Journal of Coastal Research	SI	86	138–141	Coconut Creek, Florida	2019

Usage of *Pleurotus ostreatus* for Degradation of Oxytetracycline in Varying Water Salinities in Brackishwater Aquaculture System

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ABSTRACT

Lalitha, N.; Patil, P.K.; Rajesh, R., and Muralidhar, M., 2019. Usage of *Pleurotus ostreatus* for degradation of oxytetracycline in varying water salinities in brackishwater aquaculture system. *In*: Jithendran, K.P.; Saraswathy, R.; Balasubramanian, C.P.; Kumaraguru Vasagam, K.P.; Jayasankar, V.; Raghavan, R.; Alavandi, S.V., and Vijayan, K.K. (eds.), *BRAQCON 2019: World Brackishwater Aquaculture Conference. Journal of Coastal Research,* Special Issue No. 86, pp. 138-141. Coconut Creek (Florida), ISSN 0749-0208.

The oxytetracycline is being used in aquaculture industry as a broad spectrum antimicrobial agent with bacteriostatic property. Unregulated and indiscriminate usage of antibiotics leads to their buildup in the environment. Physical and chemical methods to remediate or remove these antibiotics are ineffective. The present study aims at the mycotic bioremediation of the oxytetracycline by *Pleurotus ostreatus*. It was cultured in the potato dextrose broth for 7 to 14 days and used in the experiment with fungal load of 10^4 CFU ml^{-1}. *In vitro* soil based lab study was performed in triplicate at four different salinities 1, 15, 30 and 45 ppt, with three different concentrations of oxytetraycline (50, 150 and 250 mg L^{-1}), and treated with *P. ostreatus*. The suspended water samples were collected at periodic intervals of 2, 7 and 14 days after treatment and estimated for antibiotic in treatment and control. It was found that the *P. ostreatus* significantly ($p \leq 0.05$) degraded the antibiotic at all salinities. The reduction of drug ranged from 58-76% (2 days), 45-78% (7 days) & 36-62% (14 days) at 1ppt, 32-34% (2 days), 58-71% (7 days) & 48-80% (14 days) at 15 ppt, 52-73% (2 days), 27- 61% (7 days) & 44-62% (14 days) at 30 ppt, and 32-68% (2 days), 44-66% (7 days) & 44-64% (14 days) at 45 ppt compared to the control.

ADDITIONAL INDEX WORDS: *Aquaculture, mycotic remediation, oxytetracycline, salinity.*

INTRODUCTION

Indian brackishwater aquaculture is predominated with shrimp farming. Pacific white shrimp, *Penaeus vannamei* cultured in the country has the ability to grow in varying salinities. In aquaculture, oxytetracycline (OTC) is widely used to control pathogenic vibrios and fish bacterial diseases. The usage of antibiotics as prophylaxis measure has led to antibiotic residues in the food animal products resulted in the rejection of export fish and shrimp products (Cabello 2006). Maximum residue limit (MRL) of the OTC used as antimicrobial agent in muscle tissue of fish species and *Penaeus monodon* was 200 µg/kg (Codex Alimenrarius, 2018). The non-degradable antibiotic use in the aquaculture forces a threat to the human and animal health because of its persistence in the environment for longer duration (Manage, 2018). The OTC residue of 1.8 µg g^{-1} (10^{th} day) in tissues, 0.5 µg ml^{-1} (10^{th} day) in water, 1900 µg g^{-1} (10^{th} day) in soil was found when fed with feed @ 1% body weight in the freshwater fish rainbow trout (Williams, Bullock, and Carson, 2002). These antibiotics in the environment especially in water and soil can be remediated by using the physical, chemical and microbial methods. Bansal (2013) suggested the green remediation technology for remediation of OTC using vetiver grass, water lettuce, and sunflower. Makhijani *et al*. (2014) used the seeds of *Cicer arietiumum* as phytoremediator to remediate the tetracycline. In the river and water sediment of the aquatic environment, microbes play pivotal role in the remediation of OTC (*Dzomba, Kugara, and Zaranyika*, 2015).

