Characterization of a Novel Chromosome-Encoded AmpC β-Lactamase Gene, \textit{bla}_{PRC-1}, in an Isolate of a Newly Classified \textit{Pseudomonas} Species, \textit{Pseudomonas wenzhouensis} A20, From Animal Farm Sewage

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In this work, we characterized a novel chromosome-encoded AmpC β-lactamase gene, \textit{bla}_{PRC-1}, in an isolate of a newly classified \textit{Pseudomonas} species designated \textit{Pseudomonas wenzhouensis} A20, which was isolated from sewage discharged from an animal farm in Wenzhou, China. Susceptibility testing, molecular cloning, and enzyme kinetic parameter analysis were performed to determine the function and enzymatic properties of the β-lactamase. Sequencing and comparative genomic analysis were conducted to clarify the phylogenetic relationship and genetic context of the \textit{bla}_{PRC-1} gene. PRC-1 is a 379-amino acid AmpC β-lactamase with a molecular weight of 41.48 kDa and a predicted pI of 6.44, sharing the highest amino acid identity (57.7%) with the functionally characterized AmpC β-lactamase PDC-211 (ARX71249). \textit{bla}_{PRC-1} confers resistance to many β-lactam antibiotics, including penicillins (penicillin G, amoxicillin, and amoxicillin-clavulanic acid) and cephalosporins (cefazolin, ceftriaxone, and cefotaxime). The kinetic properties of PRC-1 were compatible with those of a typical class C β-lactamase showing hydrolytic activities against β-lactam antibiotics, and the hydrolytic activity was strongly inhibited by avibactam. The genetic context of \textit{bla}_{PRC-1} was relatively conserved, and no mobile genetic element was predicted in its surrounding region. Identification of a novel β-lactamase gene in an unusual environmental bacterium reveals that there might be numerous unknown resistance mechanisms in bacterial populations, which may pose potential risks to human health due to universal horizontal gene transfer between microorganisms. It is therefore of great value to carry out extensive research on the mechanism of antibiotic resistance.

Keywords: PRC-1, β-lactamase, \textit{Pseudomonas}, AmpC, kinetic analysis, resistance
INTRODUCTION

The genus *Pseudomonas*, first described by Professor Mikula in 1894, is one of the most common bacteria in the world and has been found in various natural environments, human clinical specimens, and infected plants (Peix et al., 2009). *Pseudomonas* is a diverse and complex genus with the largest number of known species (Lalucat et al., 2020). At present, 396 species and 21 subspecies of *Pseudomonas* are included in the List of Prokaryotic Names with Standing in Nomenclature (Parte, 2014). The genus *Pseudomonas* mainly includes species that are pathogenic to animals and humans (*Pseudomonas aeruginosa*), insects (*Pseudomonas entomophila*), and plants (*Pseudomonas syringae*); species that are plant commensals (*Pseudomonas stutzeri* and *Pseudomonas fluorescens*); and species used in bioremediation (*Pseudomonas putida*) (De Smet et al., 2017). Extensively studied and economically important species are the human opportunistic pathogen *P. aeruginosa* and the plant pathogen *P. syringae* (Silby et al., 2011). *P. aeruginosa* is the most common cause of infection among non-fermenting gram-negative bacteria, which mainly affects patients with weakened immune function (Behzadi et al., 2021).

