Korean J. Chem. Eng., 34(4), 1122-1128 (2017)
DOI: 10.1007/s11814-016-0345-6

pISSN: 0256-1115
eISSN: 1975-7220

Comparative study on degradation of norfloxacin and ciprofloxacin by *Ganoderma lucidum* JAPC1

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(*Received 11 May 2016 • accepted 10 December 2016*)

Abstract−Indiscriminate use of antibiotics and the rise in drug resistance emphasizes the immediate need for managing pharmaceutical products sensibly. The pharmaceutical products end in wastewater due to the manufacturing process of pharmaceutical industries, misuse of antibiotics and improper disposal of expired drugs, which eventually ends in sewers. In the present study we attempted to degrade fluoroquinolones by the process of mycoremediation. Hospital waste water soil sample was collected and screened for fungi which can tolerate norfloxacin. One fungal isolate was able to withstand 2 g/L of norfloxacin which was chosen for degradation studies. Degradation pattern was checked with HPLC, FTIR and SEM analysis. Kinetics studies were carried out to analyze the degradation pattern. The result confirmed that *Ganoderma lucidum* JAPC1 strain was capable of degrading norfloxacin successfully but was unable to degrade ciprofloxacin.

Keywords: *Ganoderma lucidum*, Antibiotics, Biodegradation, HPLC, Kinetic Study

INTRODUCTION

Fluoroquinolones are widely applied as they are active against many Gram negative and Gram positive pathogenic bacterial species. Among fluoroquinolones, ciprofloxacin and norfloxacin are the most widely used antibiotics [1] to treat mainly urinary, respiratory and gastrointestinal tract infections. Frequent detection of this group of antibiotics in the environment has proven to be genotoxic [2]. It is a fact that the partially digested ciprofloxacin and norfloxacin enter the environment via human excretion in sewage. Survey facts show that more than 70% of the total amount of ciprofloxacin and norfloxacin ends up in wastewater treatment plants annually and is ultimately found in the digested sludge at concentrations up to 6.3 and 8.3 mg kg^{-1}, respectively [3,4]. As fluoroquinolones have the potential to promote antibiotic resistance in bacteria, the present situation is considered a health concern. But it also has possible toxic effects on microbial activity in wastewater treatment plants [5,6].

Ciprofloxacin is the main metabolite of enrofloxacin, a commonly used veterinary fluoroquinolone. Among the total administered dose of ciprofloxacin in humans, 45-62% of unmetabolized compound is excreted via urine and 15-25% via feces [7]. Recently, a rise in ciprofloxacin concentrations of 31 mg L^{-1} has been reported [8] to end in wastewater treatment plant from pharmaceutical industries in India. Although 80-90% of ciprofloxacin is removed via sorption to sludge during wastewater treatment, the remaining compounds enter the sludge digesters. Therefore, digested sludge contains ciprofloxacin (around 3 mg kg^{-1}) [9]. In soil, the concentrations range from 0.37 mg kg^{-1} to 0.40 mg kg^{-1} [7,10], underlining the ecotoxicological relevance of ciprofloxacin in soil.

For the degradation of fluoroquinolones, fungi are least used as degrading organisms, though white rot fungi (Basidiomycetes) are proven biodegrading organisms as they possess an extracellular enzyme system. *Trametes versicolor*, *Pestalotiopsis guepini*, *Gloeophyllum striatum*, *Mucor ramannianus* are some wood rotting fungi, which are reported to degrade fluoroquinolones as well as sulfamerazine and tetracyclines. These widespread organisms have the potential to transform and even mineralize a broad spectrum of xenobiotics. These transforming activities are attributed to a nonspecific enzymatic system that includes extracellular lignin modifying enzymes (mainly laccase, lignin peroxidase, manganese dependent peroxidase) and intracellular enzymes such as the cytochrome P450 system [11]. Such enzymes catalyze degradation via diffusible oxidizing agents, e.g., aryloxy radicals, Mn31, or specific mediators. Generally, the white rot fungi contain either of the above or all the three types of above lignolytic enzymes. Most of the white rot fungal strains produce laccase as the main enzyme. One of the advantages associated with laccase is that they do not require H_2O_2 for substrate oxidation unlike peroxidases, and moreover, they have broad substrate specificity.

Ganoderma lucidum is a white rot fungus belonging to Polyporaceae family of biotechnological importance due to its therapeutic properties [12-14]. The complete lignolytic system of this fungus is still not known, as research on the biodegradation potential of environmental pollutants by this fungus is very limited [15].

There are very few works on antibiotic degradation and no reports on lignolytic enzyme system of *Ganoderma lucidum*. Our objective was to explore the capability of extracellular enzymes of *Ganoderma lucidum* for degradation of antibiotics, identification of the degraded product and study of the degradation kinetics.

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MATERIALS AND METHODS

1. Chemicals

Analytical grade Ciprofloxacin and Norfloxacin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used as standard. Technical grade of both the antibiotics was purchased from local pharmaceutical shop. All the other chemicals and solvents used in this study were of high purity and analytical grade.

