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Elucidation of prazosin biodegradation by isolated *Bacillus* spp. from the tropical environment

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Prazosin (PRZ), a drug used to treat hypertensive patients, is an emergent contaminant in water systems. PRZ is an alpha-adrenergic receptor blocker used to treat anxiety, and is believed to reach the environment through human excretion, irresponsible disposal of unused medicine, and waste products from manufacturing plants. The purpose of this research was to isolate and characterize potential microbes for PRZ biodegradation and to identify the degradation pathway. After screening, isolated strain STP3 showed a capability for PRZ degradation and was chosen for further analysis. Resting cell assays with PRZ were conducted to identify the intermediate metabolites formed from biodegradation by Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) analysis. Two metabolites degraded from PRZ by STP3 were successfully found, and as these metabolites are derived from the main structure of PRZ, their presence proved PRZ degradation. Draft genome sequencing analysis of STP3 was performed to identify potential enzymes for PRZ biodegradation based on the metabolites found.

Key Words: biodegradation; hospital wastewater; pharmaceutical residues; prazosin

Introduction

Worldwide studies have found an abundant occurrence of pharmaceuticals and personal care products in ground and surface waters throughout the world (Caliman and Gavrilescu, 2009). In previous studies, places such as North America, Europe, the United Kingdom, and Asia show an enormous amount of pharmaceutical residues, endocrine disruptors, and other organic contaminants in the effluent of wastewater treatment plants (Bartelt-Hunt et al., 2009; Bendz et al., 2005; Bester et al., 2008; Kaspryk-Hordern et al., 2009; Petrovic et al., 2004). A review by Liu and Wong (2013) asserted that the primary sources of pharmaceutical environmental contaminants are sewage systems. Based on the same source, the worldwide presence of pharmaceutical residues is reported with concentrations ranging from ng/L to μg/L, especially in developed countries. According to Afroz et al. (2014), causes of water pollution include household waste, industrial waste, and septic tanks. These sources might include expired medicines, excreted drugs, manufacturer waste effluent, and agricultural wastes. Additionally, de Jesus et al. (2015) described many types of drugs found in bottled drinking water sources and water systems. Identified drugs include caffeine, erythromycin, ibuprofen, and PRZ (Mussa et al., 2016).

This study focused on PRZ (IUPAC name of 4-(4-amino-

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6,7-dimethoxyquinazolin-2-yl) piperazin-1-yl]-(furan-2-yl) methanone (PubChem Compound Database; CID = 4893)), a pharmaceutical primarily used as an antihypertensive (Fuchs et al., 2015). PRZ is also used to treat trauma nightmares and reduce sleep disturbances (Raskind et al., 2007). It also acts as a vasodilator that reduces blood pressure and peripheral resistance around arterioles and veins while maintaining heart rate (Sultana et al., 2013). PRZ is proven to treat post-traumatic stress disorder (PTSD) (Coupland et al., 2009; Gehrman and Harb, 2016) and has been used to increase insulin sensitivity in obese patients with hypertension (Pollare et al., 1988).

Al-Qaim et al. (2014a) reported the amount of PRZ residue in sewage treatment plant effluent is as high as 117 ng/L. Even though drugs are found at trace levels, eventual accumulation will cause adverse environmental effects and impact organisms that consume it indirectly. A surface study of PRZ degradation proved that PRZ can form metabolites. Bakshi et al. (2004) proved that PRZ was highly degraded under hydrolytic stress and Shrivastava and Gupta (2012) showed that PRZ was degraded under acidic and alkaline conditions, resulting in probable metabolites like 6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine and 2-furoic acid. Another study of PRZ degradation by Erve et al. (2007) using animal liver cells proposed some of the metabolites formed from PRZ degradation and connected metabolites to elucidate metabolic pathways. All previous studies used liquid chromatography to assess PRZ degradation products. An investigation conducted by Al-Qaim et al. (2014b) validated the method of using liquid chromatography-mass spectrophotometry (LC-TOF/MS) for detecting pharmaceutical compounds polluting the environment. Mass spectrometry together with electrospray ionization can be used to identify and profile pharmaceutical metabolites (Nassar et al., 2006). Bacteria played a vital role in ecosystem preservation naturally because they can metabolize environmental contaminants using their own metabolic pathways (Caracciolo et al., 2015). The efficiency of bio-degradation was proven by Baena-Nogueras et al. (2017) when they showed that pharmaceutical residues can be degraded by up to 90% of the total amount.