β-lactamase is an enzyme that can hydrolyze the β-lactam ring and inactivate antibiotics before binding to penicillin-binding proteins (Li et al., 2007). The AmpC enzyme is a type of β-lactamase mediated by chromosomeres or plasmids in most Enterobacteriaceae and non-fermenting species, such as *P. aeruginosa*. AmpC belongs to Class C β-lactamase in the Ambler molecular structure classification and group I in the Bush-Jacoby-Medeiros functional classification of β-lactamases (Bush et al., 1995). AmpC β-lactamases are able to confer resistance to most penicillins and cephalosporins and cannot be inhibited by normal β-lactam inhibitors, such as clavulanic acid and tazobactam (Bush et al., 1995; Livermore, 1995), but it can be strongly inhibited by avibactam (Docquier and Mangani, 2018). AmpC β-lactamases are usually not expressed or are underexpressed in *E. coli* because of a weak promoter and a transcriptional attenuator preceding the *ampC* gene; however, due to mutations in β-lactamases or induction by specific β-lactams, these enzymes can be expressed at high levels and consequently increase the minimum inhibitory concentrations (MICs) of β-lactams (Fisher and Mobashery, 2014; Juan et al., 2017). This effect is attributed to specific spontaneous mutations in the promoter or attenuation consensus sequence of the *ampC* gene (Haenni et al., 2014). It is worth noting that the diversity caused by mutations and the overexpression caused by antimicrobial induction of these enzymes are evolving to confer resistance to various β-lactam antibiotics. Therefore, the development of new antibiotics and novel antibacterial therapeutic strategies is urgently needed (Bonomo, 2017).

In this study, based on the complete genome sequencing of an isolate of a newly classified *Pseudomonas* species, *Pseudomonas wenzhouensis* A20, we characterized a novel chromosome-encoded AmpC β-lactamase, designated PRC-1, and analyzed the kinetic parameters of the β-lactamase. Discovering a novel resistance gene in an environmental bacterium provides valuable information for the treatment of infectious diseases.

MATERIALS AND METHODS

Bacterial Strains

The strain *P. wenzhouensis* A20 carrying the novel β-lactamase gene *blaPRC-1* was isolated from sewage discharged from an animal farm in Wenzhou, China. It was initially identified using a bioMérieux VITEK 2 Compact Instrument (BioMérieux, Marcy L’etoile, France). Further species identification was carried out by 16S ribosomal RNA (rRNA) gene sequencing and finally verified using average nucleotide identity (ANI) (Lachance et al., 2020). The bacterial strains and plasmids used in this work are listed in Table 1.

Antibiotic Susceptibility Test

Minimum inhibitory concentrations were determined with the standard agar dilution method on Mueller-Hinton (MH) agar according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). The MICs of β-lactam inhibitors were determined by broth microdilution in cation-adjusted Mueller-Hinton broth (CAMHB). The MIC was interpreted as the β-lactam concentration at which bacterial growth was no longer observed after 20 h of incubation at 37°C. The MICs of ampicillin-avibactam and ampicillin-tazobactam were determined at a constant concentration of 4 mg/L avibactam and tazobactam, and in combination with a series of increasing concentrations of ampicillin, while for amoxicillin-clavulanic acid, a constant concentration ratio (2:1, amoxicillin-clavulanic acid) was applied. MICs for the tested antibiotics were interpreted according to the guidelines of the CLSI (2020); however, for some antibiotics without CLSI interpretation criteria for *P. aeruginosa*, breakpoints for Enterobacteriaceae in the CLSI

| Strain or plasmid | Characteristics | Source |
|-------------------|----------------|--------|
| **Strain** | | |
| A20 | The wild-type strain of *P. wenzhouensis* A20 | This study |
| DH5α | Escherichia coli DH5α was used as a host for the cloning of the *blaPRC-1* gene | Our laboratory collection |
| BL21 | Escherichia coli BL21 was used as a host for the expression of PRC-1 | Our laboratory collection |
| ATCC 25922 | Escherichia coli ATCC 25922 was used as a quality control strain for antimicrobial susceptibility testing | Our laboratory collection |
| pUCP24-*blaPRC-1*-DH5α | DH5α carrying the pUCP24 vector with the *blaPRC-1* gene with its upstream promoter region | This study |
| pCold I-*blaPRC-1*-BL21 | BL21 carrying the pCold I vector with the open reading frame of the *blaPRC-1* gene | This study |
| **Plasmid** | | |
| pUCP24 | Cloning vector for the PCR products of the *blaPRC-1* gene with its upstream promoter region, GEN¹ | Our laboratory collection |
| pCold I | Expression vector for the PCR products of the ORF of the *blaPRC-1* gene, AMP² | Our laboratory collection |

¹ Resistance; GEN, gentamicin; AMP, ampicillin.
guidelines were used as a reference. *E. coli* ATCC 25922 was used as a quality control strain. Values are the means of three independent measures.