2. Isolation and Characterization of Fungal Strain

Ganoderma lucidum was isolated by inoculating soil sample in potato dextrose agar (PDA). Soil sample was collected from sewage waste water and processed, dried and sieved. Three fungal strains were isolated, and among the three only one strain was able to withstand the antibiotics. Confluent growth of mycelia was observed on the plate after four days of inoculation. Subculture was done on PDA media and stored at 4 °C for further experiments.

Genomic DNA was isolated and purified according to the Chromous Genomic DNA Isolation Kit (Chromous Biotech Pvt. Ltd., Bangalore, India). 18S rRNA gene was amplified with the forward primer (5′-CAAGCGTTCTTCCTCGTCTC-3′) and reverse primer (5′-TCGGTCTGGAAGGTGGTATC-3′). The polymerase chain reaction amplification was carried out in a final volume of 100 µl. Briefly, the amplification reaction containing 100 ng template DNA, 400 ng each of universal primers, 2.5 mM dNTPs, and 10× Taq DNA polymerase assay buffer and Taq DNA polymerase enzyme was run on a Thermal Cycler ABI2720. The amplification reaction was cycled as follows: initial denaturation at 94 °C for 5 mins, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and final extension at 72 °C for 15 mins for 35 cycles. The product was directly sequenced with the primer using ABI 3130 Genetic Analyzer (Chromous Biotech Pvt. Ltd., Bangalore, India). The sequencing result was submitted to the GenBank National Center for Biotechnology Information (NCBI) database.

3. Experimental Procedure

3-1. Gradient Plate Technique

Gradient plate method was performed to check the antibiotic tolerance ability of the isolated strain before performing minimum inhibitory concentration (MIC). The norfloxacin and ciprofloxacin gradient plate consisted of two wedged layers of media: a bottom layer of plain PDA and top layer with specific concentration of antibiotic with PDA. The bottom layer was kept to solidify at an angle of 30° before the top layer with antibiotic was poured to establish an antibiotic gradient across the plate surface. The antibiotic in the top layer diffused into the bottom layer, producing a gradient of antibiotic concentration from low to high. The gradient plate was made by using norfloxacin and ciprofloxacin at 1 g/L concentration in PDA medium separately. Point inoculation was done on the center of the plate and incubated for seven days maximum at 30±2 °C.

3-2. Inoculum Preparation

JAPC1 strain was subcultured and maintained on PDA slants at 4 °C. Subcultures were routinely made every 30 days. All the *in vivo* degradation experiments used JAPC1 strain inoculum. The inoculum was prepared by adding 100 µl of Tween 20 in 10 ml of autoclaved dH_2O and one loop full of fresh culture was added. It was shaken gently to prepare a viable spore solution.

3-3. Minimum Inhibitory Concentration (MIC)

As the next step, MIC was performed to find the highest concentration of antibiotic tolerance by the organism and to determine the best degradation concentration. MIC was performed for the fungal isolate using broth dilution method. Two sets of 250-ml Erlenmeyer flasks containing 100 ml of the M1 medium composed of $NaNO_3$ 2 g; KCl 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; glucose 10 g; $FeCl_3$ 10 mg; $BaCl_2$ 0.2 g; and $CaCl_2$ 0.05 g per liter at pH 6.8 were spiked with increasing concentrations (1.0, 1.5, 2.0, 2.5 g/L) of ciprofloxacin and norfloxacin. The flasks were inoculated with 1 ml of JAPC1 spore suspension prepared in 0.01% Tween 20 and incubated at 30±2 °C on a rotary shaker at 120 rpm. After ten days of incubation, the flasks were observed for mycelia growth. Mycelial mass from each flask was separated by filtration using Whatman filter paper no. 1 and washed with deionized water. The dry weight of fungal biomass was determined by drying at a constant weight for 80 °C in preweighed aluminum foil cups. The MIC was noted as the concentration of antibiotic resulting in complete inhibition of mycelia growth in flasks.

3-4. *In Vivo* Degradation of Ciprofloxacin and Norfloxacin

In vivo degradation was performed using 250 ml Erlenmeyer flask containing 100 ml of PDB media. Degradation concentrations of antibiotics used were determined with MIC experiments. 100 µl spore solution of JAPC1 was added to each flask. One flask was kept as control which was not inoculated with the spore solution. To eliminate the possibility of photodegradation of ciprofloxacin and norfloxacin, all the experiments were carried out in dark. All the flasks were incubated in a temperature controlled orbital shaker at 30 °C, operating at an agitation rate of 90 rpm for ten days.

Degradation of ciprofloxacin and norfloxacin in time interval was assessed by comparing their concentration in the control with the concentration in the experimental flasks. The amount of absorbed ciprofloxacin and norfloxacin was determined by the difference in the antibiotic concentration between uninoculated and inoculated flasks.

Five ml of sample was withdrawn on 24 hrs interval for ten days. The samples were then centrifuged at 4,500 rpm for 15 mins at 4 °C. The supernatant was collected and stored under 4 °C for further analysis.