In this study, bacterial strains isolated from contaminated sites were studied for their ability to degrade PRZ under carbon starvation, and intermediate metabolites were identified along with possible enzymatic pathways.

Materials and Methods

Chemicals and materials. Pure standard PRZ was purchased from Wako Pure Chemicals (Osaka, Japan). HPLC-grade methanol (MeOH) by Fisher Scientific (Loughborough, UK), acetonitrile (ACN) and formic acid were supplied by JT Baker (Philadelphia, USA). Analytical-grade acetone, methanol, and dichloromethane were supplied by Merck (Darmstadt, Germany).

Strain isolation and resting cell assay. Sample collection was performed at a Hospital Pantai sewage treatment plant located at 3°07′13.8″ N 101°40′00.2″ E in Kuala Lumpur, Malaysia. Samples were stored at –20°C until use. Bacterial strains were isolated by soaking 5 g of 5 samples from different sampling points in 10 ml of distilled water for 30 min. Microbes were obtained by filtration and dilution (Sumintha and Sivakumar, 2013). Media were screened using solidified Minimal Salt Media (MSM) without carbon, and with the addition of 0.1 M PRZ to replace the carbon source. Pure cultures were maintained on solidified MSM without carbon at 4°C. MSM had the following composition (g L^{-1}): 6 g $Na_2HPO_4{\cdot}12H_2O$; 2 g K_2HPO_4; 0.125 g NH_4NO_3; 0.07 g $MgSO_4$; and 0.09 g $CaCl_2{\cdot}2H_2O$.

During the stationary phase of each isolate, 50 mL of Lysogeny Broth (LB) culture was removed and centrifuged for 15 min at 12 000 rpm for cell collection. The cells were mixed with 10 mL of Tris-HCL buffer with 0.1 mM PRZ. The mixtures were cultured for 5 days and 1 mL samples were taken on days 0, 3, and 5 at 1500 consistently.

HPLC UV-Vis and FT-ICR MS analysis. LC separation was performed on the 1220 Infinity II LC from Agilent Technologies (California, USA) system equipped with auto injector, degasser, gradient pump, column oven and DAD UV-Vis detector with detection wavelength range 220–400 nm. Analyses were performed on a 5 μm Eclipse Plus C18, 4.6 × 250 mm by Agilent Technologies (California, USA) column elute with mobile separation phase (A) 0.1% formic acid in ultra-pure water and (B) ACN-MeOH (3:1, v/v) with elution program as follows: 0 to 3 min, 5% to 60% B gradient, 3 to 6 min, 60% to 97% B gradient, 6 to 11 min, hold 97% B. Final selected and optimized conditions were as follows: injection volume 10 μL, mobile phase isocratic pump at flow rate 0.8 mL/min at 35°C, and detection wavelength of 275 nm (Al-Qaim et al., 2013, 2014a).

Shimadzu LC-20AD system with a binary pump from Shimadzu Co. (Kyoto, Japan) was used combined with mass spectrometry as stated below: Xbridge column was used (150 × 2.1 mm, 3.5 um) from Waters (Massachusetts, USA), the LC autosampler system was maintained at 4°C, and the column oven was maintained at 40°C. Mobile phase was (A) 0.1% formic acid in purified water and (B) acetonitrile, with a flow rate of 0.2 ml/min. The analysis started at 0% (B) and gradually increased to 100% after 30 min. Mass spectral data were gathered using a Fourier transform ion cyclotron resonance mass spectrometer (9.4 T; FT-ICR MS) by Bruker Daltonics Inc. (Massachusetts, USA). Samples were ionized using electrospray ionization methods and analyzed in both polarity modes. The spectra were scanned between 100–1000 *m/z* and the capillary voltage of the MS system was 4500 V. The dry gas flow rate was 8 L/min and the temperature was 200°C. The ESI was sprayed at 4.0 bar.