Migliore *et al*. (2012) studied the effect of *Pleurotus ostreatus* for degradation of OTC at various concentrations 50, 100 µg ml^{-1} in the culture medium showed degradation on 3rd day and removed totally on 14^{th} day. *Pleurotus ostreatus* degraded 68 to 91% of ciprofloxacin at 500ppm concentration after 14 days (Singh, Khajuria, and Kaur, 2017). Information is scanty on degradation of antibiotics in brackishwater ecosystem of varying salinities. The present study aims at evaluating the efficiency of the *P. ostreatus* as the bioremediating agent in degradation of OTC (50, 150 and 250 mg L^{-1}) at four salinities (1, 15, 30 and 45 ppt).

METHODS

The oxytetracycline hydrochloride, ≥95% (HPLC), crystalline (5-Hydroxytetracycline hydrochloride with empirical formula $C_{22}H_{24}N_2O_9$. HCl) with molecular weight 496.89 was obtained from Sigma Chemical company (St. Louis, MO 63103). *Pleurotus ostreatus* was cultured in the potato dextrose broth (Himedia Laboratories, Mumbai, India) for 7 to 14 days, incubated at 25±2°C.

DOI: 10.2112/ SI86-021.1 received 7 March 2019; accepted in revision 14 May 2019.

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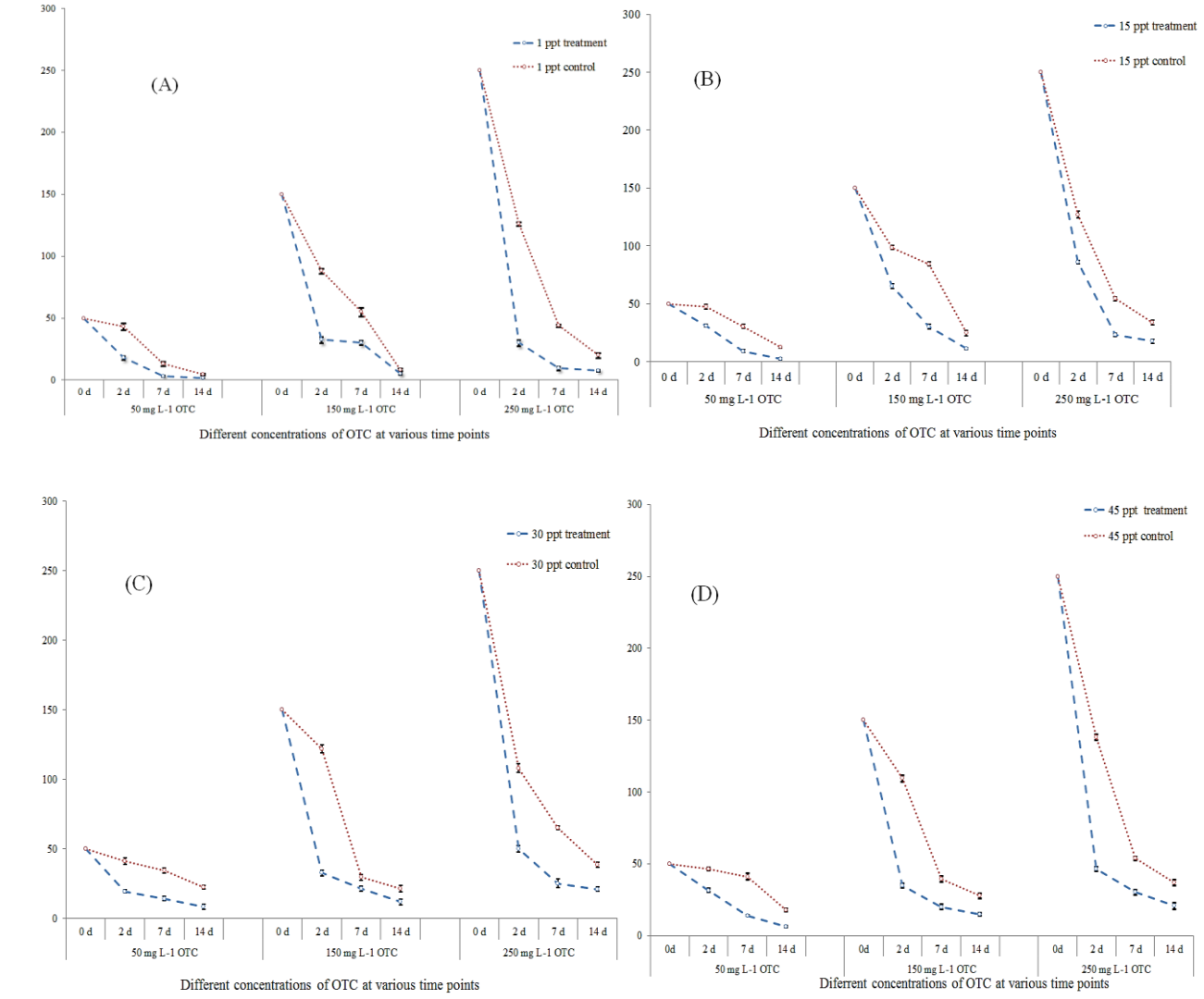

