EFFECT OF TITANIUM DIOXIDE NANOPARTICLES ON GROWTH OF Curvularia lunata
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Abstract: The effects of TiO2 nanoparticles on growth of pathogenic soil fungi, Curvularia lunata, were studied. The concentration of 0, 250, and 2500 mg/l TiO2 nanoparticles were added in spore suspension or in Potato Dextrose Agar (PDA). One set of TiO2 nanoparticles was induced with UV for 30 min and another set did not induced. The results showed that the exposure of spore to TiO2 nanoparticles did not inhibited C. lunata growth well. The growths of C. lunata were more inhibited when TiO2 nanoparticles were added in PDA. It is possible that TiO2 nanoparticles were toxic to the fungal cell when growing in media more than spore.

Introduction: The application of metal oxide nanomaterials in the antibacterial field has emerged as a successful technology in recent years. Among of those metal oxides, titanium dioxide (TiO2) has been found highly attractive due to its potential photocatalytic under ultraviolet. The antibacterial activity of TiO2 nanomaterials depends on the particle size, morphology, crystalline structure, phase composition, concentration and the used of doping metal ion. Generally, the antibacterial mechanism of TiO2 involved interaction of the hydroxyl free radical with amino group in the molecule of protein in microorganism, resulting in cell membrane damage, cause cell death.

The efficiency of TiO2 nanoparticles to kill bacteria was reported in many studied. Titanium dioxide thin film could show antibacterial activity to Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus faealis1. Also, anatase TiO2 nanocomposite with Vanadium could inhibit growth of E. coli DH 5α and Bacillus megaterium QM B15512. However, the potential use of TiO2 nanoparticles as anti-fungal compound was reported only with yeast, such as Candida albicans3 and Saccharomyces cerevisiae4. In this study, the anti-fungal of TiO2 nanoparticles were tested with Curvularia lunata which was plant pathogen and often found in soil. For example, C. lunata caused leaf blight in soghum, leaf spot on Cannabis sativa and seedling blight in Saccharum spp.5

Methodology: The synthesis procedure of TiO2 nanoparticles was reported by Wetchakun and Phanichphant6. A cellophane membrane pouche contained a mixture of a titanium isopropoxide used as a precursor was mixed with an absolute ethanol under vigorous stirring. The cellophane membrane pouche was then put in the beaker containing absolute ethanol, ammonia solution and deionized water, following heated at 70-80°C for an hour under vigorous stirring. Condensation and hydrolysis reaction of titanium ions took place instantly, forming titanium dioxide. After hydrolysis, the reaction in the cellophane membrane pouche was stopped by quenching the mixture in an ice bath and washed 3 times with milli-Q water to remove the impurities. The water in the slurry mixed solution was evaporated in an oven set at 100-110°C for a day. Finally, the powders were ground and calcined at the temperature of 400°C for 3h to obtain anatase TiO2 phase. The TiO2 nanoparticles were then characterized by using X-ray diffractrometry (XRD), transmission electron microscopy (TEM) and nitrogen adsorption (BET).

The culture of C. lunata on PDA incubated at 30±2 °C for 5 days. The spore suspension was prepared by flooding the plates with 5 ml of sterilized distilled water and the
Conidia were gently scraped with sterile inoculation needle. The titanium dioxide was dissolved in distilled water and prepared concentrations of 0, 250, and 2500 mg/l. Each concentration was induced with UV for 0, and 30 minutes before loaded in agar well. Ten µl of titanium dioxide was added into the 0.5 cm³ agar wells on PDA medium. After that, 10 µl of spore suspension was added into the agar wells on PDA medium and then incubated at 30±2 °C for 5 days. The diameter of fungal colony was measured everyday. The percentage of growth inhibition (I) were calculate as $I = (1-d_t/dc) \times 100\%$.

The PDA containing 0, 250, and 2500 mg/l of TiO₂ was prepared and then induced with UV for 0, and 30 minutes before used. The 10 µl spore suspension of C. lunata was added into the 0.5 cm³ agar wells of PDA plates and then incubated at 30±2 °C for 5 days. The diameter of fungi colony were measured and calculated as described above.

**Results, Discussion and Conclusion:**

The XRD confirmed the obtained hexagonal structure of the anatase TiO₂ (Figure 1). The surface morphology with the porous nanostructure was investigated using TEM (Figure 2). Particle with average diameter of 20 nm was observed. The specific surface area (SSA) was evaluated by nitrogen adsorption (BET analysis). The SSA of TiO₂ nanoparticle was found to be 84.30 m²/g.

![Figure 1. XRD patterns of TiO₂ nanoparticles calcined at 400°C for 3h and JCPDS file no. 78-1764 (inset)](image)
Figure 2. TEM image of TiO$_2$ nanoparticles and electron diffraction pattern (inset) after calcination at 400°C for 3h.

The presence of TiO$_2$ nanoparticles were low toxicity to *C. lunata* growth in PDA when these fungi exposed to TiO$_2$ nanoparticles in spore suspension (Figure 3 and 5). Only spores exposed to 2500 mg/l TiO$_2$ nanoparticles which induce with UV for 30 min was reduced 40% on day 2. However, the toxicity to *C. lunata* growth of 2500 mg/l TiO$_2$ nanoparticles without UV induction did not different from 250 mg/l TiO$_2$ nanoparticles.

Figure 3. Inhibition percentage of growth of *C. lunata* growing on PDA after exposed to TiO$_2$ nanoparticles in spore suspension.

When TiO$_2$ nanoparticles were added into PDA directly, the toxicity of TiO$_2$ nanoparticles to *C. lunata* growth was higher than exposed in spore suspension. All concentration of TiO$_2$ nanoparticles, with or without UV induction, could inhibit fungal growth as 60 -70 % since day 2 (Figure 4 - 5). It is possible that the hydroxyl free radical occur from TiO$_2$ nanoparticles could destroy the fungal cell when growing in media more than spore. Even though, Exposing with TiO$_2$ nanoparticles as spore suspension did not inhibited *C. lunata* growth well but the pattern of fungal colony was abnormal (Figure 5). The toxicity of TiO$_2$ nanoparticles on fungal growth at molecular level sould be studied in more detail.
Figure 4. Percentage of growth inhibition of C. lunata growing on PDA containing various concentrations of TiO$_2$ nanoparticles.

Figure 5 C. lunata growing on PDA for 6 day (left) without TiO$_2$ nanoparticles (center) exposed to 2500 mg/l TiO$_2$ nanoparticles which induce with UV for 30 min in spore suspension (right) exposed to 2500 mg/l TiO$_2$ nanoparticles which induce with UV for 30 min in PDA.

References:

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Keywords: Titanium dioxide, Curvularia, Anti-fungal activity