PRZ and all metabolites were searched in both positive and negative modes. FT-ICR MS analysis produced a Total Ion Chromatogram (TIC). Extracted Ion Chromatograms (EICs) were extracted to find metabolite and PRZ ions. After finding metabolites and PRZ ions, mass spectral properties such as retention time, M/Z, and ionization mode were extracted from the Bruker Data Analysis software. Chemical formulas were identified from the same software.

Table 1. Retention time and mass spectral properties of PRZ and its intermediates metabolites obtained via HPLC FT-ICR MS analysis.

Metabolite	RT (min)	Mass (*m/z*)	Ionization mode	Chemical formula
Prazosin	11.60	384.166	$[M+H]^+$	$C_{19}H_{21}N_5O_4$
M1	10.20	205.097	$[M+H]^+$	$C_{11}H_{13}N_2O_2$
M2	10.18	344.109	$[M+H]^-$	$C_{13}H_{18}N_3O_8$

Field Emission Scanning Electron Microscope (FESEM). Isolate STP3 was fixed onto microporous specimen capsules. The sample was fixed using McDowell-Trump fixative reagent (50 ml 0.2 M Phosphate Buffer Saline from R&M Marketing (Essex, England), 11 ml 37% formaldehyde from R&M Marketing (Essex, England), 4 ml 25% glutaraldehyde from TAAB Lab and Equipment (Berkshire, England) and 100 ml distilled water). Further dehydration was performed with an increasing concentration of ethanol (35–100%) and acetone. The sample underwent critical point drying using nitrogen gas, E3000 CPD from Quorum Technologies (Lewes, UK). The sample was then sputter-coated with platinum using an Auto-Fine Coater by JEOL (Tokyo, Japan) or viewing under FESEM. SEM imaging was achieved using the back-scattered electron emission mode with JS-7800F PRIME from JEOL (Tokyo, Japan).

Genome sequencing and data assembly. Strain STP3 was cultured in Luria-Bertoni broth at 30°C for cell amplification. Genomic DNA was extracted using a Hi Yield RBC extraction kit from RBC (Taipei, Taiwan). gDNA was enzymatically fragmented using an Ion Shear Kit from Thermo Fisher Scientific (Massachusetts, USA) and adapters were ligated. Size selection was done using Pippin Prep from Sage Science Inc. (Massachusetts, USA) to purify the selected DNA size. The library was amplified by running polymerase chain reaction (PCR) and then analyzed using 2100 Bioanalyzer by Agilent Technologies (California, USA) for fragments validation. Template preparation was done using an Ion Chef System from Thermo Fisher Scientific (Massachusetts, USA). A loaded Ion 530 chip was placed in the sequencer and sequencing was done using the Ion S5 XL from Thermo Fisher Scientific (Massachusetts, USA).

Automatic contig assembly obtained from sequencing was done using CLC Genomic Workbench from Qiagen Bioinformatics (California, USA). An assembly report for isolate STP3 showed nucleotide distribution, contig measurements, and summary statistics. Annotations of the sequence data were done against the RAST (Aziz et al., 2008) database by uploading query contigs in FASTA format to receive annotated genomic data. The annotated genomic data will provide insights into the types of enzymes or proteins produced by the isolated strain. STP3 assembled contigs were aligned using BLASTN 2.8.0+ by NCBI (Maryland, USA) against bacterial 16s rRNA database sequences to identify the isolated strain species. Protein clustering was done after obtaining amino acid sequence from RAST annotation. Clustering was done with 5 other *Bacillus* species using CD-HIT program (Li et al., 2006) with similarity (-c) of 40% (0.4). -c is one of the options in the command lines in CD-HIT program indicating the changeable sequence identity threshold with 90% similarity as default. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession QYZC00000000. The version described in this paper is version QYZC01000000.

