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In silico analysis of adenylation and ketosynthase domain related to antifungal activity of rice phyllosphere bacteria

Sri Martina Wiraswati[1*], Iman Rusmana[2], Abdjad Asih Nawangsih[3], Aris Tri Wahyudi[2]
[1]Faculty of Biology, Universitas Jenderal Soedirman
Jl. Dr. Soeparno No.63 Karangawangkal Purwokerto, Central Java, Indonesia. 53122
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University
Jl. Agatis Kampus IPB Dramaga, Bogor, West Jawa, Indonesia. 16680
[3]Department of Plant Protection, Faculty of Agriculture, IPB University
Jl. Kamper Kampus IPB Dramaga, Bogor, West Jawa, Indonesia. 16680
*Email: sri.martina@unsoed.ac.id

ABSTRACT. Adenylation (A) and ketosynthase (KS) are domain of non-ribosomal peptide synthase (NRPS) and Polyketide synthases (PKS) gene cluster which involved in bioactive compounds synthesis in microbes. The previous study, A and KS domain were amplified from four rice phyllosphere bacteria (isolate STGG 3, STGG 7, STGG 14 and SKBV 1) which actively displayed inhibition activities toward *Pyricularia oryzae* race 173. The present study aims to determine the active site of amplified A and KS domain related to antifungal activity from four isolates STGG 3, STGG 7, STGG 14 and SKBV 1**.** Analysis of active site determination were conducted by aligning the amino acid sequences of A dan KS domain using MEGA 11 program. The alignment process used amino acid sequence of gramicidin (Grs A) as template of A domain. Meanwhile, the alignment of KS domain used amino acid sequence of KS domain from *Bacillus, Pseudomonas, and Streptomyces maritimus* as template. According to the alignment results, the active site (amino acid marker) of A domain and KS domain were detected as DAKDLGVV and DTVCSSS, respectively. The similarity of KS and A domain active site of rice phyllosphere bacteria indicates the similarity of substrates and enzyme activity. Meanwhile, the differences of amino acid sequences of A domain especially in motifs A5 and A6 from bacterial isolate STGG 3 dan SKBV 1 are predicted of having an effect toward substrate adenylation activity of A domain from both bacterial isolates.

Keywords: adenylation; antifungal; ketosynthase; rice phyllosphere bacteria; *Bacillus* sp.

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INTRODUCTION

Secondary metabolites from many microorganisms possessed several biological activities which useful for many areas. According to the biosynthetic derivation, secondary metabolites are classified in to four classes: polyketides (PKs), non-ribosomal peptides (NRPS), terpenes and indole alkaloid (Courtial *et al*., 2022). Diverse secondary metabolites which belong to polyketides as well as non-ribosomal peptides compounds were produced by nature through acetate building block and amino acid assembly into polyketide and peptides compounds, respectively (Fisch, 2013). The polyketides and non-ribosomal peptides group are well known as remarkable natural product with abundant of biological activity and diversity in structure (Tambadou *et al*., 2014). Non-ribosomal peptides compounds have been being explored as toxins, siderophores, pigments, antibiotics, cytostatic, immunosuppressants or anticancer agents (Martínez-Núñez & López, 2016). Meanwhile polyketide compounds possess bioactivities as antibiotic, antifungal, antiparasitic and antitumor (Arguelles Arias *et al*., 2011) have been widely developed in pharmaceutical industry.

