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Short Communication

Efficiency of Titanium Dioxide on Mungbean Seed Sterile and their Nanotoxicity to Mungbean Growth *In Vitro*

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Abstract

Titanium dioxide (TiO_2) nanoparticles have been reported to be microbial disinfectant. Surface sterilization of explants with nanoparticles instead of high toxicity compound, such as, NaOCl, and $HgCl_2$, will be a challenge. TiO_2 nanoparticle was synthesized with sol-gel technique. This TiO_2 was tested their efficiency to sterilize mungbean seed surface at different concentration and their toxicity to mungbean seedling growth *in vitro*. There efficiency were compared with standard method used sodium hypochlorite in 10% Hyter© solution. Sterilized seeds were culture in ½ Murashigi and Skoog (MS) media for 10 d. Contaminated seed or seedlings with fungi or bacteria were counted. The result showed that the specific surface area of synthetic anatase TiO_2 contained 85.10 m^2/g with the average size of 15-30 nm. Only percentage of contamination of seeds sterilized with 50 mg/L TiO_2 induced with ultraviolet for 30 min was not significantly different to standard method. The mungbean seedling exposure to 250 – 2,500 mg/L TiO_2 in ½ MS, both of non-induce and 30 min UV-induced TiO_2, did not affect shoot and root growth, and chlorophyll content of 7-day old mungbean seedlings. © 2013 Friends Science Publishers

Keywords: Titanium dioxide; Mungbean; Surface sterilization

Introduction

Normally, surface sterilization of explant in plant tissue culture often use NaOCl or $HgCl_2$ (Baskaran and Jayabalan, 2005; Noman *et al*., 2008; Ranyaphia *et al*., 2011) in various concentration and time. However, these compounds are toxic to human and animal. Use of nanoparticle such as silver and titanium dioxide (TiO_2) with anti-bacterial properties will be an effective choice. There were several reports shown that TiO_2 nanoparticle have the anti-bacterial activity (Li *et al*., 2008). TiO_2 exhibits strong antibacterial activity which related to ROS production under ultraviolet illumination via redox pathway (Dinh *et al*., 2003). Upon the illumination of TiO_2 surface under the photon energy of 300-390 nm, inter-band transition creation the electron-hole pair (e^--H^+). Excited electrons are migrated from valence band to conduction band and leaved hole, while allowing the superoxide radicals ($O_2^{\bullet-}$). These $O_2^{\bullet-}$radicals can be further protonated to hydroperoxyl radicals ($HO_2^{\bullet}$). The overall photocatalysis reaction is promoted the reactive oxygen species (ROS), which can oxidize or reduce organic matter and further mineralized to CO_2 and H_2O.

The particle size plays an importance role in the antimicrobial activity of TiO_2. In general, the antibacterial activity increases with decreasing particle size. The photocatalytic disinfection of *P. aeruginosa* and *B. subtilis* sterilized in Ming China Lake with a thin film TiO_2 induced by UV light was studied. When the factors that affect the efficiency in killing bacteria such as temperature used in TiO_2 synthesis process were studied, it was found that the smaller particles and thinner film will be effective in degrading *P. aeruginosa* more than 95% and 75% for *B. subtilis* (Xu *et al*., 2006). Furthermore, thin film TiO_2 was used as the catalyst to kill *E. coli*, *P. aeruginisa*, *S. aureus* and *S. faealis* (Xu *et al*., 2004). Similar to those studies, *E. coli* DH 5a and *B. megaterium* QM B1551 could be killed by vanadium doped TiO_2 thin films. Titanium alloy has the ability to kill bacteria and significantly enhance microbial disinfection. This suggests that vanadium reduced the electron/hole recombination (Guifen *et al*., 2005).

However, there is a few study conducted on the surface sterilization activity of TiO_2 for plants tissue culture. There was a report showed that silver nanoparticles have a capacity to use as anti-bacterial agent for plant surface. Silver/clorox series shows great promise as a photocatalytic material for *Valeriana officinalis*. The most effective method is the use of 100 mg/L 180 min silver particles, followed by 10% clorox disinfection, which showed 11% contamination (Abdi *et al*., 2008). The probability and capacity of TiO_2 for surface sterilization will be more

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challenge than silver with their low cost for synthesis.

