Comparative genomic of biosynthetic gene cluster of andrimid antibiotic from *Serratia plymuthica* UBCF_13

R Fatiah¹, I Sulianyah¹,³,₄, D H Tjong²,⁴, J Jamsari¹,³,₄*  
¹Department of Agricultural Science, Faculty of Agriculture, Andalas University, Padang, West Sumatera, 25163 Indonesia  
²Department of Biology, Mathematics and Life Sciences Faculty, Andalas University, Padang, West Sumatra, 25163 Indonesia  
³Department of Agrotechnology, Agriculture Faculty, Andalas University, Padang, West Sumatra, 25163 Indonesia  
⁴Biotechnology Magister Program, Andalas University, Padang, West Sumatra, Indonesia  
E-mail: jamsari@agr.unand.ac.id

**Abstract.** *Serratia plymuthica* UBCF_13 was a promising bacterium to control plant pathogenic fungal. Availability of this bacterium genome and genome mining approaches has assisted to identify gene-encoded antibiotics. In this study, we identified the biosynthetic gene cluster (BGC) of andrimid and compared it with andrimid BGC from other bacterial strains and species. Andrimid is a hybrid non-ribosomal peptide-polyketide antibiotic that blocks the carboxyl-transfer reaction of bacterial acetyl-CoA carboxylase (ACC) and thereby inhibits fatty acid biosynthesis with submicromolar potency. Andrimid encoded by a gene cluster containing 20 genes, AdmA-T. Understanding the mechanism of synthesis of the non-ribosomal peptide (NRP) and polyketide assembly will assist the effort to manipulate production and increase efficacy of this antibiotic.

**Keywords:** Genome mining, andrimid, antibiotic, *Serratia plymuthica*, comparative genomic

1. Introduction
Microorganisms are an important sources of bioactive compounds and secondary metabolites that are part of important drug formation used for many fields, such as clinic field and to control plant pathogen [1]–[3]. *Serratia plymuthica* UBCF13 and other strains of this bacteria are promising biocontrol agents for pathogens [4]–[7]. It produces several compounds that have the role as biocontrol, including hydrolytic enzymes and antibiotics. In this study, we found andrimid is one of the antibiotics that potent to produce by UBCF_13. Andrimid is a promising candidate antibiotic that interferes with the β-subunit of acetyl-CoA carboxylase (ACC) enzyme through the reaction of carboxyltransferase. The ACC converts acetyl-CoA to malonyl-CoA, the substrate for fatty acid biosynthesis. Besides alpha-linolenic acid metabolism, fatty acid biosynthesis is also a critical pathway associated with lipid metabolism. Therefore, inhibition of ACC prevents cell growth [8]–[10]. Many antibiotics belong to polyketides (PKs) and nonribosomal peptides (NRPs) represent the family. This two large families have diverse structures and complex microbial metabolites that include many therapeutically valuable antibacterial drugs [11].
Andrimid had been isolated from various organisms, such as *Enterobacter sp.* that present in the egg of *Nilaparvata lugens* (brown planthopper) [12], *Pseudomonas fluorescens* [13], and *Serratia proteamaculans* [14]. Andrimid has specific activity against the white blight pathogen of rice plants, *Xanthomonas campestris* pv. *Oryzae* [12], potent in vitro inhibition of methicillin-resistant *Staphylococcus aureus* [13]. Understanding the mechanism of synthesis of non-ribosomal peptide (NRP) and polyketide assembly will assist effort to manipulate production and increase efficacy of this antibiotic. This study will show the comparison between biosynthetic gene clusters of andrimid from different species of bacteria.