The large group of polyketides and non-ribosomal peptides were synthesized by two mega-enzymes, non-ribosomal peptides synthetase (NRPS) and polyketides synthases (PKS) (Fisch, 2013). Polyketides synthases are modular enzymes with consecutive activities in the biosynthesis of bioactive compounds. The biosynthesis process are facilitated by the enzymes through the use of combinations of specific catalytic domains (Selvin et al. 2016). NRPS consist of multi-modular enzymes which catalyses each step of polypeptide chain elongation and modification to derive non-ribosomal peptides (Arguelles Arias *et al*. 2011). One NRPS module comprises at least three domains

which activate each step of non-ribosomal peptides synthesis individually (Martínez-Núñez & López, 2016). First domain, namely Adenylation (A) domain have a role in recognizing and activating amino acid residue. The activated amino acid were then transferred to peptidyl carrier protein (PCP) domain and later condensed by condensation (C) domain to form amine bond (Strieker *et al*., 2010). Another domain namely thioesterase (TE) commonly terminate the amino acid chain elongation (Tambadou *et al*., 2014).

According to the distinctive of catalytic domain, PKS are commonly divided into three types: type I, II and III. Type I and II PKS use acyl carrier protein (ACP) as catalytic domain even though the model and structure are different. Meanwhile, type III PKS which well known as chalcone synthase like PKS do not use ACP as catalytic domain (Lim *et al*., 2016; Pandith *et al*., 2020; Neves *et al*., 2022). Every module of PKS are mostly comprises both acyl carrier protein (ACP) and β-keto synthase (KS) domain in the same module which together catalyze polyketide chain elongation. Furthermore, both domain are subsequently adhere to KS domain from the next module in order to assist polyketide chain transfer (Kapur *et al*., 2010).

The existence of NRPS and PKS enzymes have been reported in the three domains of life, bacteria, eukaryote and archaea which are very common in bacteria and rare in archaea. Among bacteria domain, both enzymes abundantly find in Proteobacteria (Timmermans *et al*., 2017; Rego *et al*., 2020), Actinobacteria (Amin *et al*., 2020), Firmicutes (Aleti *et al*., 2015), and Cyanobacteria phyla and moreover, the correlation between genome size and the number of NRPS's clusters have been investigated (Wang *et al*., 2014). In most cases, bacterial NRPS modules tend to be located at smaller subunit proteins which incorporate to a larger multi-enzyme system (Winn *et al*., 2016).

In previous research, we successfully obtained four rice phyllosphere bacteria isolates with antifungal activity against *P. oryzae* race 173 both in vitro and in planta (Wiraswati *et al*., 2019; 2020). The four isolates have been identified as *Bacillus* sp. STGG 3, *B. subtilis* STGG 7, *B. subtilis* subsp. *subtilis* STGG 14 and *B. subtilis* SKBV 1. As evidence of their antifungal activities, the genes encoding the A and KS domains of the NRPS and PKS megaenzymes were successfully amplified from the four isolates. *Bacillus* sp. STGG 3, *B. subtilis* STGG 7, *B. subtilis* subsp. *subtilis* STGG 14 were proven to have A and KS domains, while *B. subtilis* SKBV 1 isolate only have A domain. According to the similarity analysis, A and KS domains of the four isolates are similar to the A and KS domains of *Bacillus* sp. and *B. subtilis* (Wiraswati *et al*., 2019). To find out the difference in function of adenylation and keto synthase domain from the bacterial isolate, *in silico* analysis is conducted toward amino acid sequence from A and KS domain. The *in silico* analysis targeted active site of amino acid sequence of KS domain and conserved core motif sequence of amino acid from A domain. The prediction of A and KS domain activities from PKSs and NRPSs could be used to predict the resulted bioactive compounds which usually beneficial to many aspects of human life such as agriculture, environment, as well as human health.

MATERIALS AND METHODS

The amino acid sequences of A domain from the four potential isolates and A domain protein sequence of *Commamonas* sp. (AHG59387.1), *Pseudomonas* sp. (WP_131178002.1), and *Streptomyces* sp. (CCF223455.1) from NCBI database were each aligned using MEGA 7 program. In addition, the A domain sequence of gene encoding the gramicidin (GrsA) from *B. brevis* (1AMU_A) was used as a template to determine the active site of A domain from potential rice phyllosphere bacteria (isolates: STGG 3, STGG 7, STGG 14 and SKBV 1) (Figure 1). The active site of the domain is located at amino acids 235, 236, 239, 278, 299, 301, 322 and 330 of the A domain in GrsA. Through this analysis, we also obtained motifs A1 to A10 of A domain from the results of the amino acid sequences alignment (Stachelhaus *et al*. 1999). The alignment results were next observed whether there are differences amino acids motifs of the A domain A from potential isolates in order to identify distinction activity of A domain.