Results

Isolation and evaluation of PRZ-degrading microbes from the effluent of hospital wastewater

Sediment samples were collected from the Hospital Pantai sewage treatment plant in Kuala Lumpur. Initial screening was carried out by the preparation of bacterial suspensions from the sediments collected, and then HPLC analysis was performed to evaluate the PRZ-degrading bacteria. The isolation process started with the sampling process in the highly suspected contaminated site. The site was deemed to be contaminated after HPLC analysis was carried out on the water samples collected. For isolate STP3, the sampling was done at the hospital sewage treatment plant in Kuala Lumpur. Another reason in choosing a PRZ contaminated site, was to ensure that the bacteria have the potentials to degrade PRZ by consuming it and breaking it down because of its ability to live in the contaminated environment. The screening was done on the MSM without carbon media. Fast growing bacteria colonies were picked individually and cultured into fresh media separately. The purification continued until a pure culture was obtained. From the same site, only 3 isolates were found to have PRZ degrading activity. Then, HPLC analysis was executed to study the effectiveness of these isolates in degrading PRZ. It was found that the isolate STP3 was the best degrader among the 3 isolates found as it degraded PRZ the most during a given period of time. Therefore, isolate STP3 was chosen for further study to identify the metabolites after degradation and to elaborate its draft genome sequence.

From our experiments, one isolates was chosen, which showed the capability to degrade PRZ and the ability to survive. This isolate was used for the resting cell assay for further evaluation of capability of degradation of PRZ. The percentage of PRZ degradability was measured using HPLC. The HPLC chromatogram (b) (Supplementary Fig. S1) shows that the isolate named as STP3 reduced the PRZ peak at 6.08 min, which corresponds to 88% PRZ degradation. PRZ content in the assay started to decrease to 48% on day 3 and decreased continuously to 12% on day 5 (data not shown). Intermediate metabolites appear in the chromatogram as smaller peaks, which indicate the degradation of PRZ into smaller compounds (Fig. S1). From the data obtained via HPLC, STP3 isolate was used for the determination of intermediate PRZ metabolites.