**Genome Sequencing**

A Generay Genomic DNA Miniprep Kit (Shanghai Generay Biotech Co., Ltd., Shanghai, China) was used to extract the total bacterial DNA of *P. wenzhouensis* A20. Genomic DNA was sequenced by both the Illumina HiSeq-2500 and PacBio RS II platforms by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The PacBio long reads were initially assembled by Canu v1.8 (Koren et al., 2017), and hybrid assembly was subsequently conducted using Unicycler v0.4.8 (Wick et al., 2017), with the contigs generated by Canu and all the sequenced reads (including short and long reads) serving as an input. The cyclization of the whole-genome assembly was confirmed through the built-in tools of Unicycler. BWA v0.7.12 (Li and Durbin, 2009) and Genome Analysis Toolkit (McKenna et al., 2010) were used for short read alignment to the draft of the whole-genome assembly to improve assembly quality. Open reading frames (ORFs) were predicted using Prokka v1.14.6 (Seemann, 2014) with default parameters and annotated by the BLAST program with an e-value threshold of 1e-5 against the non-redundant protein sequence (NR) database of the National Center for Biotechnology Information (NCBI) and the UniProt/Swiss-Prot database. Resistance genes were identified using a combination of the ResFinder database (Zankari et al., 2012) and the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al., 2013). Mobile genetic elements (MGEs) were detected using ISFinder (Siguier et al., 2006) and INTEGRALL (Moura et al., 2009) with default parameters. FastANI v1.31 (Jain et al., 2018) was used to calculate the ANI. ProtParam was used to predict the molecular weight and pI value of PRC-1. The putative signal peptide cleavage site of PRC-1 was predicted by SignalP 5.0 (Almagro Armenteros et al., 2019). Circular maps of the genome and other relations were drawn using CGView Server (Petkau et al., 2010). Multiple sequence alignments of PRC-1 and other PDC family β-lactamas were performed using MAFFT v7.475 (Katoh and Standley, 2013). A neighbor-joining phylogenetic tree including PRC-1 and other PDC family β-lactamas were reconstructed using MEGAX (Kumar et al., 2018). The resulting tree was visualized using the online tool iTol (Letunic and Bork, 2007). Other bioinformatics tools in this study were applied with Python and Biopython scripts (Cock et al., 2009).

**Cloning of the blaPRC-1 Gene and Expression and Purification of PRC-1**

The nucleotide sequence of the blaPRC-1 gene with the upstream promotor region was amplified using PrimeSTAR HS DNA Polymerase (Takara Bio Inc., Dalian, China), and *P. wenzhouensis* A20 genomic DNA was used as the template. The primers with restriction endonuclease sites (BamHI and HindIII for the forward and reverse primers, respectively) are listed in Supplementary Table 1. The PCR product and the cloning vector pUCP24 were digested with both BamHI and HindIII (Takara Bio Inc.). The resulting DNA fragments were ligated with T4 DNA ligase (Takara Bio Inc.), and the ligated product was transformed into *E. coli* DH5α with the calcium chloride method. The transformants were selected on LB agar plates supplemented with gentamicin (40 μg/mL). Single colonies were inoculated into LB medium supplemented with the same antibiotics and cultured overnight. Plasmids were extracted from the cultures using a Plasmid Mini Extraction Kit (Generay Biotech Co., Ltd.), and the inserts were verified by PCR and further by DNA sequencing (TsingKe, Shanghai, China). The same method was used to clone the ORF of *blaPRC-1* without the signal sequence into the pCold I vector (the primers are listed in Supplementary Table 1), and the recombinant plasmid (pCold I-*blaPRC-1*) was transformed into competent *E. coli* BL21 cells. The transformants were selected on LB agar plates supplemented with 100 μg/mL ampicillin. For the expression of PRC-1, the recombinant strain (pCold I-*blaPRC-1*BL21) was cultured overnight in LB medium, diluted 100-fold in fresh medium, and then incubated at 16°C and 250 rpm for 2–3 h, which was followed by the addition of 0.5 mM isopropyl-β-d-thiogalactopyranoside (IPTG, Sigma Chemicals Co., St. Louis, MO, United States) when the OD₆₀₀ reached 0.6 and further incubation for 21 h at 16°C (Choi and Geletu, 2018). His-tagged PRC-1 protein was purified by nickel affinity chromatography with BeyoGold His-tag purification resin (Beyotime, Shanghai, China) and then digested with Enterokinase (GenScript, Nanjing, China) for 40 h at 37°C to remove the His-tag. The protein was identified by SDS-PAGE using a 12% acrylamide separation gel and Coomassie blue G-250 staining.