2. Materials and methods

2.1. Identification of Andrimid Biosynthetic Gene Cluster (BGC) and Function of The Genes

The Whole genome sequence of *S. plymuthica* UBCF_13 was obtained by whole genome sequencing using Illumina platform and submitted in NCBI (accession number CP068771). A Secondary metabolite genomic-based approach was identified by using antiSMASH [15]. The function of identified andrimid biosynthetic gene cluster was analyzed using BLASTX and Conserved Domains on website of The National Center for Biotechnology Information (NCBI-https://www.ncbi.nlm.nih.gov) [16], [17].

2.2. Comparation of the BGC

The BGCs of andrimid were curated based on information from antiSMASH result. Each of gene in andrimid BGC was BLAST in NCBI. Locus of andrimid BGC from each bacterium was obtained based on BLAST information, therefore the sequences could collect. The multi alignment was obtained by alignment tool in Geneious software (version 2020.2.3, default settings). The phylogenetic construction of the BGCs was performed by MEGA X [18].

3. Results and discussion

3.1. Identification of Andrimid Biosynthetic Gene Cluster and Function of The Genes

Andrimid is a form hybrid assembly line of NRPS/PKS. Genome-based analysis using antiSMASH identified that the BGC of andrimid in UBCF_13 involves 20 genes (*admA-T*) (Figure 1a). Comparative BGC andrimids from different bacteria was shown in Figure 2. By comparing gene function of andrimid from UBCF_13 and *Pantoea agglomerans* that proposed by Jin et al. [19], the role of the genes distinguishes to be four roles (i) iterative type II PKS to form the polyunsaturated fatty acid (green), (ii) formation and insertion of β-phenylalanine (blue), (iii) construction of the succinimide precursor from valine, glycine, and C2 units from 2 equiv of malonyl-CoA (red), and (iv) host resistance and enzyme priming (yellow) (Figure 1b). Further information about the genes functions in the andrimid BGC shows in Table 1.
Figure 1. (a) The cluster of andrimid within the genome of *Serratia plymuthica* UBCF_13, (b) The scheme of andrimid synthesis and proposed biosynthesis of andrimid by Jin et al. (2006) [19]

Modular NRPS and PKS assembly lines are generally composed of multiple enzymes. They are responsible for initiation, elongation and termination of polyketide or peptide chains to produce the core scaffold of natural product [20]. Acylsuccinimide is an essential fragment of andrimid. The experiment indicated that this fragment was derived from a combination of acetate and amino acid building block. Proposed biosynthesis proceed has through a dipeptide-like intermediate formed from ɤ-amino-8-keto acids that are in turn formed from valine and glycine homologated with acetate, presumably via malonyl-CoA [13].

Figure 2. Comparative andrimid BGCs from different bacteria. Phylogenetic tree (left) and structure of andrimid BGCs from different bacteria (right). The colouring shapes are the genes in BGC. Each colour represents different role.
β-Phenylalanine is a constituent of andrimid [21]. The chemical structure of andrimid contains four characteristic subunits, including the β-amino acid (S)-β-phenylalanine, an unsaturated fatty acid chain, an L-valine-derived β-ketoamide moiety, and the pyrrolidinedione head group [22]. Inhibition of bacterial growth by pyrrolidinediones is without targeting the counterpart enzyme in the human host [22]. It makes this antibiotic to be potential broad-spectrum antibiotic with high selectivity for prokaryotic ACC. The first heterologous expression of andrimid was done by Jin et al [19]. Bacterial resistance producing adrimid is obtained by involving a 2-fold strategy of antibiotic efflux and target replacement. Heterologous expression admQ in E. coli strain caused this host to be resistant to andrimid. Based on homology analysis, this gene encode enzyme that has role as purine efflux pump. AdmT encodes the β-subunit of ACC and is likely to be an andrimid-resistant form of this enzyme [19]. Antibiotic producers have to be resistant against to their products to avoid suicide [23].

4. Conclusion
The BGC andrimid within the Serratia plymuthica UBCF_13 genome is composed of 20 genes (admA-T). All of the genes are also constituent andrimid from other species. Not all of the genes have a role in NRP/PKS assembly, some of them have the role as a resistant gene toward andrimid as a mechanism to avoid suicide.