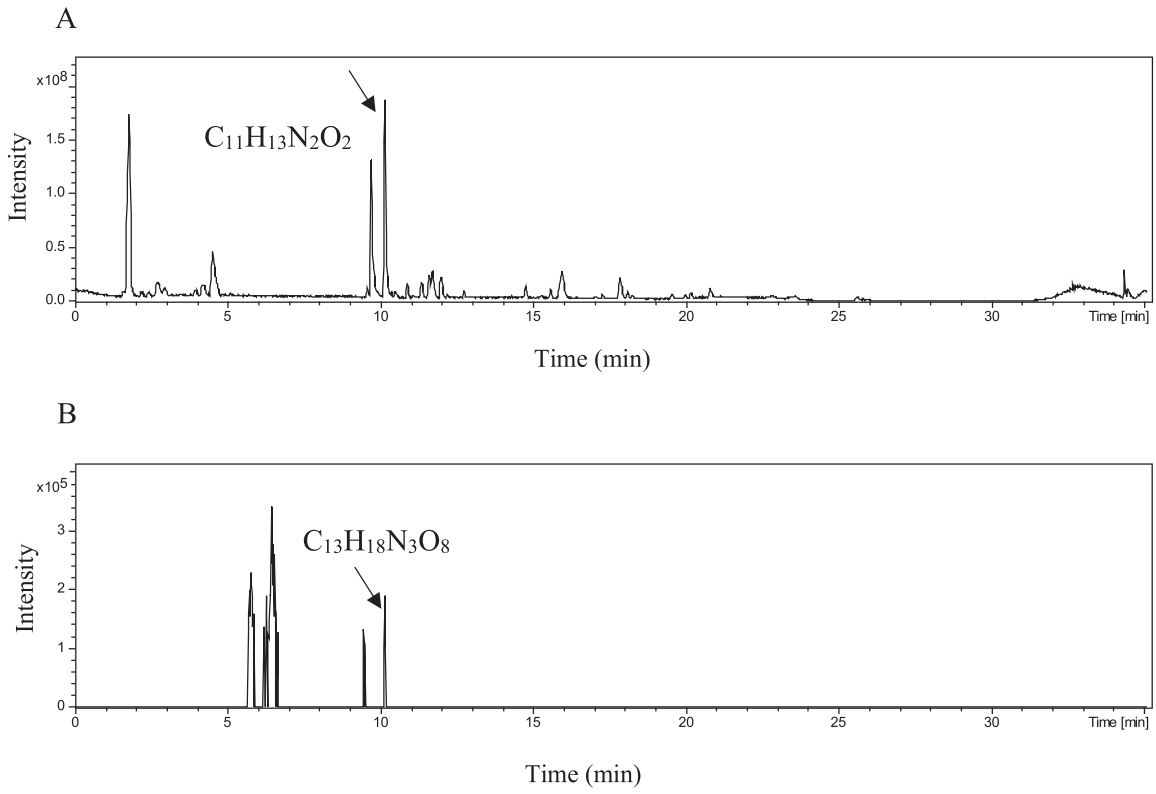

Fig. 1. Identification of intermediates metabolites of PRZ degradation by STP3.

FTMS chromatograms indicating intermediate metabolites after 7 days of resting cell assays in 10 mM Tris HCl buffer and 0.1 mM PRZ (A) as the positive mode for the detection of M1, and (B) as the negative mode for the detection of M2.

Identification of metabolites from PRZ biodegradation

In total, two intermediate metabolites were found to result from PRZ degradation, as listed in Table 1. One metabolite is from the positive mode and the other is from the negative mode. Chromatogram peaks representing intermediate metabolites showed prominent changes throughout the seven days of the resting cell assay period (Fig. 1). In the positive mode represented by chromatogram A, chromatogram peaks of metabolite M1 (1-(furan-2-carbonyl)-3,5-dimethylidenepiperazine) initially appeared during the day 0 sample and continued to increase in concentration until day 5, but decreased in intensity at day 7. In the negative mode represented by chromatogram B, the chromatogram peak corresponds to metabolite M2 (4-amino-6,6,7-trihydroxy-7-methoxy-decahydroquinazoline-5,5,8,8-tetracarbaldehyde) which exhibited immediately at the beginning of the assay with the highest intensity and continued to decrease until the peak was fully suppressed on day 5 and remained unchanged on day 7. Based on these metabolites, a proposed pathway is shown in Fig. 2, indicating that PRZ is cleaved at the C-N bond between the quinazoline and piperazine rings.

Draft genome sequence and FESEM images of STP3

Assembly results presented in Table 2 show that the isolate STP3 has a nucleotide distribution of 27.1% Adenine, 22.9% Cytosine, 23.3% Guanine and 26.7% Thymine. Additionally, it has 217 contigs with an average length of 19,131 bp, making the size of the STP3 genomic sequence 4.2 MBp. The assembly has 20, 6970 N50 contigs. Annotation done via submitting the nucleic acid sequence FASTA file to RAST gave 4,235 of the contig sequence numbers and showed 217 contigs with protein encoding genes (PEGs). Annotation results indicated the presence of 95 rRNA and tRNA genes.