The amino acid sequences of KS domain from the three potential isolates were also aligned with the KS domain of the *B. subtilis* (SAJ35050.1), *B. subtilis* KCTC 1028 (AKC47275.1), *B. subtilis* subsp. *subtilis* strain 168 (NP_389600), *B. subtilis* subsp. *subtilis* strain AG1839 (AIC44368.1), *S. maritimus* (AAF81729.1) and *Pseudomonas* sp. (BAQ74664.1) from the NCBI database. The presence of amino acid which have a role as active site were further determined from alignment results. The active side residues of KS domain are characterized by DTXCSSS amino acid sequence that are conserved both in Gram positive and Gram negative bacteria.

RESULTS AND DISCUSSION

Alignment of A domain from isolates STGG 3, STGG 7, STGG 14 and SKBV 1 A domain of Gramicidin (Grs A) as template showed that all isolates had the same 8 amino acids markers i.e. Asp235, Ala236, Lys239, Asp278, Leu299, Gly301, Val322, and Val330 (Table 1). They are located in certain sequences of A domain which have a role in determining the specificity, nature and type of A domain's substrate. Asp235 and Lys517 are two amino acids that are mostly conserved in A domain from microbes and other organisms (Di Vincenzo *et al*. 2005). According to this finding, A domain from STGG 3, STGG 7, STGG 14 and SKBV 1 isolates were sharing similar substrate in synthesizing nonribosomal peptide compounds. This finding also proved that there are no mutations occurred in the active site of A domain, so that the substrate will be perfectly attach along non ribosomal peptide compounds synthesis.

Table 1. Amino acid residue indicated active site and substrate specificity of adenylation domain from four rice phyllosphere bacteria

Bacterial isolates	Amino acid position							
	235	236	239	278	299	301	322	330
STGG 14	D	A	K	D	L	G	V	V
STGG 3	D	A	K	D	L	G	V	V
STGG 7	D	A	K	D	L	G	V	V
SKBV 1	D	A	K	D	L	G	V	V

According to KS domain alignment results, amino acid sequence of KS domain from STGG 14, STGG 3 and STGG 7 were identical (Fig. 2). The identical amino acid sequence of KS domain indicates similar activity of the ketosynthase enzyme which involved in polyketide compounds synthesis. The amino acid marker of KS domain active site i.e. DTVCSSS were also successfully determined from three bacterial isolates which are equal to the active site of other B. subtilis isolates from the NCBI database. Other conserved core motifs from KS domain were not detected in the alignment results because the limitation of primer which not amplify KS domain from N-terminal to C-terminal.

In contrast to the KS domain, A domain from isolates STGG 3, STGG 7, STGG 14 and SKBV 1 showed different amino acid sequences both in the conserved core and outside the conserved core motif. The results indicated that A domain of the rice phyllosphere bacterial isolates were thought to have different levels of enzymatic activity. Determination of conserved core motifs of A domain was successfully detected conserved motif A3 to A7 but not A8 to A10 motifs. This is because the MTF and MTR primers which are used to amplify the A domain are specifically designed to cover only A2 to A8 motifs in A domain (Tambadou et al., 2014). Among the five detected motifs, A domain from STGG 3 isolate showed differences in the 188th amino acid, namely asparagine (N) → lysine (K) which is part of the conserved core motif A5. In addition, A domain from SKBV 1 isolate also showed differences in the 251st amino acid, namely arginine (R) → glutamine (Q) which is part of the conserved core of the A6 motif (Fig. 1).