3-5. Solid Phase Extraction (SPE)

The target compounds were separated in a one-step procedure by solid phase extraction (SPE). SPE of samples was carried out with a Visiprep SPE manifold (Supelco, Bellefonte, PA, USA). The cartridges were prepared by packing 200 mg of LiChrolut EN sorbent (Merck, Darmstadt, Germany) into empty 10 ml SPE-cartridges (Isolute-XL) between two polyethylene frits (Separtis, Grenzach-Wyhlen, Germany). Prior to the extraction, a conditioning was carried out with 10 ml of methanol: acetic acid mixture (50 : 50, v/v) and 10 ml of methanol followed by 10 ml of water (pH 6.0). Sample volume of 500 µl was then applied to the cartridges, which were subsequently washed with 2 ml of Milli-Q water (pH 6.0). Finally, elution was performed by adding 12.5 ml of methanol:acetic acid mixture (50 : 50, v/v) [16]. The eluted fractions were collected and stored at 4 °C.

4. Analytical Procedures

The degradation of ciprofloxacin and norfloxacin was analyzed

by HPLC. The functional groups of the compounds were detected by FT-IR.

4-1. High Performance Liquid Chromatography (HPLC)

Degraded samples were analyzed on a Varian HPLC equipped with a binary pump, programmable variable wavelength UV detector, and ODS2 C18 reversed phase column. The antibiotic residue analyses used a gradient mobile phase of water/acetonitrile/triethylamine (80 : 20 : 0.3 v/v/v). Sample injection volume was 20 µl and the mobile phase was programmed at a flow rate of 1 ml/min. All solutions, including the mobile phase, were sonicated for 25 mins before use. The UV detection was at 280 nm and the result was recorded [17].

4-2. FT-IR

Infrared (IR) spectra of the parent compound (norfloxacin) and sample after fungal degradation were recorded at room temperature in the frequency range of 4,000-400 cm^{-1} by Fourier transform infrared (FTIR) spectroscopy (8400 Shimadzu, Japan, with Hyper IR-1.7 software for Windows) with a helium-neon laser lamp as a source of IR radiation. Pressed pellets were prepared by grinding the extracted samples with potassium bromide in a mortar with 1 : 100 ratio and immediately analyzed in the region of 4,000-400 cm^{-1} at a resolution of 4 cm^{-1} [18].

4-3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was performed on the treated and untreated fungal mycelia samples. The freeze-dried fungal mycelium treated with norfloxacin and ciprofloxacin and untreated sample were mounted on specimen stubs after 120 hrs of incubation with double-sided adhesive tape and coated with gold in a sputter coater (Hitachi, Model E-1010 Ion Sputter) to avoid charging and examined under SEM (Hitachi, Model S-3400N) [19].

5. Laccase Production Test

As antibiotic degrading fungi are reported to possess the unique laccase enzyme system, a simple test was carried out to check the laccase production ability of the isolated fungus. Point inoculation of the fungus was done on a PDA plate spiked with 0.01% of ABTS and incubated for seven days at 30 °C. Formation of a purple to blue zone on the inverse side of the plate indicates a positive result for laccase production [20].

6. Kinetic Studies

Degradation of norfloxacin in medium has been applied to various kinetic models such as zero-order, first-order, pseudo first-order, second-order and pseudo second-order to determine the rate constant (k). The times in which the antibiotic concentration in PDB was reduced by 50% (DT_{50} values), which was calculated from the linear equation obtained from the regression between C_t-C_o (zero order model), $\ln(C_t/C_o)$ (first order model), $\ln C_t$ (pseudo first-order model), 1/C (second-order model), t/C_t (pseudo second-order model) of the chemical data and time. Kinetic model equations were described by:

(i) $C_t - C_o = kt$ (zero-order model)

(ii) $C_t/C_o = e^{-kt}$ (first-order model)

(iii) $\ln C_t = -kt + \ln C_o$ (pseudo first-order)

(iv) $1/C = kt + 1/Co$ (second-order kinetic)

(v) $t/Ct = t/Ce + 1/kCe^2$ (pseudo second-order)

Where C_o, Ce are the amount of antibiotic in PDB at time zero and C_t, C, Ct are the amount of pesticide in PDB at time t. k and t are the rate constant (d^{-1}) and degradation time in days, respectively [21].

RESULTS AND DISCUSSION

In this present study, wood rotting fungus was isolated from hospital waste water soil which was chosen for degradation of ciprofloxacin and norfloxacin. The pure culture was maintained on PDA plates. The fungal strain was designated as JAPC1 and it was able to utilize ciprofloxacin and norfloxacin as carbon source.

