POTENCY OF ANTICANCER COMPOUNDS FROM THE InaCC LIPI COLLECTION *Actinomycetes* WERE ANALYZED USING antiSMASH

Annisa Khumaira[1)] Tsania Taskia Nabila[2)]
[1,2)] Biotechnology Study Program, Science and Technology Faculty, Universitas 'Aisyiyah Yogyakarta
email: annisakhumaira@unisayogya.ac.id

Abstract

Every year cancer cases have always increased. According to WHO data, in 2020, there were 19.2 million cases of cancer. Cancer treatment is still developing by exploring medicinal compounds that have a minimal risk to healthy cells but can still kill cancer cells. Secondary metabolites from the phylum Actinomycetes are often explored to determine their usefulness, one of which is an anticancer compound. The InaCC project from LIPI has several collections of Actinomycetes that have never been explored, mainly novel species obtained in the 2011-2016 timeframe. The purpose of this study was to analyze the genomic sequences of the InaCC Actinomycetes collection using antiSMASH to determine the potential of the compounds for anticancer. First, the genome sequence data search was carried out through the NCBI gene bank, then analyzed the sequences using antiSMASH 5.0, after searching for the compounds' potential through publication search using the ChEMBL Database and Google Scholar. In the NCBI database, all bacterial phylum Actinomycetes InaCC 2011-2016 have not found any genomic sequence data to use bacteria with the closest relationship for antiSMASH analysis. The closest relatives' analysis results showed that BGC compounds that can have anticancer properties in the bacterial collection of the phylum Actinomycetes InaCC 2011-2016 include terpenes butyrolactol, furans, alkyresorcinol, desferroxamine, and siderophore. These results indicate that the phylum Actinomycetes bacteria could have BGC anticancer compounds and could be developed to treat cancer. The analysis of this research needs to be confirmed again if each bacterium's genomic sequence is complete.

Keywords*: anticancer compounds, Actinomycetes, InaCC LIPI 2011-2016, antiSMASH*

1. INTRODUCTION

The World Health Organization (WHO) states that 7.6 million people died from cancer in 2005, and cancer cases increased to 19.2 million in 2020, with a death rate of 9.9 million (GLOBOCAN, 2020). The increase in cancer cases from year to year is influenced by several factors such as unhealthy food intake, hormonal fluctuations, physical activity, smoking, and others (Grosso et al., 2017). Due to the increasing number of cases, it is necessary to have an effective treatment and have a high efficacy to prevent death from cancer cases.

Cancer treatment is still developing by exploring various medicinal compounds that have a minimal risk to healthy cells but can still kill cancer cells. Various components of the compound were isolated, discovered, and tried to be explored in vitro in cancer cells, one of which was the compound obtained from bacterial metabolism, both primary and secondary metabolism. Bacteria that could produce cancer-fighting biomolecules include those included in the phylum *Actinomycetes* genus *Streptomyces*, genus *Nonomureae*, genus *Halomonas*, genus *Sulfitobacter* (Girao et al., 2019; Nakaew et al., 2009; Sagar et al., 2013). Besides, some bacteria are used in fighting cancer, not from their biomolecules but also their life cycle systems, such as the *Clostridium* bacteria's spores (Heap et al., 2014). Bacteria that can produce cancer-fighting biomolecules are isolated from various environmental ecosystems, including deep seas, caves, rivers, and mangrove forest areas (Sagar et al., 2013; Nakaew et al., 2009; Ramirez-Rodriguez et al., 2018; Chen et al., 2018).