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**Table 1.** Proposed gene function of The Andrimid BGC UBCF\_13

| Gene | Adm- | Number of Amino Acids | Protein sequence homolog | Query cover (%) | Similarity (%) | Proposed Function |
|------|------|-----------------------|--------------------------|-----------------|----------------|-------------------|
| A    | 94   | acyl carrier protein [Erwiniaeae] (WP\_187509966.1) | 100 | 93.62 | Acyl carrier protein |
| B    | 220  | AdmB [Pantoea agglomerans] (AAO39096.1) | 100 | 80.63 | Unknown |
| C    | 249  | SDR family NAD(P)-dependent oxidoreductase (FabG) [Serratia plymuthica] (WP\_062867889.1) | 100 | 99.60 | Beta-Keto acyl carrier protein reductase (BKR), involved in Type II FAS, classical (c) SDRs |
| D    | 734  | beta-ketoacyl-[acyl-carrier-protein] synthase family protein [Serratia plymuthica] (WP\_062867888.1) | 100 | 99.46 | Ketoacyl synthase |
| E    | 110  | (3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase [Serratia plymuthica] (FabZ) (VEA66623.1) | 100 | 89.09 | Dehydratase |
| F    | 274  | transglutaminase domain-containing protein [Serratia plymuthica] (WP\_062867886.1) | 100 | 99.27 | Transglutaminase |
| G    | 131  | hypothetical protein [Serratia plymuthica] (WP\_062867885.1) | 90 | 99.15 | Unknown |
| H    | 541  | phenylalanine aminomutase (D-beta-phenylalanine forming) [Serratia plymuthica] (WP\_062869696.1) | 95 | 100 | Phenylalanine aminomutase |
| I    | 87   | AdmI [Pantoea agglomerans] (AAO39103.1) | 96 | 82.56 | Acyl carrier protein |
| J    | 522  | AMP-binding protein [Serratia plymuthica] (WP\_073999736.1) | 100 | 95.59 | Adenylate forming domain |
| K    | 562  | AMP-binding protein [Erwinia persicina] (WP\_187509976.1) | 100 | 87.72 | The adenylation domain of nonribosomal peptide synthetases (NRPS) |
| L    | 135  | ester cyclase [Erwinia persicina] (WP\_187509977.1) | 100 | 95.56 | |
| M    | 877  | Beta-ketoacyl-acyl-carrier-protein synthase I [Serratia plymuthica] (VE120163.1) | 100 | 89.05 | Ketoacyl synthase |
| N    | 104  | Dabb family protein [Serratia plymuthica] (WP\_126528357.1) | 100 | 87.50 | Stress responsive A/B Barrel Domain |
| O    | 969  | Beta-ketoacyl-acyl-carrier-protein synthase I [Serratia plymuthica] (VEA66611.1) | 100 | 91.33 | Acyl transferase domain in polyketide synthase (PKS) enzymes |
| P    | 574  | AMP-binding protein [Serratia plymuthica] (WP\_062867877.1) | 100 | 98.78 | The adenylation domain of nonribosomal peptide synthetases (NRPS) |
| Q    | 402  | Purine efflux pump PbuE [Serratia plymuthica] (VEA66608.1) | 98 | 96.71 | Transporter |
| R    | 237  | 4'-phosphopantetheinyld transferase superfAMILY protein [Serratia plymuthica] (WP\_062867875.1) | 100 | 99.16 | Phosphopantetheinyl transferase |
| S    | 295  | Transglutaminase domain-containing protein [Burkholderia ubonensis] (WP\_059981348.1) | 93 | 34.77 | Transglutaminase |
| T  | 304 | acetyl-CoA carboxyltransferase subunit beta [Serratia plymuthica (WP_062867873.1)] | 100 | 100 | Acetyl-CoA carboxylase subunit beta |