The molecular characterization based on 18S rRNA sequence analysis was used to identify JAPC1 isolate. BLAST result of the 18S rRNA gene sequence of JAPC1 isolate exhibited close relation-

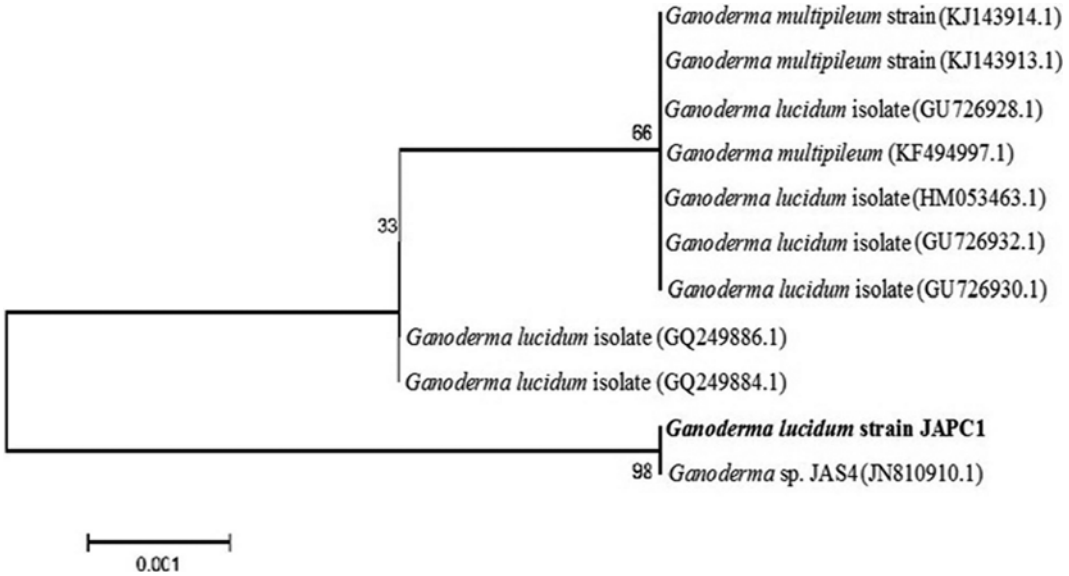

Fig. 1. Phylogenetic tree of *Ganoderma lucidum* JAPC1 strain.

ship with 100% similarity to that of the 18S rRNA gene of *Ganoderma* sp. JAS4 (GenBank accession no. JN810910.1), 91% similarity to that of the 18S rRNA gene of *Ganoderma lucidum* isolate Gl-44 (GenBank accession no. HM053463.1), 99% similarity to that of the 18S rRNA gene of *G. lucidum* isolate GL-22 (Gen-Bank accession no. GU726932.1), 91% similarity to that of the 18S rRNA gene of *G. lucidum* isolate GL-22 (GenBank accession no. GU726932.1), and 91% similarity to that of the 18S rRNA gene of *G. lucidum* strain GL-20 (GenBank accession no. GU726930.1). The partial nucleotide sequence of 18S rRNA of JAPC1 isolate showed the highest similarity to *G. lucidum*. Multiple sequence alignments and phylogenetic analysis (Fig. 1) revealed that the strain JAPC1 cluster exhibited *G. lucidum* 91% similarity. Therefore, the JAPC1 isolate was identified as *Ganoderma lucidum* and it was designated as *Ganoderma lucidum* JAPC1. The GenBank accession number for the 18S rRNA sequence of *G. lucidum* JAPC1 isolate is KM655758.1.

Gradient plate method was performed to check the antibiotic concentration range sustained by isolated *Ganoderma lucidum* JAPC1 strain. A gradual decrease of mycelial growth along with the increasing antibiotic concentration was observed (Fig. 2). The performed antibiotic concentration was chosen for MIC determination step. MIC was determined by the dry weight of fungal mycelia. The metabolism of ciprofloxacin and norfloxacin by the fungus *Ganoderma lucidum* JAPC1 in PDB broth was indicated by a visible increase in mycelia growth within days. After four days, visible increase in growth was observed in both norfloxacin and ciprofloxacin batches. Highest growth was observed and determined by dry weight in 2 g/L concentration flask for norfloxacin and in 1 g/L concentration for ciprofloxacin. The organism was able to tolerate 2.5 g/L concentration of norfloxacin; however, luxuriant growth was observed in 2 g/L concentration. Upon exposure to ciprofloxacin, the organism was able to grow at 1 g/L was able to tolerate up to 1.5 g/L. Hence, 2 g/L of norfloxacin concentration and 1 g/L of ciprofloxacin were chosen for further studies.

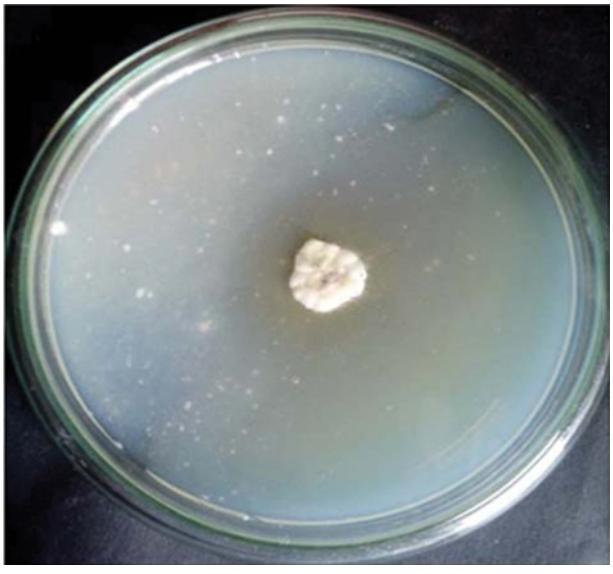

Fig. 2. Gradient plate of *Ganoderma lucidum* JAPC1 strain showing no growth around the antibiotic zone.