**Enzyme Kinetic Analysis**

Kinetic parameters of hydrolysis for β-lactams by the novel β-lactamase PRC-1 were determined on a UV-VIS spectrophotometer (U-3900, HITACHI, Japan) at 37°C in 10 mM phosphate buffer (pH 7.4) in a final reaction volume of 200 μl. The steady-state kinetic parameters (kcat and Km) were determined by non-linear regression of the initial reaction rates with the Michaelis-Menten equation in Prism (version 8.0.2) software (GraphPad Software, CA, United States) (Chen et al., 2019). For poor substrates (cefepime, imipenem, and aztreonam), the tested antibiotics were treated as competitive inhibitors of the AmpC enzyme and nitrocefin as a reporter substrate, and the inhibition constants were determined by observing the apparent Km at various concentrations of inhibitor. Data were analyzed to obtain the values of Vmax and Km by GraphPad Prism with the competitive model inhibition/non-linear regression curve fit method. kcat values were determined from the initial rates calculated at saturating substrate concentrations (Faheem et al., 2013). The concentrations of the β-lactamase inhibitors avibactam and clavulanic acid leading to a 50% reduction in hydrolysis of nitrocefin (IC50) were measured after 5 min of preincubation of the enzymes with the inhibitors at 37°C and nitrocefin as the substrate at 100 μM. The IC50 values were determined by non-linear regression analysis (GraphPad Prism, version 8.0.2) using log (inhibitor) vs. response – (three
parameters) (Chen et al., 2019). Values are the means of three independent measures.

**Nucleotide Sequence Accession Numbers**
The chromosome and \( \text{bla}_{PRC-1} \) gene sequences of \( P. \text{wenzhouensis} \) A20 have been deposited in GenBank under accession numbers CP072610 and MW854031, respectively.

**RESULTS AND DISCUSSION**

**Genomic Properties of \( P. \text{wenzhouensis} \) A20**
The strain \( P. \text{wenzhouensis} \) A20 was isolated from sewage discharged from an animal farm in Wenzhou, China. 16S rRNA homologous gene analysis demonstrated that \( P. \text{wenzhouensis} \) A20 showed the closest relationship with the strain \( P. \text{hydrolytica} \) DSWY01 (NR_170428.1), at 98.50% identity and 100% coverage. The taxonomy of the genus \( P. \text{wenzhouensis} \) was developed mainly through phenotypic and biochemical properties (Palleroni, 2010), whereas currently, molecular analysis, as a good supplement, is particularly important. According to previous studies on the phylogeny of \( P. \text{wenzhouensis} \) based on 16S ribosomal DNA (rDNA) sequences, there was not enough discrimination at the species level (Mulet et al., 2010),

| TABLE 2 | General features of the \( P. \text{wenzhouensis} \) A20 genome. |
|-----------------|-----------------|
| **Chromosome**  | **Size (bp)**   |
|                 | 4,452,100       |
| GC content (%)  | 62.20           |
| CDS             | 4,063           |
| Known proteins  | 3,601 (88.63%)  |
| Hypothetical proteins | 462 (11.37%) |
| Protein coding (%) | 88.12          |
| Average ORF length (bp) | 970            |
| Average protein length (aa) | 321          |
| tRNAs           | 83              |
| rRNA operons    | (16S-23S-5S)*4 |

