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NOTA CORTA / SHORT NOTE

NEW METHOD OF SULFADIAZINE RESIDUE BIODEGRADATION IN POULTRY MANURE BY SPORE-BOUNDING LACCASE

Nuevo método de biodegradación de residuos de sulfadiazina en estiércol de aves de corral mediante lacasa de esporas

Pimprapa CHAIJAK[1*]

[1.] Department of Biology, Faculty of Science and Digital Innovation, Phatthalung, Thaksin University, Thailand

[*] *For correspondence: chaijak.pimprapa@gmail.com*

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ABSTRACT

Antibiotics have been used in livestock farming worldwide. In poultry farming, sulfonamide antibiotics are mainly used to inhibit microbial infection. Sulfadiazine (SDZ) is one type of sulfonamide that is secreted into the ecosystem through feces and urine owing to its low adsorption and degradation in the animal intestine. In this study, the spore-bound laccase from the *Bacillus* sp. strains was investigated for its potential for degradation of SDZ. The highest laccase activity was selected to degrade the SDZ residue in the poultry feces. The results demonstrated that the spore-bound laccase of *Bacillus* sp. PM45 successfully reduced the residue of SDZ in poultry manure by 98.00±0.50 %. This work gained new knowledge and the method is cost-effective and more eco-friendly for antibiotic residue treatment.

Keywords: Antibiotics, Biodegradation, Laccase, Poultry, Sulfadiazine

RESUMEN

Los antibióticos se han utilizado en la ganadería de todo el mundo. En avicultura, los antibióticos sulfonamídicos se utilizan principalmente para inhibir la infección microbiana. La sulfadiazina (SDZ) es un tipo de sulfonamida que se segrega al ecosistema a través de las heces y la orina debido a su baja adsorción y degradación en el intestino animal. En este estudio, se investigó la actividad de la lacasa unida a esporas de cepas de *Bacillus* sp. y su potencial de degradación de SDZ. Se seleccionó la mayor actividad de lacasa para degradar los residuos de SDZ en las heces de las aves de corral. Los resultados demostraron que la lacasa unida a esporas de *Bacillus* sp. PM45 reducía con éxito el residuo de SDZ en el estiércol avícola en un 98,00±0,50 %. Este trabajo aportó nuevos conocimientos y el método es rentable y más ecológico para el tratamiento de residuos de antibióticos.

Palabras clave: Antibióticos, Biodegradación, Lacasa, Aves de corral, Sulfadiazina

Antibiotics are successfully used in both veterinary and human drugs to treat microbial infections and boost the growth of animal farming (Bai et al., 2019). In 2010, the antibiotics consumed for livestock production, such as poultry, swine, and cattle, were predicted to be approximately 63.151 tons and are estimated to rise by 67 % in 2030 worldwide (Van Boeckel et al., 2019). Sulfonamide (SA) antibiotics such as sulfamethazine (SMZ), sulfadimidine (SM2), and sulfadiazine (SDZ) are the general antibiotics applied in livestock growth promoters (Oberoi et al., 2019). The sulfonamide metabolites secreted by the livestock in the form of manure or urine can be released into the environment (Weng et al., 2012). However, the non-absorbable form of sulfonamide remains in the livestock digestive tract in the active form (Zhang et al., 2017). Approximately 62 – 94 % of sulfonamide has been reported in the groundwater (Chen et al., 2015). Therefore, the contaminated sulfonamide drug residue causes antibiotic resistance, resulting in a high rate of human infection and death (Cañas and Camarero, 2010). The SA removal has been reported through various processes, including physical, chemical, and biological methods (Xu et al., 2007). Additionally, degradation potential and genes in the SA degrading bacteria are more interested owing to low operating cost and environmentally friendly process (Zhou et al., 2018).

Laccase has shown a high prospective in the bioremediation of various pollutants using crude enzymes and free enzymes. It is also reported to be used for SA degradation through an oxidation reaction. However, its application in bioremediation and biodegradation at a large scale is restricted by the complex substrate structure and extreme environmental conditions (salt concentration, pH, temperature) that disturb enzyme stability, recycling, and recovery. These concerns are also related to operational problems and the requirement for large-scale laccase production (Arregui et al., 2019; Ding et al., 2016). Hence, the environmentally tolerant laccase is interested in improving this problem.

The spore-bound enzyme has been initially proposed for the development of several biotechnological applications owing to its remarkable and high stability in a broad range of conditions (Mattossovich et al., 2017). Spore-bound laccase of *Bacillus* sp. has successfully been applied in the biotechnological process owing to its high thermo-stability and pH-stability (Wang et al., 2011). Thus, we hypothesize the development of the spore-bound laccase to degrade the SDZ residue found in poultry manure.

All chemicals used in this experiment are analytical grade and were purchased from Sigma-Aldrich, United States. A total of 50 samples of *Bacillus* sp. isolated from poultry manure were acquired from the Microbial Fuel Cell & Bioremediation Laboratory, Thaksin University. All *Bacillus* sp. strains were used to study the spore-bound laccase activity and SDZ removal potential in the next section.