The biodegradation of clinical grade norfloxacin and ciprofloxacin by *Ganoderma lucidum* JAPC1 strain was analyzed by high performance liquid chromatography (HPLC). HPLC was used to monitor the disappearance of norfloxacin in the degraded sample. Absence of peaks for norfloxacin was first observed on the fourth day of analysis when compared against the standard chromatogram. The elution time of norfloxacin metabolite was 5 mins and the total peak area was 3.6%. The comparison in test sample chromatogram and standard chromatogram is presented in Fig. 3. The HPLC analysis showed that *Ganoderma lucidum* JAPC1 strain is successful in degrading the antibiotic norfloxacin at a concentration of 2 g/L after four days incubation. Regarding ciprofloxacin degradation, the presence of peak at the same retention time compared to the standard sample indicated that the organism was not able to degrade ciprofloxacin. Since the experimental conditions were identical in both ciprofloxacin and norfloxacin experiments, it is hypothesized that the highest degradability observed for norfloxacin could be due to the difference in the molecular structure of the antibiotics.

In comparison to ciprofloxacin, which has a cyclopropyl group, norfloxacin has an ethyl group, which is reported to be more suitable for degradation by laccase and laccase-mediator system rather than that contained in ciprofloxacin [22,23]. As observed, ciproflox-

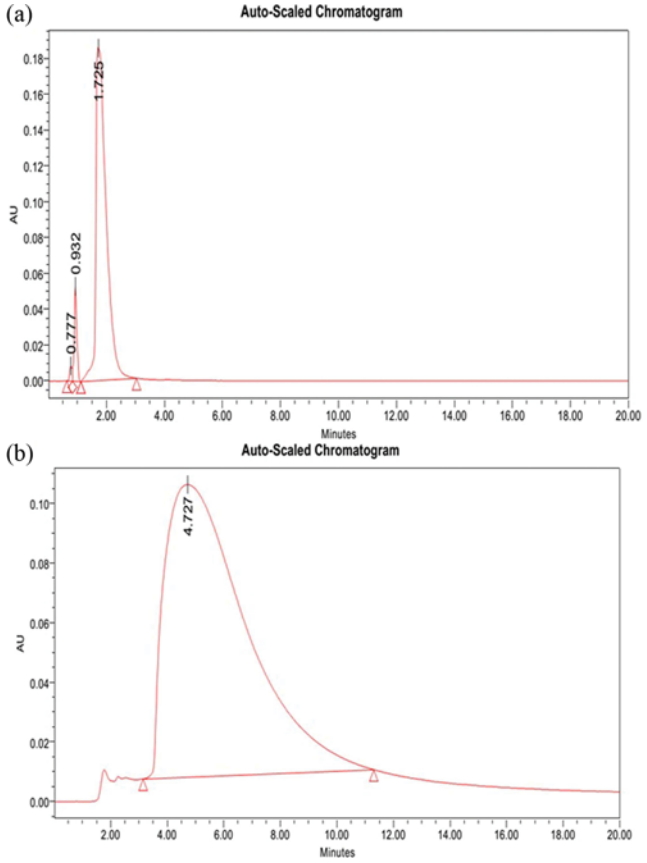

Fig. 3. HPLC chromatogram of norfloxacin degradation by *Ganoderma lucidum* JAPC1 strain, (a) represent the chromatogram for standard norfloxacin sample, whereas (b) represents the chromatogram of degraded norfloxacin sample by *Ganoderma lucidum* JAPC1 strain on fourth day.

acin and norfloxacin share a common degradation pathway via oxidation of the piperazinyl substituent. A net loss of C_2H_2 at the piperazinyl substituent of norfloxacin resulted in the generation of 7-((2-aminoethyl)amino)-1-ethyl-6-fluro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Nor-1) that appeared in the earlier stages of the incubation [24]. It is reported that the changes were produced in the piperazinyl substituent, since the typical loss of C_2H_4N from norfloxacin was replaced by the loss of CH_4N in the spectra of NH_2 for Nor-1. In the case of norfloxacin, intermediate 7-((2-Acetamidoethyl)amino)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Nor-3) appeared simultaneously to Nor-1 and involved the opening of the piperazinyl ring with formation of a carboxylic acid in the amino group. The main reaction catalyzed by the cytochrome P450 enzymes was the epoxidation of C=C double bounds and subsequent aromatic hydroxylation. Therefore, this enzyme may be responsible for either catalyzing the breakdown of the piperazinyl substituent following the mechanism stated above or catalyzing the hydroxylation in norfloxacin molecular moieties. It is reported that these initial degradation intermediates disappeared due to a further oxidation of the piperazinyl substituent with a loss of a C_2H_5N fragment producing 7-Amino-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Nor-2) that accumulated in the medium. The above pathway is presented in Fig. 4, which shows no loss of fragments for Nor-2 of the piperazinyl group. This reported pathway confirms and supports that the ethyl group of norfloxacin was degraded completely [25].