**FIGURE 1 | Comparative chromosome map of the \( P. \text{wenzhouensis} \) A20 and 20 other \( P. \text{wenzhouensis} \) strains using A20 as the reference.**

Circle 1 (from inside to outside) shows the scale in kb. Circles 2 and 3 show the GC content and GC skew, respectively. Circles 4–25 are the regions homologous to A20 and the other 20 strains, respectively. Similar parts are represented by lines of different shades, and the regions without similar hits leave blank. The strains used for comparison in this study are listed in Supplementary Table 2.
The Resistance Profile of Pseudomonas wenzhouensis A20

Antibiotic susceptibility testing showed that A20 exhibited intermediate resistance to nalidixic acid. P. wenzhouensis A20 had the highest MIC levels for fosfomycin (>512 µg/mL), cefazolin (256 µg/mL), and cefoxitin (128 µg/mL) and higher MIC levels for aztreonam (32 µg/mL) and cefotaxime (8 µg/mL) (Table 3). As the breakpoints for fosfomycin, cefazolin, cefoxitin, aztreonam, and cefotaxime were not available for P. aeruginosa in CLSI interpretation criteria, the breakpoints for Enterobacteriaceae in the CLSI guidelines were referred to, and the MIC values of P. wenzhouensis A20 were equivalent to those of resistant enterobacteria for these five antimicrobial agents. The isolate was susceptible to some third- and fourth-generation cephalosporins (e.g., ceftazidime, cefoperazone, cefoselis, and cefepime) and carbapenems (such as meropenem). When analyzing the resistance mechanism of the bacterium, especially for β-lactam antibiotics, we found that only one predicted ampC β-lactamase gene was annotated within the whole genome, which showed an identity of more than 50% with the functionally characterized resistance gene blaPRC-1 (57.4%, MF281075.1). The predicted gene was then cloned, and the resistance function was further determined.

Functional Characterization of the PRC-1 β-Lactamase

To determine the resistance characteristics of blaPRC-1 to β-lactam antibiotics, the coding sequence of blaPRC-1 together with its upstream promoter region was amplified and cloned into the pUCP24 vector and then transformed into E. coli DH5α. The results revealed that blaPRC-1 conferred resistance to some penicillins and first- and third-generation cephalosporins (Table 3). Compared with the control strains (DH5α and pUCP24/DH5α), the recombinant strain (pUCP24-blaPRC-1/DH5α) exhibited increased MIC levels for ampicillin, cefotaxime, and amoxicillin by 8-, 8-, and 32-fold, respectively. The MICs of ceftriaxone, cefazolin, penicillin G, and cefotaxime exhibited a lower increase of 4-fold. However, the recombinant strain did not show any MIC level changes for carbapenems or monobactams. The activity of PRC-1 was poorly inhibited by classical class A β-lactamase inhibitors such as clavulanic acid.

| Antibiotic   | P. wenzhouensis A20 | pUCP24-blaPRC-1/DH5α | pUCP24/DH5α | MHIC (µg/mL) |
|--------------|---------------------|----------------------|-------------|--------------|
| Ampicillin   | 16                  | 16                   | 2           | 4            |
| Cefazolin    | 256                 | 4                    | 1           | 1            |
| Cefoxitin    | 128                 | 4                    | 2           | 4            |
| Cefotaxime   | 1                   | 0.5                  | 0.25        | 0.25         |
| Cefepime     | 0.125               | 0.125                | 0.06        | 0.125        |
| Cefoperazone | 8                   | 0.5                  | 0.5         | 0.5          |
| Ceftriaxone  | 2                   | 0.125                | 0.03        | 0.03         |
| Cefotaxime   | 8                   | 0.25                 | 0.03        | 0.03         |
| Cefoselis    | 1                   | 0.125                | 0.06        | 0.125        |
| Aztreonam    | 32                  | 0.125                | 0.06        | 0.25         |
| Meropenem    | 0.25                | 0.03                 | 0.015       | 0.015        |
| Amoxicillin  | 8                   | 32                   | 1           | 2            |
| Amoxicillin-clavulanic acid | 8 | 32 | 1  | 1 |