Indonesia's diverse landscapes make it possible to discover many new *Actinomycetes* species from various ecosystems. LIPI (Lembaga Ilmu Pengetahuan Indonesia), as a research institute under the Indonesian government,

collects *Actinomycetes* found in the Indonesian region, various Actinomycetes were collected from 2001 to 2016. In the 2011-2016 period, LIPI recorded several *Actinomycetes* novels through the InaCC SATREPS Project (Indonesian Culture Collection Partnership for Sustainable Development), These species have never been explored to determine their biomolecular potential (Ratnakomala et al., 2016).

antiSMASH (antibiotics and Secondary Metabolites Analysis Shell) is an open-access tool on the internet that could be used for rapid identification, annotation, and analysis of secondary metabolites in bacteria and fungi' genomes. antiSMASH also uses several open sources tools such as NCBI BLAST, HMMer 3, Muscle 3, FastTree, PySVG, and JQuery SVG. antiSMASH currently used is version 5.0 with more detailed predictive capabilities and has added several detection clusters (Blink et al., 2019). This article will discuss the anticancer compounds in secondary metabolites from the LIPI *Actinomycetes* collection from 2011-2016, which were analyzed using antiSMASH 5.0.

2. MATERIALS AND METHODS

Genome Sequence Data Mining

NCBI (National Center for Biotechnology Information) is one of the open-access gene banks that could be accessed to obtain bacterial genome sequences. NCBI gene bank could be accessed via https://www.ncbi.nlm.nih.gov/genbank/. InaCC LIPI 2011-2016 data for the novel *Actinomycetes* could be seen in table 1.

Table 1. Novel Taxa Discovered during Exploration of Actinomycetes In Indonesia (Ratnakomala et al., 2016)

Family	Species	Similarity with the closest species	References
Pseudonocardiaceae	*Actinophytocola timorensis* sp.nov	A.*oryzae* GMKU367 (98,1%)	Otogura et al., 2011
	Actinophytocola corallina sp.nov	A.*oryzae* GMKU367 (98,2%)	
Cellulomonadaceae	*Tropicihabitans flavus gen* sp. Nov	*Sediminihabitans luteus* (97,94%)	Hamada et al., 2015a
Beutenbergiaceae	*Serinibacter tropicus* sp.nov	*Serinibacter salmoneus* Kis4-28 (99,6%)	Hamada et al., 2015b
Cryptosporangiaceae	*Cryptosporangium cibdasense* sp nov	C. *minutisporangium* NBRC15962	Nurkanto et al 2016b
Actinoplanaceae	*Actinoplanes tropicalis* sp nov	A.*ferugineus* NBRC15555 (98,2%)	Nurkanto et al., 2015
	Actinoplanes cibodanensis sp nov	A.*durhamensis* JCM7625 (97,7%)	
	Actinoplanes bogoriensis sp nov	*Actinoplanes abujensis* A4029 (99,3%)	Nurkanto et al., 2016

antiSMASH Analysis

The genomic data of each species obtained from the NCBI gene bank was then inserted into antiSMASH 5.0. Open the antiSMASH web https://antismash.secondarymetabolites.org/#!/start then enter the genome's reference number, or it could be fasta data, GenBank, or EMBL. After that, it will be analyzed by antiSMASH. At the top, the user will be asked for an email address that will be used to send the analysis results. The result is an overview of the gene region and BCG (Biosynthetic Gene Cluster) predictions.

Search for Potential Compounds against Cancer Cells

After knowing the BGC of each species, the potential of these compounds is searched. Potential searches are carried out by searching for data through the ChEMBL Database https://www.ebi.ac.uk/chembl/ and Google Scholar. From there it will be known publications on the potential use of compounds against cancer.

3. RESULTS AND DISCUSSIONS

Genome Sequence Data Mining

From the data of InaCC Ratnakomala et al., 2016, displayed in table 1, it searched on NCBI to get the genome reference number that will be entered into antiSMASH. All species shown in table 1 are novel *Actinomycetes*, so that after searching at NCBI, no genes or bacterial genomes of these species have been found. The alternative is to use an available bacterial genome with the closest similarity level. The genome obtained from its relatives is also in the incomplete genome category. The gene data used are shown in table 2.