The nano-toxicity will be an important concern for nanoparticle application (Bystrzejewska-piotrowska *et al*., 2009; Ahmed *et al*., 2010). There have been some reports showed that they were toxic to plant growth. For example, silver nanoparticles inhibit seedling growth of *Phaseolus radiatua* and *Soghum bicolor* and accumulate in plant tissue (Lee *et al*., 2012). On the other hand, spinach seeds immersed in 0.25- 0.6% nano and non-nano particleTiO_2 under natural sunlight for 48 h was not retarding seed germination and their seedling growth (Zheng *et al*., 2005). Also, 1 – 100 mg/L TiO_2 without UV induction was no acute toxicity to growth willow tree cutting (Seeger *et al*., 2009). There have been no exactly known about plant nanotoxicity of UV-induced TiO_2. Because plant toxicity of UV-induced TiO_2 will limit benefit for surface sterilization, the nanotoxicity of TiO_2 suspension induced by ultraviolet radiation to seedling growth *in vitro* was examined. The results of this study will serve as a knowledge base to study the possible use of titanium dioxide as a disinfectant.

Materials and Methods

TiO_2 Synthesis and Characterization

The anatase TiO_2 nanoparticles were prepared by procedure described previously (Wetchakul and Phanichphant, 2006). Titanium isopropoxide, absolute ethanol and ammonia were introduced in the cellophane membrane, which putted in the beaker containing ethanol and deionized water and vigorously stirred at the temperature of 80ºC for an hour. The mixture of precursor was quenched in an ice bath and subsequently washed residual away with 250 mL distilled water for 3 times. Then, those mixtures were dried and heated at the temperature of 110ºC and 400ºC, respectively with increasing of 2ºC/min. The crystallinity of TiO_2 nanoparticles were identified by X-ray diffraction (XRD). The morphology was examined by scanning electron microscope (SEM) and transmission electron microscope (TEM). The specific surface area was evaluated by nitrogen desorption technique.

Efficiency for Seeds Sterilization

Mungbean seeds (commercial seeds from Nakhonsawan Province, Thailand) were rinsed with tap water and detergent. After that, 2.5, 25 and 50 mg/L TiO_2 were induced with ultraviolet for 30 min and were stirred with magnetic bar for thoroughly exposed to UV. Seeds were immersed in each TiO_2 solution for 30 min after exposed to UV without stirring. The efficiency of sterilization was compared with seeds sterilized with 10% Hyter © (containing 6% NaOCl) for 20 min and then in 5% Hyter © for 10 min and seed without any surface sterilized. The sterilized seeds were rinsed with sterile distilled water three times and then inoculated in ½ MS media. These seeds were cultured at 25^0C and received light 16h per day. The contaminated seeds with either bacteria or fungi were count at day 5 and day 10 after inoculation.

Nanotoxicity to *In Vitro* Plant Growth

Mungbean seeds were rinsed with tap water and detergent. After that, these seeds were sterilized with 10% Hyter ©for 20 min and then in 5% Hyter © for 10 min. The sterilized seeds were rinsed with sterile distilled water three times and then inoculated in ½ MS media containing 0, 250, 2500 mg/L TiO_2 induced or non induce with ultraviolet for 30 min before use. These seeds were cultured at 25ºC and received light 16 h per day. After 7 days, all seedlings were removed and shoot length, root length, shoot fresh weight, shoot dried weight, root fresh weight, and root dried weight were measured. Chlorophyll a and chlorophyll b content in seedling leaves were measure follow Huang *et al*. (2004).

Statistical Analysis

ANOVA was used to test for statistically significant differences between treatments. One way ANOVA was used to examine the sterilization efficiency. Two ways ANOVA was used to examine the nanotoxicity of TiO_2 to seedling growth followed by LSD's test.

Results and Discussion

Efficiency for Seeds Sterilization

The TiO_2 specific surface area of 83 m^2/g was determined by using BET adsorption of nitrogen gas. The particle size of TiO_2 was found to be in the range of 15-30 nm (Fig. 1A). TEM observations showed spherical shape and highly porous of the particles (Fig. 1B). The electron diffraction patterns indicated the intensity of polymorphic discrete ring of good the crystalline of the synthesized particle process as show in Fig. 1C (inset). Fig. 1D shows the XRD patterns of TiO_2 sample. The distinctive peaks at 2θ = 25.3º, 37.8º, 47.7º, 54.0º and 62.4º, indicated that the position and intensity of characteristic peaks of anatase crystal planes and was confirmed with the JCPDS file no. 76-318.