Isolate STP3 has a 99% similarity with *Bacillus velezensis* sp. with a 0 E-value, based on alignment in BLASTN 2.8.0+. Annotation results matched the cleavage of PRZ compounds; thus, the possible enzymes that cleave the C-N bond were identified. In the tabulated results shown in Supplementary Table S1, 26 enzymes belonging to the nitrogen metabolism category, including cytochrome P450 and nitrate reductase, were annotated as being involved in the breakdown of nitrogen. The metabolism of aromatic compounds was identified by RAST, with 15 enzymes annotated as functioning to metabolize aromatic compounds, including monooxygenases, decarboxylases, and hydroxylases.

Putative protein annotated by RAST was clustered in finding unique genes responsible with PRZ degradation. The outcome from clustering annotated protein is tabulated in Supplementary Table S2, which shows 78 distinctive gene features of isolated STP3 when compared with 5 other *Bacillus* strains (*Bacillus velezensis* strain 10075, *Bacillus velezensis* strain CGMCC 11640, *Bacillus velezensis* strain DSYZ, *Bacillus velezensis* strain Lzh-a42 and *Bacillus velezensis* strain W1) for which their complete genome sequences are available at NCBI. Seventy eight unique PEGs consisting only of long amino acid se-

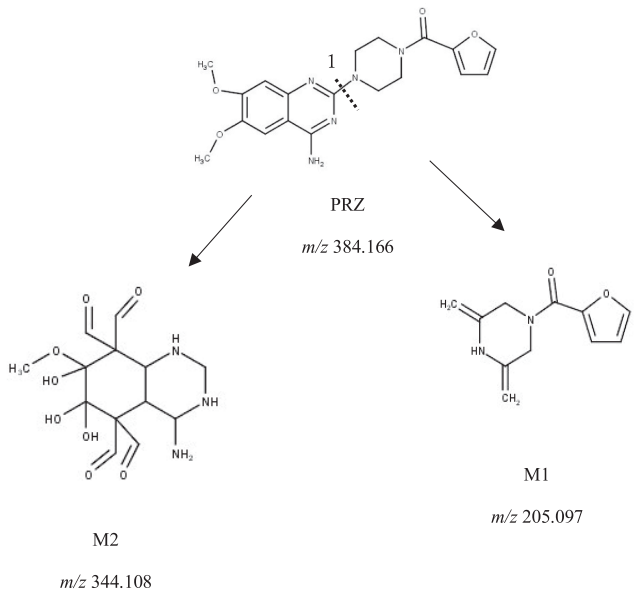

Fig. 2. Proposed pathway of PRZ degradation by *Bacillus* sp. STP3.

quences, ranging from 100aa until 1768aa, were extracted from genes of isolate STP3, when compared with 5 other different strains of *Bacillus velezensis* (Table S2). These five strains were chosen mainly because they have no reported history of degrading PRZ and the comparisons were made for clearer information about the gene features of isolate STP3 in degrading PRZ. The clustering program used in this study was CD-HIT and the parameter of similarity (-c) was set at 40% (0.4), and, therefore, the clustering will start with a 40% similarity until a similarity of 100% is reached. This was set in such a way in order to achieve a stricter selection in clustering. Consequently, 78 PEGs were discovered as probable genes in producing enzyme responsible in PRZ degradation, because they are unique compared with other non-PRZ degrading *Bacillus* strains.

SEM imaging showed that the isolate was a rod-shaped bacterium with a rough venous surface (Fig. S2). STP3 is estimated to be roughly 0.7 μm in size, and exhibits protruding flagella. Isolate STP3 was observed to have viscous, cloudy, creamy colonies, almost liquid in form with inconsistent shapes. FESEM analysis shows the isolate has peritrichous flagella for motility, a grooved cell surface and sticky characteristics that enable the bacteria to stick to each other. Described by Ruiz-Garcia et al. (2005), *Bacillus velezensis* is a rod-shaped Gram-positive bacterium, 0.5 × 1.5–3.5 μm in size, occurring both singly and in pairs, and occasionally in short chains.