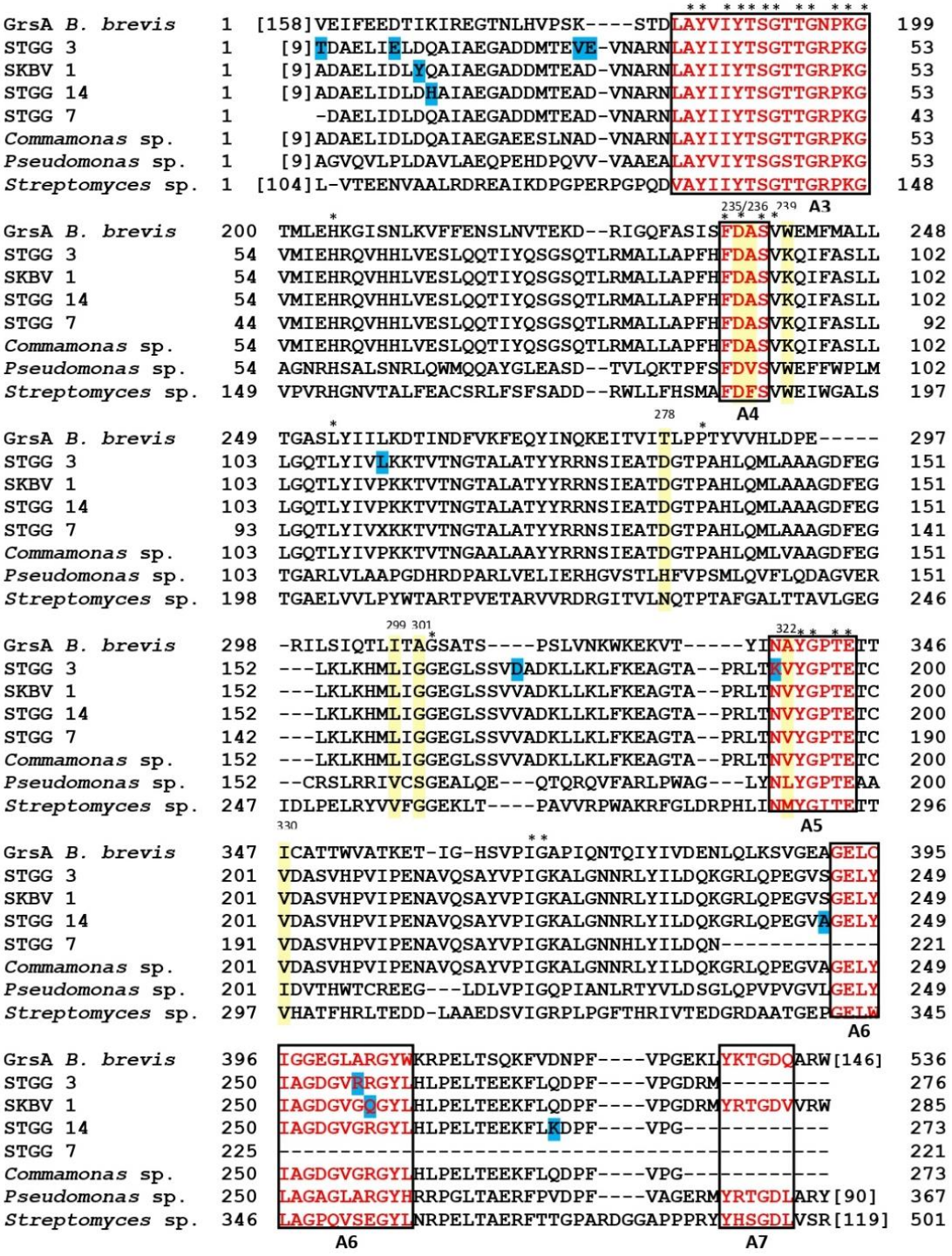

Fig. 1. Amino acid sequence alignment of adenylation domain among rice phyllosphere bacteria and *Commamonas* sp., *Pseudomonas* sp., *Streptomyces* sp. which are using GrsA template. Yellow background colour indicates amino acid residue of active site and substrate specificity of A domain. The black boxes indicate amino acid motif of A domain. Blue background colours show different amino acid sequences among rice phyllosphere bacteria.

