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Antimicrobial Activity of Actinomycetes

Highlights

Duchenne Muscular Dystrophy

Isolation Selection and Antimicrobial

Discovering Thoughts, Inventing Future

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A New View Duchenne Muscular Dystrophy

By Leonora Grinio Ph

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Abstract- Duchenne Muscular Dystrophy is the result of mutation gene-dystrophine, product - protein-dystrophin presents in organism as the complexes proteins placing everywhere, their role unclear. Suppose all dystrophine complexes work as one functional System D, thanks signal ability complexes. Suppose the System D the ancient and appeared when the gene dystrophin-utrophin divided into two genes dystrophin and utrophin at early vertebrates. Perfect this System made the gene the longest in human genome. The surprising activity creat inkinase-21-23 000 ME, found by author, make to think of the damage much membranes-damage System. Destroy System D is beginning of the disease, finishing apoptosis-general destructive factor. Two factors determinate the disease–damage the system D and apoptosis.

Keywords: *dystrophin, creatine kinase, metabolism, apoptosis.*

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I. Introduction

At the middle of 19th century Guillaume- Benjamin Duchenne studied an unusual form skeletal muscular pathology in boys and named it "Pseudohypertrophic Paralysis" because the patients looked as athletes , but could not walk and were intellectual be backward. He did not find pathology the central nervous system, hypertrophies of skeletal muscles turned out pseudohypertrophies skeletal muscles and G.B. Duchenn called disease as pathology skeletal muscles , later name of the disease progressive muscular atrophy or muscular dystrophy. Consider the disease as the pathology skeletal muscles delay its studying.

In 1968 L.Kunkel (1) described the gene-dystrophin. This gene the longest in the human genome, encompassing 2, 6 million base pairs of DNA and containing 79 exons. The product of the gene –protein-dystrophin (D) described in 1987 y. E. Hoffman(2). There are much information D, it don't exist isolated, forming tightly associated complexes with other proteins membrane and plasma The dystrogluco protein complex – DGC- the most studying, its plays a mechanical function in stabilizing the sarcolemma during muscles contraction; role scaffold in neuromuscular junctions. The general function DGC in skeletal muscles - the connection the cytoskeleton to the extracellular matrix. There are the popular scheme DGC through laminin has connection with sarcolemma and links with contractile apparatus. DGC forming numerous proteins including syntrophin, sarcoglucan, sarcospan, dystrobrevin find in skeletal muscles and brain. The deficiency D skeletal muscles reduces muscle stiffness, increases sarcolemma deformability, membranes abnormal permeability.(3-13) It is known that DGC present in the brain among the cortical neurons, hyppocamp, Purkinje cells, astrocytes, blood-brain barrier, choroid plexus, glial but its function is unclear.D-complexes found in internal organs (kidney, liver, lungs), periphery nerves, acustic and optic analyzators (14-19).

The function D repeat Utrophin (U) which encoded by the UTRN- autosomal gene. Studying the models DMD show complexes with U instead DGC, the same changes observed in patients.DMD has three clinical symptoms: damage skeletal muscles, brain, heart, but every symptom is studying apart, the great attention devote skeletal muscles. The disease has not clear pathogenesis and effective treatment.

II. Material and Method (20)

The time onset pathologic process disease has the important meaning, because permit understand essence a disease. Traditionally the first criterion onset of the disease was appearing clinical symptoms of the muscular weakness of the patients 3-5 years old as difficulties up stairs.Later the high activity some enzymes, especially creat inkinasa(CK), become the test for this disease. There are little information of early period the disease because the most patients in clinic loss walking and the parents of the patients rarely early address. Summarized the results biochemical investigation 34 patients 3-5 years of life present scheme 1 (20,21).

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Schema 1: Biochemical parameters the patients 3-5 years old with DMD (20)

Tissue	Increase	Decrease
Blood	total lipids, activity of enzymes: creatinkinasa, aldolasa, Hormones: ACTG, cortisol	phospholipides
Muscles	collagen, total lipids	carnosin, myosin, myoglobin, phospholipides
Urine	hyperaminoaciduria creatinuria	

The scheme shows the deep changes of metabolism: decreasing true muscle proteins, phospholipids, increasing hormones, enzymes in blood, appearing hyperaminoaciduria. I was shocked when I saw the loss contractility muscles, grey color during biopsy at patient 4 age old. The presented data shows that this period is not the onset of the disease, these changes are typical for destruction metabolism.