Table 2. Reference Sequence Number

Species	Similarity with the closest species in NCBI data	Reference	Reference Sequence Number
Actinophytocola timorensis sp.nov	*Actinophytocola oryzae* (98,1%)	Otogura et al. 2011	NZ_SOCP00000000.1
Actinophytocola corallina sp.nov			
Tropicihabitans flavus gen sp. Nov	*Sediminahabitans luteus* (97,94%)	Hamada et al., 2015a	NZ_PGFE00000000.1
Serinibacter tropicus sp.nov	*Serinibacter salmoneus* (99,6%)	Hamada et al., 2015b	NZ_PDJD00000000.1
Cryptosporangium cibdasense sp nov	*Cryptosporangium arvum* (98,69%)	Nurkanto et al., 2015	NZ_JFBT00000000.1
Actinoplanes bogoriensis sp nov	*Actinoplanes abujensis* A4029 (99,3%)	Nurkanto et al., 2016	NZ_JACHMF000000000.1

The sequences in NCBI for each species are not complete, so that only part of it is obtained.

AntiSMASH analysis

antiSMASH could directly analyze from the existing database, or the user can input data into antiSMASH. The results obtained were in gene region, BGC, the similarity of BGC with a compound, and others. The results of the antiSMASH analysis could be seen in table 3.

Search for Potential Compounds against Cancer Cells

Apart from obtaining BGC data from antiSMASH, data on the similarity of gene regions with other compounds were also obtained. In this case, it will be searched in the ChEMBL, and Google Scholar databases, namely BGC and other compounds with similarity above 50% that have been published have potential as anticancer. The results of the search can be seen in table 4. The compounds in table 4 have the structure BGC and are shown in table 5 and DNA sequence shown in supplemantary data.

After investigating, not all BGCs were reported to have anticancer potential. Table 4 shows some BGCs reported to have anticancer potential. In the *Actinophytocola oryzae* species, there is a BGC terpene studied by Li et al., 2017 to have anti-proliferative potential in gastric cancer cell culture. The tarpaulin used is a coumarin monoterpene synthesis which is biotransformed by *Mucor polymorphosporus*. Even though there are many types of terpenes, it is possible that the

terpenes from the InaCC *Actinomycetes* collection have almost the same potential. In table 3 it can be seen that BGC terpenes were found in all species analyzed using antiSMASH so that it is possible that all species of the phylum *Actinomycetes* InaCC 2011-2016 have the potential to synthesize terpenes.

Table 3. Number of Region and BGC

Species	**Number of Region**	**Type of BGC**
Actinophytocola oryzae	44	amglyccyl, arylpolyene, betalactone, ectoine, hgIE-KS, indole, ladderane, lanthipeptide-class-i, lanthipeptide-class-ii, lanthipeptide-class-iii, lanthipeptide-class-iv, LAP, lassopeptide, NAPAA, NRPS, NRPS-like, PKS-like, redox-cofactor. RiPP-like, RRE-containing, siderophore, T1PKS, T2PKS, T3PKS, terpen, thioamide-NRP, thiomitides
Sediminahabitans luteus	5	furan, hgIE-KS, NAPAA, siderophore, T3PKS, terpen,
Serinibacter salmoneus	3	NRPS, T3PKS, terpen
Cryptosporangium arvum	18	betalactone, NRPS, NRPS-like, ranthipeptide, redox-cofactor, RiPP-like, RRE-containing, siderophore, T1PKS, T3PKS, terpen, thiomitide
Actinoplanes abujensis	13	betalactone, LAP, NAPAA, NRPS, NRPS-like, RiPP-like, siderophore, T1PKS, T3PKS, terpen, thioamide

Butyrolactol in *Actinophytocola oryzae*, according to Singh et al., 2019, has a fairly good cytotoxic effect on MOLT-4 leukemia cell culture and can cause apoptotic bodies. This butyrolactol in table 5 shows that it has the terpene, T1PKS, and NRPS-like BGC regions where the three BGCs have 80% similarity to butyrolactol. From the analysis result allows these species to produce butyrolactol if further investigation is carried out.