The differences of amino acid sequences in conserved core motifs A5 and A6 of A domain from STGG 3 and SKBV 1 isolates were thought to affect substrate adenylation activity by A domain. According to Marahiel *et al*. (1997), the motifs of A domain display important role in ATP binding, hydrolysis and adenylation of amino acid substrates. The activation and adenylation process of amino acids in A domain depends on the presence of Mg2+-ATP, so the ability of A domain to bind ATP is important to the presence of specific substrates. Therefore, the distinction A5 and A6 motifs of A

domain from STGG 3 and SKBV 1 isolates were expected to affect the ability of ATP binding activity by STGG 3 and SKBV 1 isolates.

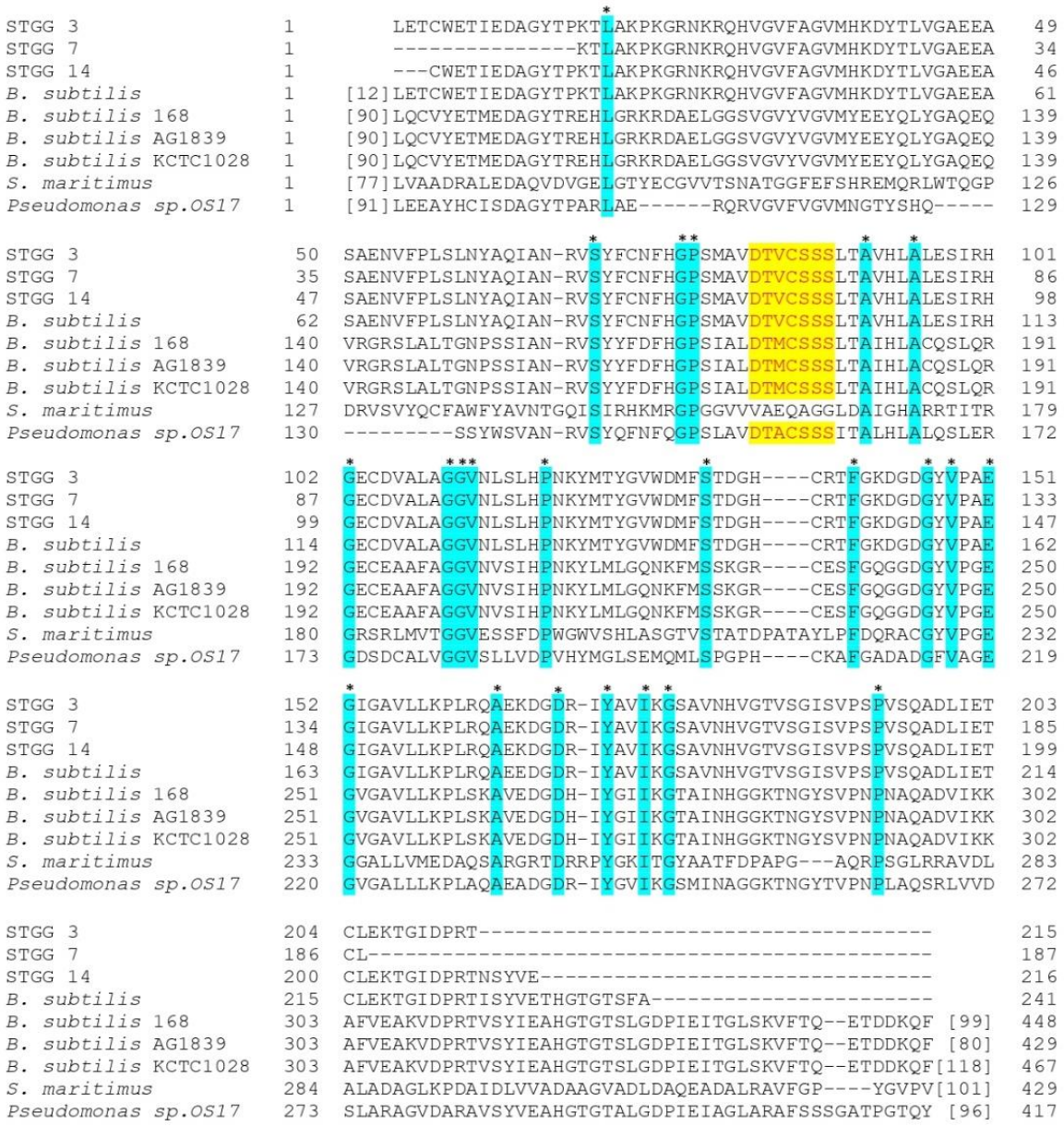

Fig. 2. Amino acid sequences alignment of ketosynthase domain from three rice phyllosphere bacteria (STGG 3, STGG 7 and STGG 14), *Bacillus* sp., *S. maritimus* and *Pseudomonas* sp.. Yellow background colour indicates amino acid residue of KS domain active site. Blue background colour indicated identical amino acid from all aligned sequence of each bacteria

The disturbance of ATP binding process results in the inhibition of antifungal compounds synthesis that play important role in suppressing the growth of P. oryzae race 173. Meanwhile, the difference amino acids outside the conserved core motifs A5 and A6 of STGG 14 isolates were predicted to have no effect on the ATP binding activity of A domain. The process of synthesizing non ribosomal peptide compounds that are antifungal can occur in STGG 14 isolates so that this isolate has the highest antifungal activity compared to other isolates.

CONCLUSION