Figure 1. Effect of *Pluerotus ostreatus* on degradation of oxytetracycline in varying salinities.

In vitro soil based lab study was performed in triplicate at four varying salinities (1, 15, 30 and 45 ppt) at three different concentrations of OTC (50, 150 and 250 mg l^{-1}), with and without *P. ostreatus* following completely randomized design. Varying salinities water (1, 15, 30 and 45 ppt) 250 ml and 100 g L^{-1} of soil was autoclaved in 500 ml Duran bottle and then added with different concentrations of OTC 50, 150 and 250 mg L^{-1} in the respective treatments.

Pleurotus ostreatus with fungal count of 10^4 CFU ml^{-1} (Amuneke, Dike, and Ogbulie, 2011) was added in the treatment bottles. After addition of *P. ostreatus*, the suspended water samples were collected and estimated for OTC at periodic intervals on 2nd, 7th and 14th day of the experiment in treatment and control. The OTC estimation in the water samples was carried out using the LC MS MS (Liquid chromatography Mass Spectrophotometry). Sample of 0.5 ml was taken in a centrifuge tube, mixed with 5ml of Mcllvaine buffer, vortexed, and incubated for 10 minutes. To the solution 20 ml Mcllvaine buffer was added, vortexed and centrifuged for 2 minutes at 6500 rpm at 4°C. The extract was filtered through Whatman No.1 filter paper and washed with Mcllvaine buffer and the filtrate was collected. The eluent was evaporated to dryness under nitrogen at 50°C. The residues were dissolved in 1ml of water, filtered through 0.22 µm membrane filter and injected in LC MS MS.

The operating conditions of the instrument were mobile phase (A) 0.1% formic acid in water, mobile phase (B) methanol, flow rate 0.4 ml min^{-1}; injection volume 10 µl,

column-Agilent Eclipse plus C18 4.5 mm X 50 mm, 3.5 µm and column temperature 30°C. The significance of difference between the mean OTC degradation at different treatments of various factors (salinity, time) are tested by one factor ANOVA using DUNCAN test (SPSS version 17.0) .

RESULTS

The effect of *P. ostreatus* on degradation of different concentrations of OTC at varying salinities is shown in Figure 1. Significant ($p \leq 0.05$) difference in the efficiency of OTC degradation was observed at varying salinities (1, 15, 30 and 45 ppt) and at the concentrations studied (50, 150 and 250 mg L^{-1}). At lower salinity (1 ppt) 50 mg L^{-1} OTC was degraded to 18.06, 3.34 and 1.93 mg L^{-1} while untreated controls were 43.35, 13.16 and 4.84 mg L^{-1} on 2nd, 7th and 14th days respectively.

Similarly, at OTC concentration of 150 mg L^{-1} the degradation was 63.14, 44.83 and 36.21% on 2nd, 7th and 14th day post treatment, respectively. The group with higher concentration of OTC (250 mg L^{-1}) showed significantly higher rate of reduction (76.15, 78.26 and 61.68%) at the same period of time.