Sediminahabitans luteus has BGC furan reported by Costa et al., 2018 to have a cytotoxic effect on Caco-2 colorectal cancer cell culture and could inhibit topoisomerase I and IIα. Furan is an aromatic compound, some of which have an aromatic structure. Not all of the species tested using antiSMASH had BGC furan; only *Sediminahabitans luteus* had furan, so from the InaCC collection, the possibility of producing furans could be seen in table 1 is *Tropicihabitans flavus* sp. nov.

S. *luteus* also has alkyresorcinal 100% similarity to BGC T3PKS (table 5), as well as *Cryptosporangium arvum*. Alkylresorcinol reported by Kruk et al., 2017 can reduce colon cancer and endometrial cancer and inhibit the growth of human colon, breast, lung, central, nervous system, adenocarcinoma, hepatocarcinoma, cervix squamous carcinoma, and ovarian cancer cell lines. Alkylresorcinol is commonly found in cereals such as wheat, rye, barley, and triticale (Kruk et al., 2017). If it can be produced from microorganisms, it can be produced in large quantities and made in medicinal products or supplements to be given to people with cancer.

Desferorroxamine has only a 50% similarity with the BGC of the siderophore (table 5). Still, here desferroxamine is an interesting compound because it is one of the iron chelators compounds or iron carriers and the siderophore. Chelator iron suppresses cancer cells' growth by depleting iron or causing oxidative stress due to redox disturbances in cells or the environment because iron is an essential element (Buss et al., 2003). One publication was written by

Wang et al., 2019 iron chelator desferroxamine combined with cisplatin can inhibit the growth of ovarian cancer and siderophore reported by Saha et al., 2019 *Enterobacter* is a cathecolate siderophore bacteria that can suppress the proliferation of RAW264.7 and J774A.1 cell line, which is a culture of leukemia cells and reticulum sarcoma. Besides, siderophore can increase ROS (Reactive Oxygen Species) so that it can induce apoptosis. The five species tested using antiSMASH, only *Serinibacter salmoneus* did not have siderophore BGC, so it can be seen in table 2 of the 2011-2016 InaCC collection that only *Serinibacter tropicus* sp. now which does not have the potential to have BGC siderophore.

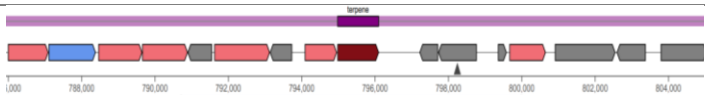
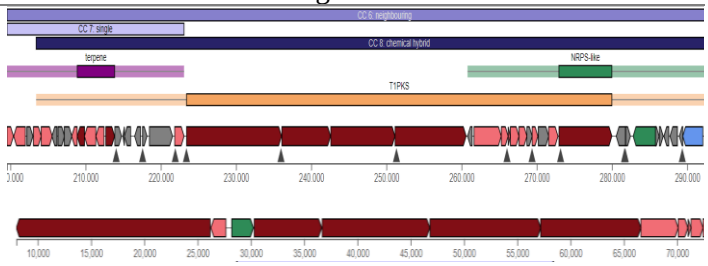
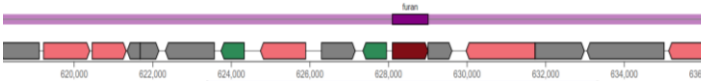
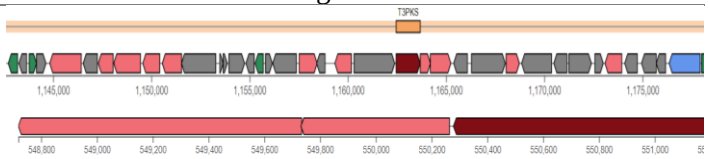
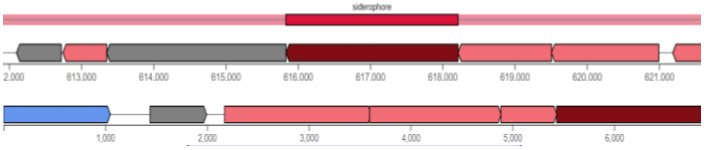
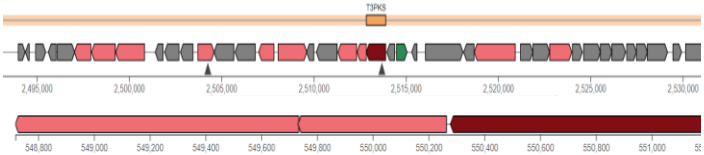
Table 4. Anticancer Potency Compound