KS domain from all isolates are predicted having similar activity in peptide compounds synthesis and conserved core motif A3 – A7 is successfully identified from amino acid sequences of A domain. In addition, A domain from STGG 3 and SKBV 1 is predicted having different activity in ATP binding, hydrolysis and substrate adenylation. Furthermore, all bacterial isolates showed similar substrate specificity amino acid. Prediction of enzymes structure and their effect toward enzymes activities need to be conducted in further research.

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REFERENCES

Aleti G, Sessitsch A, Brader G. 2015. Genome mining: prediction of lipopeptides and polyketides from Bacillus and related Firmicutes. *Computational and Structural Biotechnology Journal*. vol 13: 192-203. doi: https://doi.org/10.1016/j.csbj.2015.03.003.

Amin DH, Abdallah NA, Abolmaaty A, Tolba S, Wellington EM. 2020. Microbiological and molecular insights on rare Actinobacteria harboring bioactive prospective. *Bulletin of the National Research Centre*. vol 44(1): 1-12. doi: https://doi.org/10.1186/s42269-019-0266-8.

Arguelles Arias, A., Craig, M. & Fickers, P., 2011. Gram-positive antibiotic biosynthetic clusters: a review. *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*, pp.977–986.

Courtial, J. et al., 2022. Characterization of NRPS and PKS genes involved in the biosynthesis of SMs in Alternaria dauci including the phytotoxic polyketide aldaulactone. *Scientific Reports*, 12(1), pp.1–20. doi: 10.1038/s41598-022-11896-0.

Fisch, K.M., 2013. Biosynthesis of natural products by microbial iterative hybrid PKS-NRPS. *RSC Advances*, 3(40), pp.18228–18247. doi: 10.1039/c3ra42661k.

Kapur, S. et al., 2010. Molecular recognition between ketosynthase and acyl carrier protein domains of the 6-deoxyerythronolide B synthase. *Proceedings of the National Academy of Sciences of the United States of America*, 107(51): 22066–22071. doi: 10.1073/pnas.1014081107.