The FT-IR spectrophotometer gives a clear picture of antibiotic degradation by *Ganoderma lucidum* JAPC1. The IR spectrum of norfloxacin standard and degraded products of norfloxacin is presented in Fig. 5. A change in IR spectra between the standard sample and degraded sample clearly dictates the degradation of norfloxacin. The IR spectra of norfloxacin show bands at 3,626.17 cm^{-1} and 2,335 cm^{-1} which are characteristic of O-H carboxylic acid. Bands at 2,299 cm^{-1}, 2,002 cm^{-1}, 1,928 cm^{-1} are characteristic of C≡N stretch nitrile group. Band at 1,874 cm^{-1} is characteristic of C=O carboxylic acid, while 1,807 cm^{-1} and 1,693 cm^{-1} are characteristic of C=O esters. The band at 1,625 cm^{-1} is characteristic of N-H bend amides and 1,408 cm^{-1} is characteristic of N=O nitro groups, while 1,247 cm^{-1} and 1,163 cm^{-1} are characteristic of C-O

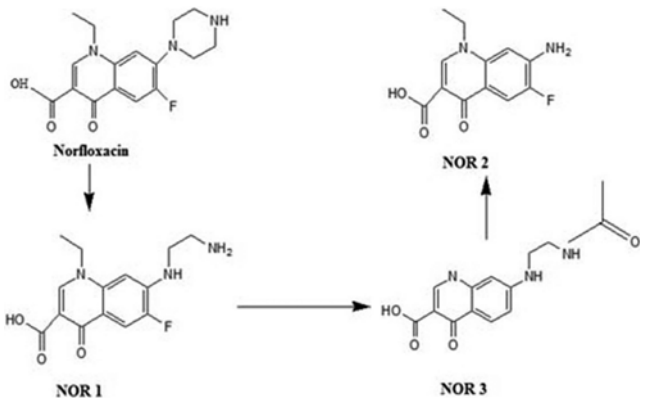

Fig. 4. Proposed degradation pathway of norfloxacin indicating the transformation of the antibiotic into the metabolites.

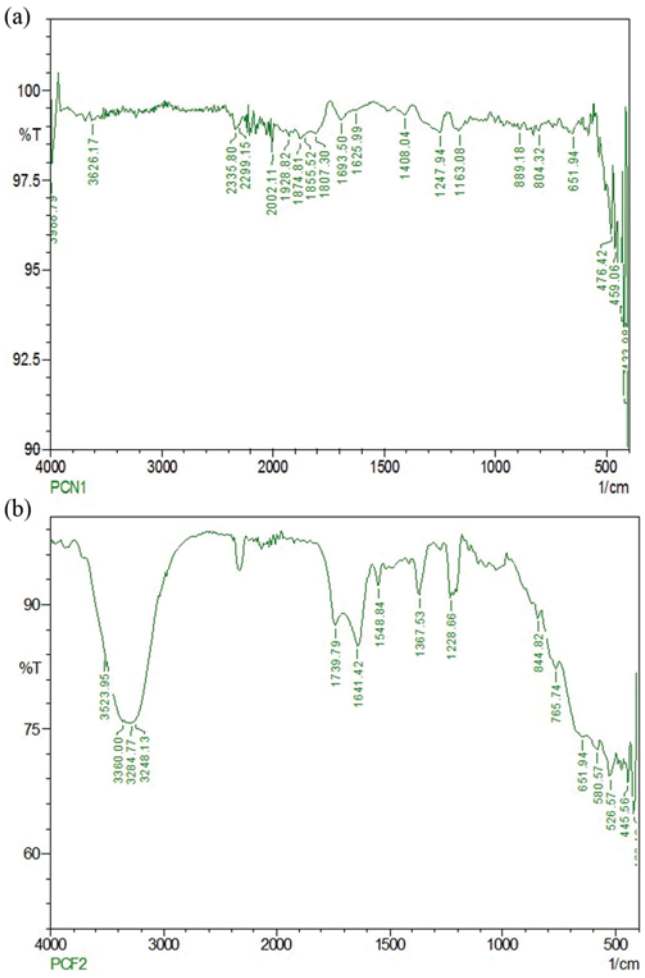

Fig. 5. FT-IR image of (a) standard norfloxacin and (b) degraded norfloxacin sample by *Ganoderma lucidum* JAPC1 strain.

stretch of ethers. Bands at 889 cm^{-1} and 804 cm^{-1} are characteristics of C-H aromatics.

In the degraded sample, the shifting of the corresponding bands from that of the standard explains the presence of metabolites. Ketone (C=O) groups were shifted to 1,737 cm^{-1} and nitro (N=O) groups were shifted to 1,548 cm^{-1} and 1,367 cm^{-1}. The band at 3348 was characteristic of ≡C-H stretch of alkynes while the band at 1,641 cm^{-1} was characteristic of C-C=C symmetric stretch of alkanes. The overall result confirms the norfloxacin degradation in the sample.

For further analysis, the degraded sample was subjected to SEM analysis for morphological changes in the surface of treated sample and the standard sample. The magnified image of *Ganoderma lucidum* JAPC1 mycelia shows that the smooth surface has been transferred to rough edges. The surface image of the mycelia of the organism is presented in Fig. 6(a), while the rough edges of the treated sample surface is presented in Fig. 6(b).