TiO_2 Nanoparticle could sterilize mungbean seed when induced with UV for 30 min. Percent of mungbean seed contamination sterilized with 50 mg/L TiO_2 (16%) was not significant from standard method with NaOCl 10% (6%). However, when lower amount of TiO_2 (2.5 – 25 mg/L) were used, their efficiency were significantly lower than standard method (Fig. 2). All seeds without bacterial or fungal contamination were germinated. The effective concentration of UV-induced TiO_2 (50 mg/L) was lower than the effective method of silver nanoparticle (100 mg/L) without UV induction and effective exposure time of UV-induced TiO_2 (30 min) was shorter than silver nanoparticle (180 min)

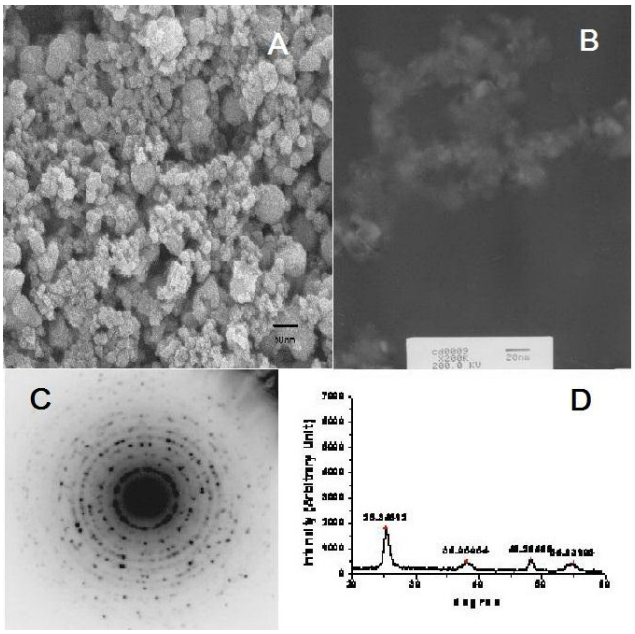

Fig. 1: Fig. 1: SEM Bar = 50 nm (A) TEM Bar = 20 nm (B) electron diffraction patterns (C) and X-ray diffraction image (D) of TiO2 nanoparticle used in this experiment

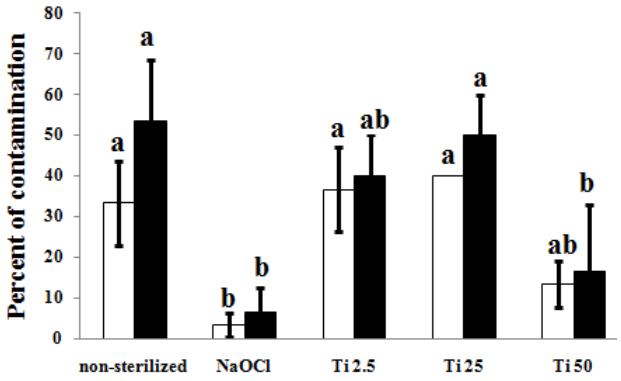

Fig. 2: Percentage of mungbean seed contamination after sterilized with UV-induced TiO_2 various concentration and cultured in ½ MS media. Symbols; □ day 5 after sterilization, ■ day 10 after sterilization. Different lower case letter showed significant difference between treatment on same day *(P<0.05)*. There was no significant difference between days of all treatments

(Abdi *et. al*., 2008). Disinfection mechanism of both silver nanoparticles and TiO_2 is related to reactive oxygen species (ROS), but TiO_2 requires UV excitation. Silver ions directly interact with thiol group in bacterial cell, and leading to inactivation of respiratory enzymes. Penetration through cell membrane of silver ions was inhibited bacterial growth (Abdi *et al*., 2008; Li *et al.,* 2008). Meanwhile, TiO_2 can kill