Discussion

Pharmaceutical products have been listed as emergent pollutants with trace levels detected worldwide (Geissen et al., 2015). The concentration of these pharmaceutical residues continues to increase over time and has a hazardous effect on the aquatic environment, as well as humans through drinking water contamination. The sample collection location at the hospital sewage treatment plant, and the river located near the hospital, predisposes the samples to a high possible presence of PRZ. It is important to prove that there is PRZ contamination in the water system, which could lead to accidental consumption. Accidental consumption of PRZ leads to adverse effects such as palpitations (Brogden et al., 1977), and PRZ is toxic to humans when consumed together with durian (Chua et al., 2008); these adverse effects might prove fatal to a healthy person.

Table 2. Genome features of isolate STP3.

Features	
Genome size (bp)	4,151,459
G+C content	46.2%
N50	206,970
Number of contigs with protein encoding genes	217
Number of coding sequences	4235
Number of RNAs	95

The current study successfully isolated a native bacterium, which can degrade PRZ at a 0.1 mM concentration in a culture without a carbon source. Therefore, PRZ degradation by the isolate STP3 is likely to occur when the microbes consume PRZ as their sole carbon source. PRZ degradation is proven by the decreasing amount of PRZ over the course of treatment, detected using HPLC analysis. In previous studies, PRZ was degraded chemically (Shrivastava and Gupta, 2012), physically (Al-Qaim et al., 2018), and biologically (using animal cells) (Erve et al., 2007), and was proven to produce metabolites as detected by liquid chromatography. This study is the first to report that PRZ can be degraded biologically using bacteria isolated from a contamination site. Although PRZ degradation was detected with photolytic and thermal degradation, the control set allowed quantification of these factors. Via FTMS, the control sample showed that PRZ was detectable throughout the 7 days of the resting cell assay. MS analysis of the intermediate metabolites (IM) of PRZ biodegradation was performed to elucidate and propose the chemical structure of the metabolites based on the chemical formula obtained.

PRZ ($C_{19}H_{21}N_5O_4$) has a retention time (RT) of 11.60 min and appeared in the positive mode with $[M + H]^+$ ions and an *m/z* ratio of 384.166. This shows the presence of PRZ in the sample, and indicates that biodegradation of PRZ is occurring. The first metabolite, M1, was eluted in the positive mode for STP3 treated samples at an RT of 10.20 min. The $[M + H]^+$ ion was extracted at *m/z* 205.097 and showed 179 Da less than the PRZ ion. The process appears to be as follows: a hydrolysis reaction occurred at the nitrogen ion of the piperazine ring. Then dehydrogenation occurred at both of the adjacent carbons with the substitution of another carbon linked with double bonds. Alkylation also manifested at the two carbons on the piperazine ring. Additionally, amination occurred on the nitrogen with the addition of hydrogen. Dehydrogenase and hydrolase could be responsible for this reaction. The M1 metabolite has the chemical formula $C_{11}H_{13}N_2O_2$.

The second metabolite (M2), found in the STP3-treated sample in the negative mode, has a retention time of 10.18 min and *m/z* of 344.108. The ion $[M + H]^-$ *m/z* measured is 40 Da less than the PRZ ion and has the chemical for-

mula $C_{13}H_{18}N_3O_8$. The proposed chemical structure implies hydrolysis occurred, separating the main 2 aromatic rings from one another and reducing the carbon double bond to a single bond. Hydroxylation also occurred with the addition of the -OH group, which eliminated the double bond; the elimination occurred via alkylation. In addition, oxygenation occurred that replaced hydrogen with oxygen on the alkyl groups. Hydroxylase and oxygenase are two key enzymes in initiating the formation of this metabolite. Since this study emphasizing the importance of carbon-lacking media in ensuring PRZ degradation, the carbon source for the alkylation process might come from the free radicals of the PRZ degradation process especially from the O-dealkylation at the quinazoline ring. This is supported with the function of cytochrome P450, which also found in the annotated gene within the genomic sequence of isolate STP3, in catalyzing O-dealkylation as studied by Lee et al. (2013). The mechanism consists of two major steps of hydrogen abstraction and oxygen rebound, as explained by Totah (2013). Then, the methyl radicals would be the main source of carbon for the alkylation to form elucidated metabolites.