Table 3 | Minimum inhibitory concentrations of antimicrobials for P. wenzhouensis A20, the recombinants, and the control strain (µg/mL).

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and tazobactam, while a significant decrease in resistance to ampicillin was observed in the presence of avibactam.

Multiple sequence alignment of the deduced amino acid sequence of PRC-1 with those of functionally characterized β-lactamases revealed that PRC-1 had identities of 57.7, 57.5, 57.3, 57.1, 57.0, 56.8, and 56.8% with PDC-211, PDC-241, PDC-7, PDC-68, PDC-3, PDC-1, and PDC-315, respectively. The deduced amino acid sequence carries the characteristic catalytic residues of the serine active site of β-lactamases, including motifs of S-X-X-K (serine-isoleucine-serine-lysine) with the initial amino acid sequence number at positions 64–67, Y-S-N (tryptophan-serine-asparagine) at positions 150–152, and K-T-G (lysine-threonine-glycine) at positions 315–317 (Figure 2).

Because no detailed resistance spectrum is available for blaPDC-3, blaPRC-1 exhibited a relatively narrow resistance spectrum and did not confer resistance to piperacillin, cefepime, ceftazidime, or even aztreonam. The resistance spectrum of PRC-1 was compared with those of PDC-211, PDC-241, PDC-7, PDC-68, and PDC-3. PDC-3 (ACQ82808) is a chromosomal AmpC β-lactamase of P. aeruginosa with an amino acid identity of 57.0% (216/379) with PRC-1. Unlike PDC-3, PRC-1 exhibited a relatively narrow resistance spectrum and did not confer resistance to piperacillin, cefepime, ceftazidime, or even aztreonam.

### TABLE 4 | Kinetic parameters of PRC-1 for β-lactam antibiotics.

| Substrate       | $K_m$ or $K_i$ (µM)$^a$ | $k_{cat}$ (s$^{-1}$)$^b$ | $k_{cat}/K_m$ (µM$^{-1}$·s$^{-1}$) |
|-----------------|------------------------|--------------------------|-----------------------------------|
| Benzylpenicillin| 126 ± 18               | 123 ± 17                 | 98 × 10$^{-2}$                     |
| Ampicillin      | 65 ± 7                 | 23 ± 2                   | 35 × 10$^{-2}$                     |
| Cefazolin       | 210 ± 31               | 463 ± 28                 | 220 × 10$^{-2}$                    |
| Cefotaxime      | 529 ± 81               | 156 ± 10                 | 29 × 10$^{-2}$                     |
| Ceftriaxone     | 68 ± 14                | 32 ± 4                   | 47 × 10$^{-2}$                     |
| Ceftazidime     | 253 ± 20               | (249 ± 31) × 10$^{-2}$   | 1 × 10$^{-2}$                     |
| Aztreonam       | (307 ± 21) × 10$^{-3}$ | NH$^b$                   | –                                 |
| Imipenem        | (134 ± 20) × 10$^{-3}$ | NH$^b$                   | –                                 |
| Cefepine        | (72 ± 7) × 10$^{-3}$   | NH$^b$                   | –                                 |

$^a$ Values are means ± standard deviations.