For spore preparation, all strains were inoculated onto nutrient agar (10.0 g/L peptone, 10.0 g/L beef extract, 8.0 g/L NaCl and 15.0 g/L agar) supplemented with 0.63 g/L $CuSO_4$ modified from the previous study (Wang et al., 2010) and incubated at 37 °C for seven days. The spores of *Bacillus* sp. were removed from the agar surface with 0.85 % sterile NaCl and resuspended in 0.1 Mol/L sodium phosphate buffer (pH 6.8).

For laccase activity, the 100 µL of spore suspension with 1.0 x 10^7 spore/mL was added into 2.9 mL of 0.1 mmol/L ABTS contained sodium acetate buffer, pH 6, and were incubated for 3 min. The oxidation of ABTS was detected by measuring the absorbance at 420 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). One unit (U) of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per minute. A *Bacillus* sp. with spore-bound laccase activity was selected.

In vitro SDZ biodegradation, the 10 % (v/v) of spore-suspension (1.0 x 10^7 spore/mL) was inoculated into 100 mg/L SDZ solution incubated for 3 hr at room temperature. All samples were collected and centrifuged at 12000 rpm for 10 min. Then the supernatants were filtered through the 0.22 µm filter. The filtrated liquids were measured by UV-Vis spectrophotometry at 458 nm (Mohammed and Zebary, 2013). Briefly, the 1.0 mL of filtered liquid was transferred into a 25 mL volumetric flask. The 0.5 mL of 1M HCl solution and 0.5 mL of 1.0 % (w/v) sodium nitrate solution were added. The reactions were incubated at room temperature for 1 min. Then the 1.0 mL of 3.0 % (w/v) sulfamic acid solution was added and mixed thoroughly for 1 min. The 1.0 mL of 1M NaOH was added, the solution was mixed well. The 20.5 mL of deionized water was added. The colorimetric method was determined at 458 nm. The SDZ removal was calculated. The calibration curve of SDZ was created by varying SDZ concentration ranges 10 – 100

Table 1 Using microbial laccase for SDZ biodegradation.

Microbe	Laccase activity (U/mL)	Enzyme form	Initial SDZ Conc. (mg/L)	Removal (%)	Reference
Bacillus sp. PM45	8.12±0.61	Spore-bound	100	98.00±0.50	This study
Bioengineering *Escherichia coli*	2.00	Cell-bound	500	58	(Li et al., 2021)
Pleurotus eryngii	NA	Extracellular	0.03	100	(García-Delgado et al., 2018)
Trametes versicolor	0.53	Extracellular	0.15	70	(Ding et al., 2016)