From the identified intermediate metabolites above, a metabolic pathway of PRZ biodegradation is proposed in Fig. 2. The proposed pathway states that the isolate STP3 fabricated a degradation pathway leading to a cleavage point and produced two different intermediate metabolites. The isolate STP3, which cleaved PRZ at site 1, shows that hydrogenation splits PRZ into 2 major parts: M1 (IUPAC name 1-(furan-2-carbonyl)-3,5-dimethylidenepiperazine) and M2 (IUPAC name 4-amino-6,6,7-trihydroxy-7-methoxy-decahydroquinazoline-5,5,8,8-tetracarbaldehyde).

Identifying the possible enzymes involved in cleaving PRZ is an important aspect of this research. Next generation sequencing is able to yield elaborate information on genomic analyses and identify the possible enzymes involved. The STP3 assembled FASTA file obtained is used in NCBI blastn against the 16S rRNA database to identify the species of the isolate. The outcome is 99% similarity with *Bacillus velezensis* strain number FZB42, which strongly indicates the species of isolate STP3. Annotation was carried out using RAST to identify possible enzymes involved in PRZ breakdown at cleavage site 1. The bond cleaved at site 1 is between carbon and nitrogen (C-N); therefore, the enzymes involved in nitrogen metabolism are suspected in degrading PRZ at that site. There are 26 enzymes responsible for nitrogen metabolism, including cytochrome P450 hydroxylase. In addition to producing cholesterol, cytochrome P450 enzymes are known to function as drug metabolizers (Lynch and Price, 2007). Therefore, this finding shows that isolate STP3 can biodegrade PRZ by producing the enzyme cytochrome P450 hydroxylase. Another group of enzymes found by annotation is responsible for metabolizing aromatic compounds. As PRZ consists of 4 aromatic compounds, making the structure highly stable and strong, the 15 enzymes found under this category act to break apart PRZ at site 1 between the two quinazoline and piperazine segments, which consist of two aromatic rings each. The monooxygenases found in the listed enzymes suggest the removal of double bonds and the addition of oxygen ions on M2.

In previous research by Ruiz-Garcia et al. (2005), *Bacillus velezensis* was first found in a river as a bacterium that produces a surfactant and has anti-microbial properties. This species was also reported by Bafana et al. (2008), to produce azoreductase, which is responsible for degrading congo red dyes contaminating rivers. In addition, *Bacillus velezensis* also manifests powerful resistance against plant pathogens and human pathogenic bacteria (Liu et al., 2017).

Isolate STP3 was observed to have viscous, cloudy, creamy colonies, almost liquid in form with inconsistent shapes. FESEM analysis shows the isolate has peritrichous flagella for motility, a grooved cell surface and sticky characteristics that made the bacteria stick to each other. Described by Ruiz-Garcia (2005), *Bacillus velezensis* is a rod-shaped Gram-positive bacterium, 0.5×1.5–$3.5\ \mu$m in size, occurring both singly and in pairs and occasionally in short chains. Isolate STP3 viewed under FESEM is approximately $0.7\ \mu$m.

This study is the first to prove the ability of a bacterial strain to degrade the drug PRZ. Isolate *Bacillus* sp. STP3 was proven to degrade PRZ via a pathway that breaks down the C-N bond between the quinazoline and piperazine-furan rings. Therefore, enzymes belonging to the categories of nitrogen and aromatic compound metabolism are likely to be involved in breaking down PRZ compounds. Further studies, such as the identification of the enzyme for first step cleavage and metabolites degradation, are needed to confirm the biodegradation of PRZ by our isolate STP3.

Acknowledgments

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Supplementary Materials

Supplementary figures and tables are available in our J-STAGE site (http://www.jstage.jst.go.jp/browse/jgam).

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