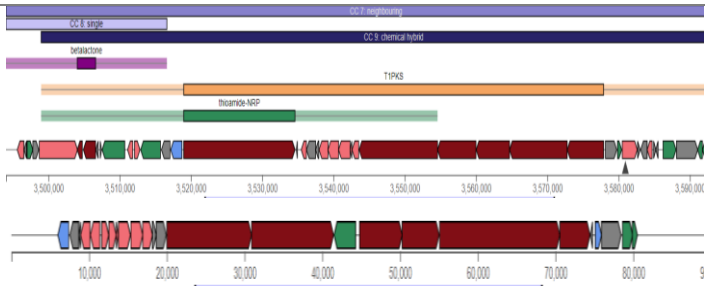
Species	**BGC or Another Compound with Similarity**	**Anticancer Potency**	**Reference**
Actinophytocola oryzae	terpene	apoptosis induction in gastric cancer cell culture MGC-803	Li et al., 2017
	butyrolactol 80%	showed a cytotoxic effect with IC50 15µg / mL, induced to apoptotic bodies in MOLT-4 cell culture (leukemia)	Singh et al., 2019
Sediminahabitans luteus	furan	Potency of cytotoxic effect on Caco-2 cells (colorectal cancer), in silico results show it can block topoisomerase I and IIα	Costa et al., 2018
	alkyresorcinol 100%	- could reduce colon cancer and endometrial cancer - could inhibit human colon, breast, lung, central, nervous system, adenocarcinoma, hepatocarcinoma, cervix squamous carcinoma, and ovarian cancer cell lines	Kruk et al., 2017
	desferroxamine	iron chelator that could be combined with the chemotherapy drug cisplatin to inhibit ovarian cancer	Wang et al., 2019
Cryptosporangium arvum	alkylresorcinol 100%	- could reduce colon cancer and endometrial cancer - could inhibit human colon, breast, lung, central, nervous system, adenocarcinoma, hepatocarcinoma, cervix squamous carcinoma, and ovarian cancer cell lines	Kruk et al., 2017
Actinoplanes abujensis	scheliprolactam 52%	a compound type prolacatam could be used as nanogels for breast cancer drug delivery	Rejinold et al., 2015

There is an interesting compound in the *Actinoplanes abujensis* species, namely sceliprolactam with a similarity of 52% in BGC beta lactone (table 5), T1PKS, and

thioamide-NRP. The prolactam group Rejinolds et al., 2017 can be used for drug delivery in the form of nanogels, in this case, Reynolds et al., 2017 using N-vinyl Caprolactam. Reynolds et al., Used it to deliver 5-fluorouracil and megestrol acetate. In vitro tests on breast cancer cell cultures showed cytotoxic effect and induced apoptosis, while in vivo tests, the drug was released within three days. There have been no publications regarding sceliprolactam in its potential to help suppress cancer, but these publications suggest it may have the same potential.

Table 5. Genetic BGC Structure

Species	BGC or Another Compound with Similarity	Genetic BGC Structure
Actinophytocola oryzae	Terpene	 Region 1.3
	Terpene T1PKS NRPS-like butyrolactol 80%	 Region 2.3 GenBank NZ_BBOK01000014.1.
Sediminahabitans luteus	Furan	 Region 1.1
	T3PKS, alkyresorcinol 100%	 Region 1.3 GenBank AP009493.1.
	siderophore, desferroxamine 50%	 Region 2.1 GenBank LT989883.1.
Cryptosporangium arvum	T3PKS alkylresorcinol 100%	 Region 4 GenBank AP009493.1.