Similar trend was observed at 15 ppt salinity, where 50 mg L^{-1} group reduced by 34.43, 70.69 and 80.24% post 2nd, 7th and 14th days of treatment. The group with 150 mg L^{-1} showed rate of degradation as 33.93, 64.34 and 53.81% and higher OTC concentration of group (250 mg L^{-1}) showed the reduction at the rate of 32.26, 57.57 and 47.91% on 2nd, 7th and 14th days of observations respectively.

Interestingly, at salinity of 30 ppt rate of degradation for 50, 150 and 250 mg L^{-1} was 52.38 to 61.65%, 73.01 to 43.69%, 53.79 to 45.4% respectively during the period of observation. Contrary to the lower salinities, the group with higher salinity (45 ppt) showed the rate degradation at 32.00 to 64.08%, 68.15 to 46.09% and 66.45 to 43.78%, respectively, during 2nd, 7th and 14th day post treatment sampling.

DISCUSSION

The present study showed that the *P. ostreatus* significantly degraded oxytetracyline in all salinities. This finding concords with findings that fungus *P. ostreatus* treatment that enhanced the degradation of oxytetracyclinbe by 23% and <3% of spiked concentration of OTC at 7 and 14 days, respectively (Migliore *et al*., 2012); *P. ostreatus* 14 days post treatment degraded ciprofloxacin 95.07% in 500 ppm ciprofloxacin, 82.3% in 100 ppm ciprofloxacin (Singh, Khajuria, and Kaur (2017); *P. eryngii* extract within microcapsules removed OTC 95.3 to 99.2% compared to suspended extract with 87.8 to 92.1% (Chang and Ren, 2015); yeast *Candida* sp. after 6 days degraded the third generation antibiotics of Cephalosporins *viz*., cefotaxime @ 61% and cefoperazone 64% in 200 mg l^{-1} and 150 mg l^{-1} concentration, respectively (Selvi and Das, 2014).

In aquatic environment some fungi play the major role in the degradation of the antibiotics viz., ciprofloxacin and erythromycin compared to the bacteria (Nnenna, Lekiah, and Obemeata, 2011). Mycorrhizal fungus and earthworm augmented the degradation of OTC in soil (Cao *et al*., 2018). Bacterial degradation by *Bacillus* sp. consortia showed the ability to degrade norfloxacin in 26 days by 75% in 5.5ppm concentration (Jałowiecki, Żur, and Płaza, 2017). *Ochrobactrum* sp. had the ability to degrade OTC and remediation of nitrogenous metabolites ammonia and nitrite in aquaculture wastewater (Shao *et al*., 2018).

In seawater, usage of microalga *Phaeodactylum tricornutum* remediates OTC economically (Santaeufemia *et al*., 2016). Enzyme degradation by Glutathion S-Transferases addition showed reduction of 30% tetracycline in 100 mg L^{-1} and 60-70% sulfathiazole and ampicillin in 100 mg L^{-1} and 50 mg L^{-1} (Park and Choung, 2007). Thus, in the aquatic environment, microbes play pivotal role in the degradation of OTC (Dzomba, Kugara, and Zaranyika, 2015). From this study it was found that the *P. ostreatus* has the potential to degrade OTC in varying salinities (1, 15, 30 and 45 ppt) and can be exploited in brackishwater aquaculture systems.

CONCLUSION

The *P. ostreatus* has the potential to degrade the oxytetracycline in 1, 15, 30 and 45 ppt salinities and can be a possible method to be used as the bioremediating agent for the removal of antibiotic residues in the environment. Further research and more studies have to be done in the direction of utilization of *P. ostreatus* for mycotic bioremediation of antibiotics in the environment and large scale production of the *P. ostreatus* through execution of pilot scale plants for its industrial production.

ACKNOWLEDGMENTS

The authors acknowledge funding agency Indian council of Agricultural Research for All India Network Project on Fish Health and the Director, ICAR-CIBA for the facilities provided to carry out the research work.

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