$^b$ NH, no detectable hydrolysis.
aztreonam. In addition, \textit{bla}_{PRC-1} showed lower MIC values than \textit{bla}_{PDC-3} against other β-lactam antibiotics (Barnes et al., 2018). Additionally, we found that the \textit{bla}_{PRC-1} gene did not show any resistance to cefoxitin but showed a relatively lower MIC level for ceftazolin, even though \textit{P. wenzhouensis} A20 showed much higher MIC levels for them. This finding may indicate the existence of unknown resistance mechanisms within \textit{P. wenzhouensis} A20.

The novel β-lactamase gene \textit{bla}_{PRC-1} is 1140 bp in size and encodes a 379-amino acid putative protein. The mature protein has a predicted molecular weight of 41.48 kDa and a predicted pl of 6.44. The secretory precursor peptide, which consists of 22 amino acids, in PRC-1 is defined by alanine residues at positions 22 and 23. The molecular weight of the protein without the signal peptide is 39.07 kDa. The purified protein PRC-1 exhibited a single band on SDS-PAGE, and its molecular size was in agreement with the predicted one. The kinetic parameters of PRC-1 were determined by measuring the rates of catalysis for various β-lactam antibiotics at different substrate concentrations. The results demonstrated that PRC-1 was a typical cephalosporinase with a high \textit{k}_{cat} and strong hydrolytic activity (\textit{k}_{cat}/\textit{K}_{m} ratios were \textit{2} \times \textit{10}^{-2} \text{µM}^{-1} \text{s}^{-1}) for the first-generation cephalosporin ceftazolin. PRC-1 showed moderate hydrolysis activities against some third-generation cephalosporins (cefotaxime and ceftriaxone) and penicillins (ampicillin and benzylpenicillin) but very poor hydrolytic activity against ceftazidime (the third-generation cephalosporin) (Table 4). However, the result of the enzyme kinetic hydrolytic activity test was not completely consistent with the MIC level change of the recombinant strain (pUCP24-\textit{bla}_{PRC-1}/DH5α) in the antimicrobial susceptibility test (Table 3). For example, PRC-1 showed hydrolytic activity against ceftazidime, but the recombinant strain carrying \textit{bla}_{PRC-1} did not exhibit a significant change in the MIC of ceftazidime compared with that for the control bacteria. This result may be attributed to its low activity in vitro. A similar case in a previous study reported that BAT-2 and BSU-2 exhibited slight hydrolytic activities against ampicillin, but the two genes did not show detectable

![FIGURE 3](image-url) Phylogenetic analysis of PRC-1 with other putative class C β-lactamases (>85% amino acid similarity). PRC-1 from this study is represented with a red dot.
resistance activities to ampicillin in the recombinant strains carrying them (Toth et al., 2016). We also found that the catalytic efficiency ($k_{cat}/K_m$) of ceftazidime was the lowest among all the tested substrates. Moreover, the affinity of poor substrates, such as aztreonam, cefepime, or imipenem, was higher than those of other substrates in the competitive inhibition test with nitrocefin; the $K_i$ values for aztreonam, cefepime, and imipenem were $(307 \pm 21) \times 10^{-3}$, $(134 \pm 20) \times 10^{-3}$, and $(72 \pm 7) \times 10^{-3}$ µM, respectively, and no hydrolysis was determined for these substrates at saturating concentrations (Table 4), which was in line with the susceptibility test results. Conversely, PDC-3, which shares 57.0% global amino acid identity with PRC-1, exhibited hydrolytic activities against cefepime and imipenem (Rodriguez-Martinez et al., 2009). Both β-lactamases showed similar catalytic efficiencies for benzylpenicillin, whereas PRC-1 displayed higher $K_m$ and $k_{cat}$ values, revealing that higher turnover numbers are compensated by higher $K_m$ values (Table 4). The IC50 (50% inhibitory concentration) of β-lactamase inhibitors showed that avibactam (IC50: 0.0003922 µM) has a strong inhibitory effect on PRC-1, while clavulanic acid (IC50: 23.43 µM) has a weaker inhibitory effect. This result is in line with the properties of β-lactam inhibitors for AmpC enzymes.