In this study, colored indicators (ABTS) have been used as it makes possible visual recognition of lignolytic enzymes actions; this made a straightforward way of enzymes screening as no measurement is necessary. In solid media of PDA supplemented with ABTS substrate, the organism formed pale green up to dark purple zone (Fig. 7) which is due to the oxidation activity of the enzyme [26]. The

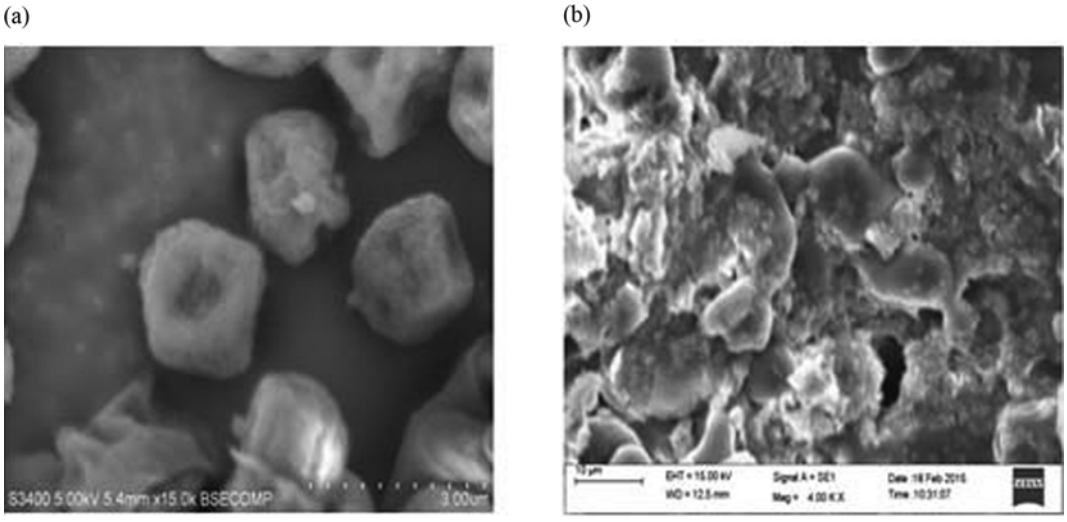

Fig. 6. SEM result of (a) *Ganoderma lucidum* JAPC1 strain and (b) after antibiotic treatment.

Fig. 7. Greenish blue colored zone showed the positive result for laccase production by *Ganoderma lucidum* JAPC1 strain.

use of ABTS as substrate for laccase provides for the rapid visual expression for the positive result of laccase production by *Ganoderma lucidum* JAPC1 strain.

Treatment of norfloxacin under specified conditions resulted in a gradual degradation in all conditions. It may be assumed that the degradation of norfloxacin followed first-order kinetics as a linear relationship between logCt of norfloxacin remaining and time, having good correlation coefficients. First-order term defines a chemical reaction in which the rate of decrease in the number of molecules in the substrate is proportional to the concentration of the substrate remaining. In first-order reactions involving two substances, only one of the concentrations affects the reaction rate. Rate constant (K), time left for 50% potency ($t_{1/2}$) and time left for 90% potency (t_{90}) for each condition were calculated.

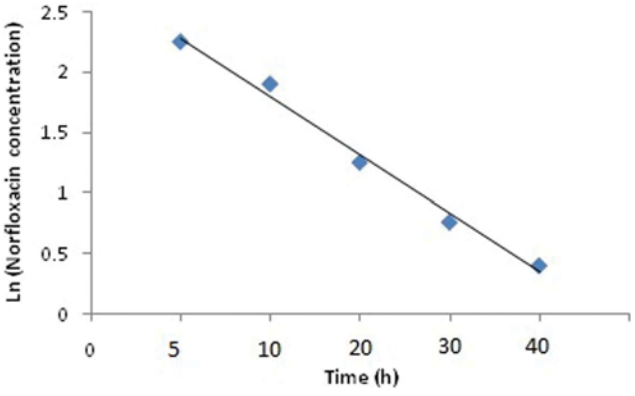

Fig. 8. Kinetic study of norfloxacin degradation by *Ganoderma lucidum* JAPC1 strain.

Kinetic studies on norfloxacin degradation best describe the experimental kinetic data results. The obtained data was fitted with a different kinetic model, namely zero-order, first and pseudo first-order, second- and pseudo second-order models.

The R^2 values for the first and pseudo first order model were higher than the zero-order, second- and pseudo second-order. This may be because the degradation data collected fits well with the first-order. The degradation process of norfloxacin was done under three different pH conditions (pH 4, pH 7 and pH 10) to fit the best kinetic model. The fastest degradation (0.6 d) was observed at pH 7. Kinetic data showed that norfloxacin degradation process was observed for strain JAPC1 in broth following the first-order model, which was characterized by the rate constant (k) of 1.394 d^{-1}. The time within which the initial norfloxacin concentration was reduced by 50% (DT50) was 0.6 d for strain JAPC1. The value of R^2 was determined to be 0.979 (Fig. 8). The result showed that pH is an important parameter in norfloxacin degradation. Norfloxacin is a zwitterion (contains both positive and negative charge, but electrically neutral in total) and its isoelectric point (7.38) is closer to pH 7, and it can be the reason for faster degradation at this pH range than pH 4 and pH 10 (data not shown) and Zhang

et al. reported similar results [27].