Lim YP, Go MK, Yew WS. 2016. Exploiting the biosynthetic potential of type III polyketide synthases. *Molecules.* vol 21(6): 1-37. doi: https://doi.org/10.3390/molecules21060806.

Marahiel, M.A., Stachelhaus, T. & Mootz, H.D., 1997. Modular peptide synthetases involved in nonribosomal peptide synthesis. *Chemical Reviews*, 97(7), pp.2651–2673. doi: 10.1021/cr960029e.

Martínez-Núñez, M.A. & López, V.E.L. y, 2016. Nonribosomal peptides synthetases and their applications in industry. *Sustainable Chemical Processes*, 4(1), pp.1–8. doi: 10.1186/s40508-016-0057-6.

Neves RPP, Ferreira P, Medina FE, Paiva P, Sousa JPM, Viegas MF, Fernandes PA, Ramos MJ. 2022. Engineering of PKS Megaenzymes—A Promising Way to Biosynthesize High-Value Active Molecules. *Topics in Catalysis*. vol 65: 544–562. doi: https://doi.org/10.1007/s11244-021-01490-5.

Pandith SA, Ramazan S, Khan MI, Reshi ZA, Shah MA. 2020. Chalcone synthases (CHSs): the symbolic type III polyketide synthases. *Planta.* vol 251: 1-29. doi: https://doi.org/10.1007/s00425-019-03307-y.

Rego A, Sousa AG, Santos JP, Pascoal F, Canário J, Leão PN, Magalhães C. 2020. Diversity of bacterial biosynthetic genes in maritime antarctica. *Microorganisms*. vol 8(2): 1-36. doi: https://doi.org/10.3390/microorganisms8020279.

Selvin, J. et al., 2016. Ketide Synthase (KS) domain prediction and analysis of iterative type II PKS gene in marine sponge-associated actinobacteria producing biosurfactants and antimicrobial agents. *Frontiers in Microbiology*, 7(FEB), pp.1–12. doi: 10.3389/fmicb.2016.00063.

Stachelhaus, T., Mootz, H.D. & Marahiel, M.A., 1999. The specificity-conferring code of adenylation domains in nonribosomal peptide synthetases. *Chemistry and Biology*, 6(8), pp.493–505. doi: 10.1016/S1074-5521(99)80082-9.

Strieker, M., Tanović, A. & Marahiel, M.A., 2010. Nonribosomal peptide synthetases: Structures and dynamics. *Current Opinion in Structural Biology*, 20(2), pp.234–240. doi: 10.1016/j.sbi.2010.01.009.

Tambadou, F. et al., 2014. Novel nonribosomal peptide synthetase (NRPS) genes sequenced from intertidal mudflat bacteria. *FEMS Microbiology Letters*, 357(2), pp.123–130. doi: 10.1111/1574-6968.12532.

Timmermans ML, Paudel YP, Ross AC. 2017. Investigating the biosynthesis of natural products from marine Proteobacteria: a survey of molecules and strategies. *Marine Drugs*. vol 15(8): 1-36. doi: https://doi.org/10.3390/md15080235.

Di Vincenzo, L., Grgurina, I. & Pascarella, S., 2005. *In silico* analysis of the adenylation domains of the freestanding enzymes belonging to the eucaryotic nonribosomal peptide synthetase-like family. *FEBS Journal*, 272(4), pp.929–941. doi: 10.1111/j.1742-4658.2004.04522.x.

Wang, H. et al., 2014. Atlas of nonribosomal peptide and polyketide biosynthetic pathways reveals common occurrence of nonmodular enzymes. *Proceedings of the National Academy of Sciences of the United States of America*, 111(25), pp.9259–9264. doi: 10.1073/pnas.1401734111.

Winn, M. et al., 2016. Recent advances in engineering nonribosomal peptide assembly lines. *Natural Product Reports*, 33(2), pp.317–347. doi: 10.1039/c5np00099h.