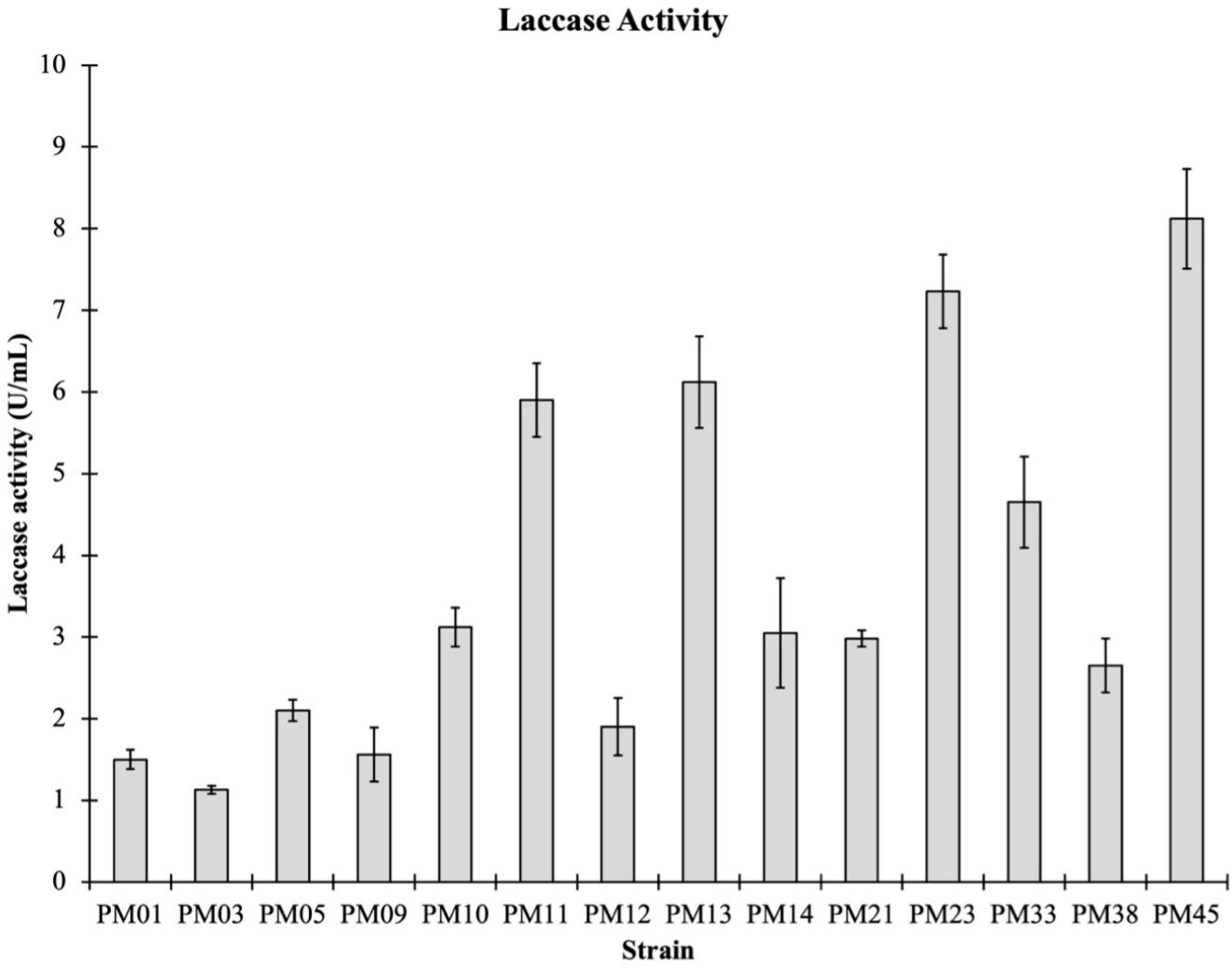

Figure 1. The laccase activity of the bacterial strains in this experiment.

mg/L. The *Bacillus* sp. with 50 % SDZ removal was selected to be used in the next section.

In poultry manure, the 10 % (v/v) of spore suspension (1.0 x 10^7 spore/mL) of *in vitro* selected *Bacillus* sp. was inoculated into the fresh poultry manure (10.52±0.50 % dry weight of moisture content, 90.11±5.02 % dry weight of total solid, 62.30±0.51 % of volatile solid, 59.00±1.00 g/kg of organic carbón, 7.01±0.50 g/kg of TKN, 50.00±1.00 mg/kg of SDZ, pH 6.90±0.10). It was mixed and incubated at room temperature for 3 hr. All samples were collected and centrifuged at 12000 rpm for 10 min. Then the supernatants were filtered through the 0.22 µm filter. The filtrated liquids were measured by UV-Vis spectrophotometry at 458 nm (Mohammed and Zebary, 2013). The highest SDZ removal strain was selected.

The effect of spore concentration was studied. The various spore concentrations were studied. The 10 % (v/v) of spore suspension concentration between 0.5 to 10.0 x 10^7 spore/mL was inoculated into the fresh poultry manure. It was mixed and incubated at room temperature for 3 hr. The SDZ removal was determined.

The results showed that among 50 *Bacillus* sp. strains, only 14 strains showed spore-bound laccase activity. The highest laccase activity was found in *Bacillus* sp. PM45 at 8.12±0.61 U/mL followed by *Bacillus* sp. PM23 at 7.23±0.45 U/mL (Fig. 1). The initial 100 mg/L SDZ solution was used to study the SDZ degradation potential of spore-bound laccase. Only the strain with 50 % SDZ removal potential was selected. Among 14 laccase-producing strains, only five strains showed an SDZ removal of more than 50 %. The maximum SDZ removal of 80.82±1.33 % was obtained by strain PM45, followed by PM23, PM13, PM11, and PM33 of 72.02±3.90 %, 70.64±2.45 %, 69.90±5.21 %, and 50.29±0.90 % respectively.

To prove the SDZ degradation ability of spore-bound laccase in real poultry manure. The 1.0 x 10^7 spore/mL of

candidates were used. The results showed the maximum SDZ removal of 75.02±1.13 % was gained by strain PM45.

To find the optimum spore concentration for the SDZ removal from fresh poultry manure, whereas the initial SDZ concentration was 50 mg/kg manure. The spore-bound laccase of selected *Bacillus* sp. PM45 was used. (Fig. 4) shows the SDZ removal by the various spore concentrations. The results showed the optimum spore concentration for 50 mg/kg manure SDZ was 8.0 x 10^7 spore/mL with 98.00±0.50 % SDZ removal.

A spore-bound laccase is a typical bacterial laccase. Their results showed that laccase is one of the integral proteins located on the spore membrane. The spore-bound laccase has shown its advantages for the bioremediation of industrial textile dye (Loncar et al., 2014; Lu et al., 2012). The use of microbial laccase for SDZ and other antibiotic degradation has been reported. The use of microbial laccase for SDZ degradation was shown in (Table 1). According to the results in the table, we found that microbial laccases have been successfully used in the SDZ degradation. Moreover, our study provides the first report about the use of spore-bound laccase for SDZ degradation.

In conclusion, the pore-bound laccase from *Bacillus* sp. isolated from poultry manure improves the degrading efficiency of SDZ. The 8.0 x 10^7 spore/mL of spore from the *Bacillus* sp. PM45 was successfully applied in SDZ residue degradation of 98.00±0.50 % from the poultry manure. This work gained new knowledge and the method is cost-effective and more eco-friendly for antibiotic residues directly from poultry manure.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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