol-gel) 製備二氧化鈦奈米薄膜，探討光觸媒二氧化鈦薄膜對茭白細菌性基腐病菌之抑菌效果，並以 2,3,5-triphenyl- tetrazolium chloride (TTC) 作爲細菌生長指示劑。實驗結果顯示二氧化鈦薄膜經 UV-A 30 分鐘照射後，其對茭白細菌性基腐病菌與大腸桿菌 (*Escherichia coli* BCRC 10450) 之殺菌率分別達 26.3% 與 31.8%，而經 60 分鐘照射後，對茭白細菌性基腐病菌與大腸桿菌之殺菌率皆達 99.5%以上。因此，利用光觸媒二氧化鈦薄膜技術對植物病原菌之抑菌作用，將具有作爲未來水耕栽培系統之植物病害防治新工具的潛力。

關鍵字：光觸媒殺菌、二氧化鈦、茭白細菌性基腐病菌

長久以來，化學農藥對防治作物病蟲害，保證糧食生產和推動農業的發展做出了很大的貢獻，但是長期大量的使用化學農藥，其無法克服的弊端逐漸被人們所認識，即害蟲產生抗藥性；污染環境並危害人類健康；農藥殺死害蟲的同時也能大量殺死天敵，使害蟲失去了自然控制。爲解決化學農藥帶來的各種問題，各國紛紛尋求非化學防治方法的新途徑，以謀求降低化學藥劑殘毒之危害[11,13,15]。爲解決此問題，除優良抗病品種 (系) 的選拔外，篩選拮抗微生物或研發分析新的抑制病原菌生長的替代方法，以作爲病害防治之用，已成爲防範植物病害重要的課題。據研究指出奈米二氧化鈦光觸媒對許多細菌、眞菌和病毒具有抑制作用，包括 *Escherichia coli*、*Lactobacillus acidophilus*、*Serratia marcescens*、*Pseudomonas aeruginosa*、*P. stutzeri*、*Bacillus pumilus*、*Streptococcus mutans*、*S. rattus*、*S. cricetus*、*S. sorrinus* AHT、*Deinococcus radiophilus*、*Saccharomyces cerevisae*、Phage MS2 及 Qβ、Polivirus 1，與降解水中有機污染物等能力[1-3,5-10]，但目前尚無應用於植物病原菌之抗菌報告，故本文主要探討奈米二氧化鈦光觸媒對茭白細菌性基腐病菌之抑制活性。

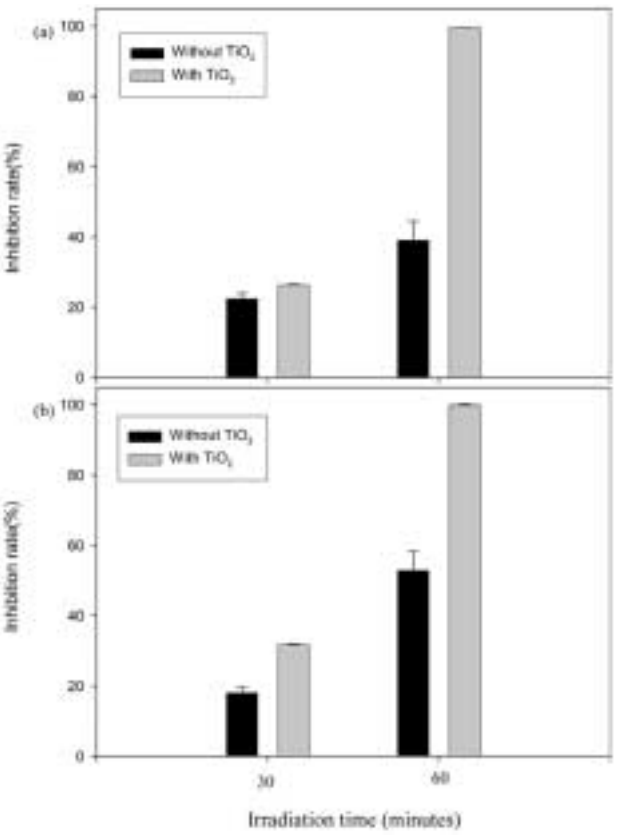
首先利用溶膠凝膠法 (sol-gel) 將 Titanium IV butoxide (0.02 mol)、n-Butanol (0.08 mol)、acetic acid (0.08 mol) 混合後，製備奈米二氧化鈦粉末[14]，再以旋轉塗佈法 (spin coating) 且經 500℃ 燒後製備之奈米二氧化鈦薄膜，並以 X-Ray 繞射儀進行晶相分析，結果顯示在 A(101) 相有一強度明顯較強的吸收峰，即爲得到具高純度與光觸媒活性之銳鈦礦相 (anatase) 的二氧