**Taxonomic Distribution of blaPRC-1**

To analyze the possible origin of blaPRC-1, a total of 40 predicted proteins with an amino acid similarity ≥85% were retrieved from the NCBI-nr database, and all of them were from the genus Pseudomonas. The phylogenetic tree showed that PRC-1 was closest to a putative AmpC β-lactamase found in P. mendocina (WP_147810921.1), and they shared the highest amino acid similarity (91.29% identity and 100% coverage) (Figure 3). These findings indicate the importance of Pseudomonas as a reservoir for PRC-1-like relatives, and additional Pseudomonas genomes must be sequenced to find proteins with higher identities with PRC-1.

**Analysis of Genetic Environment of blaPRC-1**

To analyze the genetic environments of blaPRC-1 and its relatives, nine sequences of approximately 20 kb in length with blaPRC-1-like genes at the center were retrieved from the NCBI nucleotide database, and these blaPRC-1-like genes shared over 85% amino acid similarities with PRC-1. No mobile genetic element was predicted in its surrounding area. Comparative genomic analysis of the 10 sequences (including the one from this work, P. wenzhouensis A20) revealed that the upstream regions (from folD to acoR) of the blaPRC-1 and blaPRC-1-like (ampC) genes of all 10 sequences have a conserved structure in terms of gene context and gene order. Three sequences (P. mendocina S5, P. mendocina 5, and Pseudomonas sp. B11D7D) have the most similar structure to the sequence from this study, except two additional genes (dmlR and bdcA) downstream.
of the bla<sub>PRC-1</sub>-like (ampC) genes that were found in these three sequences. Three sequences (P. mendocina NEB698, P. mendocina NK-01, and P. mendocina strain NCTC 10897) had an extra two or more genes upstream of the bla<sub>PRC-1</sub>-like (ampC) genes. The remaining three sequences had the same (P. sediminis B10D7D) or nearly the same (P. alcaliphila JAB1 and P. sihuiensis 32246) gene context as that of the one from this work in the regions downstream of bla<sub>PRC-1</sub>; however, the upstream regions of these three sequences were totally different from P. wenzhouensis A20. All 10 sequences were from the genus Pseudomonas, and the three sequences with the most similarity with P. wenzhouensis A20 were from P. mendocina (P. mendocina S5 and P. mendocina CP5) and an unclassified Pseudomonas (P. sp. B11D7D) (Figure 4). These results suggested that the gene context of bla<sub>PRC-1</sub> and its relatives is conserved in species of the genus Pseudomonas.

CONCLUSION

In this work, we characterized a novel AmpC β-lactamase-encoding gene, bla<sub>PRC-1</sub>, in the chromosome of P. wenzhouensis A20, an isolate of a newly classified species of the Pseudomonas genus. bla<sub>PRC-1</sub> shared the highest amino acid similarity (57.7%) with the functionally characterized AmpC enzyme PDC-211 from P. aeruginosa and conferred resistance to β-lactam antibiotics, including some cephalosporins, such as cefazolin, ceftriaxone, and cefotaxime. Similar to other AmpC β-lactamases, the novel β-lactamase is strongly inhibited by avibactam, but inhibitors of class A enzymes such as clavulanic acid have a weaker inhibitory effect against it. bla<sub>PRC-1</sub>-like genes with amino acid similarities of more than 85% were identified in many bacteria of different species, and further research would be carried out to determine the functions of these potential resistance genes. Deciphering more antibacterial resistance mechanisms will be crucial to assist clinics in using effective antibiotics to treat infections caused by unusual pathogens.

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DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

HZ, MZ, and QB: conception and design of the study. JLL, XL, WS, MG, and PR: acquisition of data. PZ, XD, KZ, JLL, CF, XL, and KL: data analysis and interpretation. PZ, XD, KZ, JWL, QB, and HZ: drafting of manuscript. PZ, XD, WS, MG, QL, and XZ: performed the experiments. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.732932/full#supplementary-material

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