Species	BGC or Another Compound with Similarity	Genetic BGC Structure
Actinoplanes abujensis	betalactone T1PKS thioamide-NRP, scheliprolactam 52%	 Region 7 GenBank KX230849.1.

4. CONCLUSIONS

The LIPI InaCC collection of phylum *Actinomycetes* bacteria could produce or synthesize compounds that have anticancer potential. Among the five species in the collection of InaCC in 2011-2016, *Serinibacter tropicus* sp. nov does not yet have the potential for anticancer compounds. This could be due to the incomplete sequence of the Serinibacter salmoneus genome analyzed by antiSMASH. BGC compounds that could have anticancer properties in the collection of phylum *Actinomycetes* InaCC 2011-2016 include terpenes, butyrolactol, furans, alkyresorcinol, desferroxamine, and siderophore. Further research is needed, such as research in a wet lab; besides that, when each bacterium's genome is known and complete, it is necessary to re-analyze it to get more accurate results.

5. REFERENCES

Blin, K., Shaw, S., Steinke, K., Villebro, R., Ziemert, N., Lee, S. Y., Medema, M. H., and Weber, T. 2019. antiSMASH 5.0: updates the secondary metabolite genome mining pipeline. Nucleic. Acid. Res. 47: W81-W87.

Buss, J. L., Torti, M. F., and Torti, S. V. 2003. The role of iron chelation in cancer therapy. Curr. Med. Chem. 10:1021-1034.

Chen, C., Ye, Y., Wang, R., Zhang, Y. Wu, C., Debnath, S.C., Ma, Z., Wang, J., and Wu, M. 2018. *Streptomyces nigra* sp. nov. is a novel *Actionobacterium* isolated from mangrove soil and exerts a potent antitumor activity in vitro. Front. Microbiol. 9:1587.

Costa, D. C. S., de Almeida, G. S., Rabelo, V. W., Cabral., L. M., Sathler, P. C., Abreau, P. A., Ferreira, V. F., da Silva, L. C. R. P., da Silva, F. C. 2018. Synthesis and evaluation of the cytotoxic activity furanapthoquinones tethered to 1H-1,2,3-triazoles in Caco2, Calu-3, MDA-MB231 cells. Eur. J. Med. Chem. 156:524-533.

Girao, M., Riberio, I., Riberio, T., Azevedo, I. C., Pereira, F., Urbatzka, R., Leao, P.N., and Carvalho, M.F. 2019. *Actinobacteria* isolated from *Laminaria ochroleuca*: a source of new bioactive compounds. Front. Microbiol. 10:683.

GLOBOCAN. 2020. Latest global cancer data in 2020. <https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf>. WHO. Accesed February, 1st 2021.

Grosso, G., Bella, F., Godos., J., Sciacca, S., Del Rio, D., Ray, S., Galvano, F., and Giovannuci, E. L., 2017. Possible role of diet in cancer: systematic review and multiple meta-analyses of dietary patterns, lifestyle factors, and cancer risk. Nutr. Rev. 75(6):405-419.

Hamada, M., Shibata, C., Nurkanto, A., Ratnakomala, S., Lisdiyanti, P., Tamura, T., and Suzuki, K. 2015a.

Tropicihabitans flavus gen. nov., sp., nov., a new member of the family *Cellulommonadacease*. A. Van. Leeuw. J. Microb. 107:1299-1306.

Hamada, M., Shibata, C., Nurkanto, A., Ratnakomala, S., Lisdiyanti, P., Tamura, T., and Suzuki, K. 2015b. *Serinibacter tropicus* sp. nov., an actinobacterium isolated from the rizhosphere of a mangrove, and emended description of the genus *Serinibacter*. Int. J. Syst. Evol. Microbiol. 65(Pt4):1151-1154.