化鈦薄膜(圖一)。進一步利用 Trapalis *et al*. (2003)[12] 之 antibacterial drop-test 法，測試奈米二氧化鈦薄膜在紫外光 UV-A (360nm) 照射下，對菠白細菌性基腐病菌之抑制活性，並以大腸桿菌作爲對照組。分別經30與60分鐘之 UV-A (360nm) 照射處理後，以 1ml (0.05% Tween-20) 溶液將菌液從二氧化鈦薄膜表面洗出後，再將 100 μl 菌液移入 24 孔的培養盤中，每孔加入 900 μl Luria-Bertani (tryptone 1%, yeast extract 0.5%, sodium chloride 1%, pH 7.0) 培養基，並以 0.05% 之 TTC (2,3,5-triphenyltetrazolium chloride) 作爲細菌之生長指示劑[4]，置於 32℃ 之培養箱中培養 overnight。再加入 0.04 mol/L 酸異丙醇 (acid-isopropanol) 終止反應及溶解其結晶物 (triphenyl formazon)，並在波長 540 nm下測試其吸收值 [A]。抑菌率 (%) = $\frac{[A]_i - [A]_t}{[A]_i}$ *100%，$[A]_i$ 爲未經過二氧化鈦薄膜處理之對照組菌液在波長 540 nm 之吸收值。結果顯示在經二氧化鈦薄膜處理 30 分鐘後，對菠白細菌性基腐病菌及大腸桿菌之抑菌率分別爲 26.3%、31.8%，而經 60 分鐘處理後，對菠白細菌性基腐病菌及大腸桿菌之抑菌率皆達到 99.5% 以上 (圖二)。由實驗結果得知，利用奈米二氧化鈦光觸媒對菠白細菌性基腐病菌具有顯著之抑菌作用。因此，利用光觸媒二氧化鈦薄膜技術對植物病原菌之抑菌作用，將具有作爲未來水耕栽培系統之植物病害防治新工具的潛力。



圖一、經溶膠凝膠製程與旋轉塗佈法處理並經 500℃ 鍛燒後之 TiO_2 薄膜的 X-ray 繞射分析圖譜。

Fig. 1. X-ray diffractometry on TiO_2 thin film prepared by sol-gel and spin coating process and followed by 500°C calcinations.



圖二、光觸媒二氧化鈦薄膜經紫外光 A (360nm) 照射後對 (a) 菠白細菌性基腐病菌，(b) 大腸桿菌之抑菌作用。

Fig. 2. Photocatalytic inactivation of TiO_2 thin film against (a) *Enterobacter cloacae* SM1 and (b) *Escherichia coli* BCRC 10450, exposed to UV-A irradiation (360 nm) with and without TiO_2 thin film.

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ABSTRACT

Yao, K. S. [1,4,] Wang, D. Y.[2], Yan, J. J.,[2], and Tzeng, K. C.[3] 2005. Photocatalytic inactivation of *Enterobacter cloacae* SM1 by TiO_2 thin film under UV-A light irradiation *in vitro*. Plant Pathol. Bull. 14:265-268. ([1] Department of Post-Modern Agriculture, MingDao University, Changhua, Taiwan; [2] Institute of Material and System Engineering, Mingdao University, Changhua, Taiwan; [3] Department of Plant Pathology, Chung Hsing University, Taichung, Taiwan; [4] Corresponding author, E-mail: ksyao@mdu.edu.tw, Fax: +886-4-8783783)

To solve the problems of synthetic pesticide residues and environmental pollutions, it is necessary to obtain an alternative method for the control of plant diseases. In recent years, the technology of photocatalytic inactivation of microorganisms with UV-A/TiO_2, has been proposed as an innovative method, because it does not produce toxic compounds and has high efficiency of disinfection. It has been reported that *Enterobacter cloacae* SM1 causes bacterial basal rot of *Zizania latifolia* and leads to product losses. Photocatalytic inactivation of *E. cloacae* SM1 was therefore measured by TiO_2 thin films prepared by sol-gel process on glass substrates. The bactericidal effect was examined using 2,3,5-triphenyl- tetrazolium chloride (TTC) as a bacterial growth indicator. The results showed that the inhibition rate of TiO_2 thin film against bacteria of *E. cloacae* SM1 and *Escherichia coli* irradiation with UV-A (360 nm) for 30 min was 26.3% and 31.8%, respectively. However, after 60 min irradiation the bactericidal rate of thin film was more than 99.5% for tested bacteria. Therefore, the photocatalytic inactivation of phytopathogen with TiO_2 thin films has potential as a new tool for plant protection of hydroponics in the future.

Key words: Photocatalytic inactivation, TiO_2, *Enterobacter cloacae* SM1