CONCLUSION

The degradation of norfloxacin by *Ganoderma lucidum* JAPC1 and the analysis of their transformation products were demonstrated in liquid medium. The evidence of their degradability is of interest since norfloxacin is mostly sorted in the digested sludge of wastewater treatment plants. Therefore, inoculation of biopile composting of sewage sludge with *Ganoderma lucidum* JAPC1 strain degrades fluoroquinolones, as well other pharmaceutically active compounds can be considered as an alternative to physicochemical treatments. Although degradation of ciprofloxacin by *Ganoderma lucidum* JAPC1 strain was not achieved, more research is needed to quantify the residual activity of the byproducts identified in the study.

REFERENCES

1. Y. Pico and V. Andreu, *Anal. Bioanal. Chem*., **387**, 1287 (2007).
2. K. A.Kummerer, Al-Ahmad and V. Mersch-Sundermann, *Chemosphere*., **40**, 701 (2000).
3. W. Giger, A. C. Alder, E. M. Golet, H. P. E. Kohler, C. S. McArdell, E. Molnar, H. Siegrist and M. J. F. Suter, *CHIMIA*, **57**, 485 (2003).
4. R. H. Lindberg, U. Olofsson, P. Rendahl, M. I. Johansson, M. Tysklind and B. A. Andersson, *Environ. Sci. Technol*., **40**, 1042 (2006).
5. H. A. Duong, N. H. Pham, H. T. Nguyen, T. T. Hoang, H. V. Pham, V. C. Pham, M. Berg, W. Giger and A. C. Alder, *Chemosphere*., **72**, 968 (2008).
6. T. Schwartz, H. Volkmann and S. Kirchen, *FEMS Microbiol. Ecol*., **57**, 158 (2006).
7. E. M. Golet, I. Xifra, H. Siegrist, W. Giger and A. C. Alder, *Environ. Sci. Technol*., **37**, 3243 (2003).
8. D. G. Larsson, C. Pedro and N. Paxeus, *J. Hazard. Mater*., **148**, 751 (2007).
9. E. M. Golet, A. Strehler, A. C. Alder and W. Giger, *Anal. Chem*., **74**, 5455 (2002).
10. E. Martínez-Carballo, C. González-Barreiro, S. Scharf and O. Gans, *Environ. Pollut*., **148**, 570 (2007).
11. M. Asgher, H. N. Bhatti, M. Ashraf and R. L. Legge, *Biodegradation*., **19**, 771 (2008).
12. Q. H. Fang and J. J. Zhong, *Biochem. Eng. J*., **10**, 61 (2002).
13. D. Sliva, C. Labarrere, V. Slivova, M. Sedlak, F. P. Lloyd and N. W. Ho, *Biochem. Biophys. Res. Commun*., **298**, 603 (2002).
14. R. Fujita, J. Liu, K. Shimizu, F. Konishi, K. Noda, S. Kumamoto, C. Ueda, H. Tajiri, S. Kaneko, Y. Suimi and R. Kondo, *J. Ethnopharmacol*., **102**, 107 (2002).
15. K. Perumal, K. Murugesan and P. T. Kalaichelvan, *Indian J. Exp. Biol*., **38**, 385 (2000).
16. E. A. Turiel and E. Martin. *J. Chromatogr*., **1172**, 97 (2007).
17. N. M. Kassab, A. K. Singh, E. R. M. Kedor-Hackmam and M. I. R. M. Santoro, *Braz. J. Pharm. Sci*., **41**, 507 (2005).
18. S. Silambarasan and J. Abraham, *PloSone*., **8**, e77170 (2013).
19. C. Sammon, J. Yarwood and N. Everall, *Stabil*., **67**, 149 (2000).
20. E. M. Ko, Y. E. Leem and H. T. Choi, *Appl. Microbiol. Biotechnol*., **57**, 98 (2001).
21. S. Silambarasan and J. Abraham, *J. Taiwan. Inst. Chem. E*., **44**, 438 (2013).
22. T. Paul, P. L. Miller and T. J. Strathmann, *Environ. Sci. Technol*., **41**, 4720 (2007).
23. P. Wang, Y. He and C. Huang, *Water. Res*., **44**, 5989 (2010).
24. I. A. Parshikov, T. M. Heinze, J. D. Moody, J. Freeman, A. Williams and J. Sutherland, *Appl. Microbiol. Biotechnol*., **56**, 474 (2001).
25. A. Prieto, M. Möder, R. Rodil, L. Adrian and E. Marco-Urrea, *Bioresour. Technol*., **102**, 10987 (2011).
26. H. Y. Alfarra, N. H. M. Hasali and M. N. Omar, *Int. Res. J. Biological Sci*., **2**, 51 (2013).
27. B. Li and T. Zhang, *Environ. Sci. Technol*., **44**, 3468 (2010).