Heap, J.T., Theys, J., Ehsaan, M., Kubiak, A.M., Dubois, L., Paesmans, K., Van Mellaert, L., Knox, R. Kuehne, S.A., Lambin, P., and Minton, N.P. 2014. Spores of *Clostridium* engineeried for clinical efficacy an safety cause regression an cure of tumors in vivo. Oncotarget. 5(7):1761-9.

Kruk, J., Aboul-Enein, B., Bernstein, J., and Marchlewicz M. 2017. Dietery alkylresorcinols and cancer prevention: a systematic review. Eur. Food. Res. Technol. 243:1693-1710.

Li, G., Wang, J., Li., X., Xu, J., Zhang, Z., and Si, J. 2018. A new terpene coumarin microbial transformed by *Mucor polymorphosporus* induces apoptosis of human gastric cancer cell line MGC-803. Arch. Pharm. Res. 41(6):646-654.

Nakaew, N., Pathom-aree, W., and Lumyong, S. 2009.Generic diversity of rare *Actinomycetes* from Thai Cave soils and their possible use as new bioactive compounds. Actinomycetologica. 23:21-26.

Nurkanto, A., Lisdiyanti, P., hamada, M., Ratnakomala, S., Shibata, C., and Tamura, T. 2015. *Cryptosporangium cibodasense* sp. nov., isolated from leaf litter in Indonesia. Int. J. Syst. Evol. Microbiol. 65(12):4632-4637.

Nurkanto, A., Lisdiyanti, P., Hamada, M., Ratanakomala, S., Shibata, C., and Tamura, T. 2016. *Actinoplane bogoriensis* sp. nov., a novel actinomycete isolated from leaf litter. J. Antibiot. 69(1):26-30.

Otogura, M., Yamamura, H., Tamura, T., Irzaldi, R., ratnakomala, S., Ridwan, R., Kartina, G., Triana, E., Nurkanto, A., Lestari, Y., Lisdiyanti, P., Widyastuti, Y., and Ando, K. 2011. *Actinophytocola timorensis* sp. nov. and *Actinophytocola corallina* sp. nov., isolated from soil. Int. J. Syst. Evol. Microbiol. 61(Pt4):834-838.

Ramirez-Rodriguez, L., Stepanian-Martinez, B., Morales-Gonzalez, M., and Diaz L. 2018. Optimazation of the cytotoxic activity of three *Streptomyces* strains isolated from Guaviare river sediments (Colombia, South America). Bio. Med. Resr. Int. ID2839356.

Ratnakomala, S., Lisdiyanti, P., and Widyastuti, Y. 2016. Collection of Indonesian *Actinomycetes* and its uses. LIPI Press. 177-198.

Rejinold, N, S., Baby, T., Chennazhi, K. P., and Jayakumar, R. 2015. Multi drug loaded-thermo-responsive-fibrinogen-graft-poly (N-vinyl caprolactam) nanogels for breast cancer drug delivery. J. Biomed. Nanotechnol. 11(3):392-402.

Sagar, S., Esau, L. Hikmawan, T., Antunes, A., Holtermann, K., Stingl, U. Bajic, B.V., and Kaur M. 2013. Cytotoxic and apoptotic aveluations of marine bacteria isolated from brine-seawater interface of the Red Sea. BMC. Compl. Altr. Med. 13:29-36.

Saha, P., Yeoh, B. S., Xiao, X., Golonka, R. M., Kumarasamy, S., and Vijay-Kumar, M. 2019. Enterobactin, an iron chelating, bacterial siderophore, arrests cancer cell proliferation. Bichem. Pharm. 168:71-81.

Singh, A., Masoodi, M., Nabi, N., and Ashraf, I. 2019. Medicinal plants as combating strategy against cancer: a review. UGC. J. 7(4).

Wang, L., Li, X., Mu, y., Lu, C., Tang, S., Lu, K., Qiu, X., Wei, A., Cheng, Y, and Wei, W. 2019. The iron chelator desferrioxamine synergizes with chemotherapy for cancer treatment. J. Trace. Elem. Med. Bio. 56:131-238.