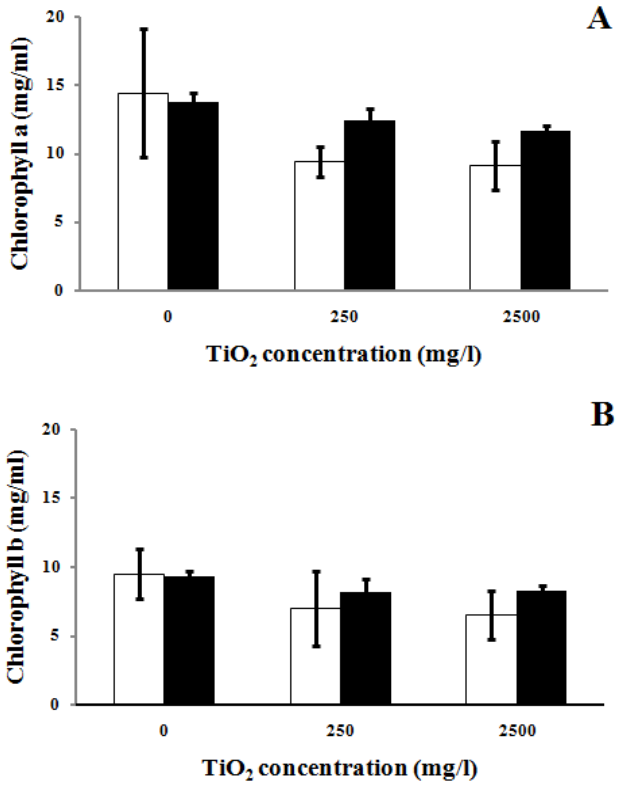

Fig. 3: Content of chlorophyll *a* (A), and chlorophyll *b* (B) of mungbean seedling cultured in ½ MS media + TiO_2. Symbols; □ non-expose with UV, ■ exposed with UV for 30 min. There were no significant difference between treatment (P<0.05)

both gram positive and gram negative bacterial by free hydroxyls radical and peroxide generated via oxidative and reductive pathways, respectively (Li *et al*., 2008). This result showed that UV-induced TiO_2 should be an effective choice for sterilization

Nanotoxicity to *In Vitro* Plant Growth

TiO_2 Nanoparticle clearly showed that there was no toxicity to mungbean growth *in vitro*. The percent of germination were 100% in all treatments (data not shown). Shoot and root growth of mungbean seedling growing in ½ MS + 250 – 2,500 mg/L TiO_2 were not significantly different from those growing in ½ MS without TiO_2 (Table 1). Also, UV-induced TiO_2 did not affect to plant growth differently when compared with non-induced TiO_2 as same concentration. The chlorophyll a and b content did not affect by TiO_2 also (Fig. 3).

The sterilization of *Valeriana officinalis* with silver nanoparticle which exposure time more than 180 min made the explants to bleach (Abdi *et al*., 2008). Also, the growth of plant exposed to silver nanoparticle was reduced without UV induction (Lee *et al*., 2012). It seems to be that TiO_2 was the less toxic nanoparticle to use with plant. The

Table 1: Growth of mungbean seedling in ½ MS media containing vary concentration of Titanium dioxide for 1 week. Values are the mean ± SD

$[TiO_2]$ (mg/L)	Shoot length (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root length (cm)	Root fresh weight (mg)	Root dry weight (mg)
0	8.2 ± 3.0a*	275.1 ± 42.6a	25.1 ± 4.2a	6.0 ± 2.1a	85.5 ± 20.0a	4.5 ± 0.8a
250	8.6 ± 3.9a	224.5 ± 61.6a	22.5 ± 6.2a	6.0 ± 1.a	83.2 ± 22.3a	4.2 ± 1.0a
2,500	7.9 ± 3.1a	244.8 ± 47.5a	24.1 ± 5.0a	4.3 ± 1.6a	81.6 ± 14.0a	4.6 ± 1.3a
0 + UV 30 min	7.2 ± 3.2a	265.2 ± 53.1a	23.9 ± 4.9a	5.2 ± 1.9a	84.5 ± 19.6a	4.5 ± 1.1a
250+ UV 30 min	6.3 ± 2.3a	257.4 ± 63.3a	27.5 ± 4.5a	4.8 ± 2.8a	82.0 ± 19.6a	4.4 ± 1.2a
2,500+ UV 30 min	6.5 ± 2.8a	260.8 ± 56.0a	23.2 ± 4.9a	4.5 ± 1.4a	81.3 ± 22.8a	5.2 ± 1.3a

*Values followed by different letters are statistically different (P< 0.05)

antimicrobial activity of silver is concerned to the number and the rate of silver released (Rai *et al*., 2009). Meanwhile, the physicochemical properties of TiO_2 such as concentration, particle size, light intensity and UV wavelength play an important role in the antimicrobial activity (Li *et al.*, 2008). Even though, UV induction will increase cost of TiO_2 but there were some reports shown that UV in natural sunlight could induce photocatalysis reaction of TiO_2. An alternative feature of TiO_2 photocatalytic antibacterial activity is can be activated under sunlight. By visible light, metal doped TiO_2 has been used to improve visible light absorbance of TiO_2. TiO_2 is believed to enhance the capacity to destroyed cellular morphology and DNA of *E. cartovora* by metal dopand under visible light irradiation (Yao *et al*., 2007). This is challenge topic to develop TiO_2 to be more cheap and effective technology for sterilization.

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