The onset of the disease revealed during my scientific travel at retired places. Trying to reveal ill boys in the large families with DMD I used CK test and found the highest activity 23 000 and 21 000 ME in 4 boys 14-24,months old; later the genetic analysis confirmed DMD in these boys. One family is russian, another tadjik See scheme 2. which shows rapid fall activity CK in blood during the disease.

The presented facts point to the time of the onset the disease and rapid course pathologic process in preclinical period. Activity CK was defined by standard spectrophotometric method (norma 100 ME) This exponent was surprising, because usually the maximal activity CK 10 000.- 15 000 ME in the patients 3-5 years old, 3 000-5000 ME - 7-9 years old and 1000-500 ME - 12 years old. The onset of the disease is in preclinical period.

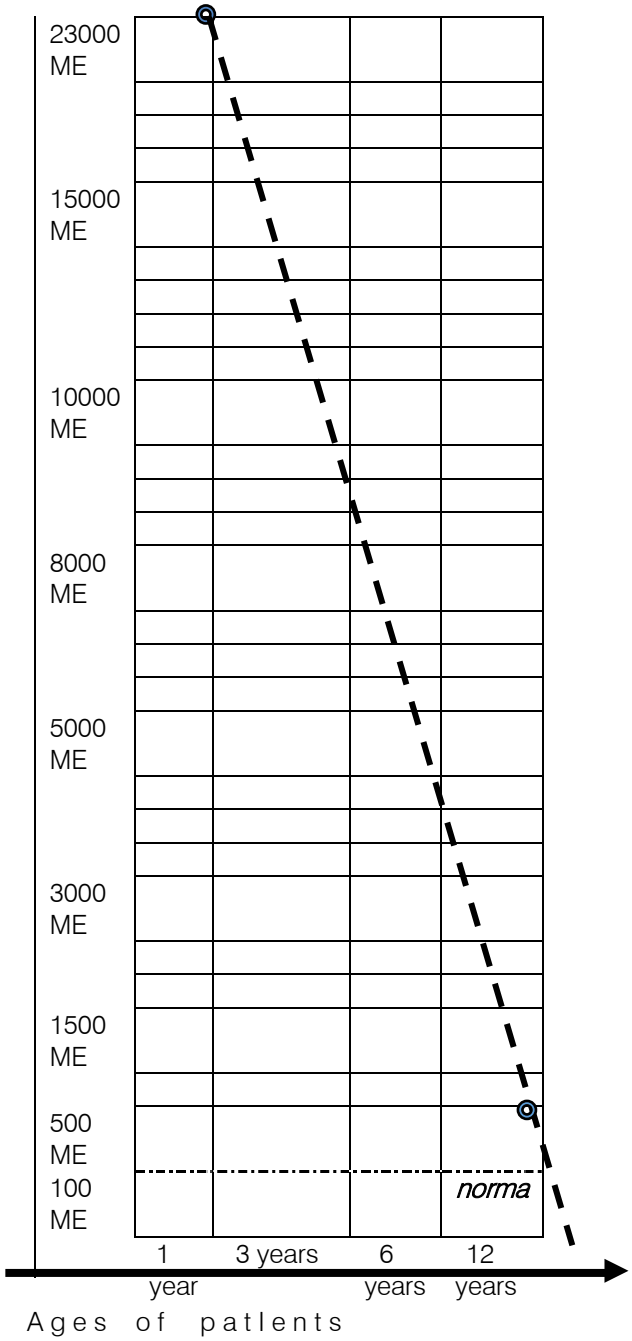
A new hypothesis

The surprising activity CK must to think of damage many membranes during physical stress, because learning walk is the intensive work patients this age. The calculation done by J. Dreyfus and G. Shapira show that exponent significant exceed CK which can way out from skeletal muscles. System unites all D complexes thanks its signal abilities.

Existence System D help to understand present D at optical and acoustic analyzators, which signals can to increase or stopmovement, touch internal organs as lien, lungs, liver. D-system has onset one year of life and suppose the end 60-70 years old because manifestations of the myopathy of old ages repeat the same symptoms muscular weakness and damage coordination.

Damage System D determinates permeability membranes–destroy metabolism- appear apoptosis. Apoptosis - general factor rapid course the disease, destruction skeletal muscles, System D. Studying the disease by the stages help to reveal the logic biochemical changes. The most important the first stage – time damage System D and appearing apoptosis.

Scheme 2: ACTIVITY CREATINKINASA( )the patients with Duchenne Muscular Dystrophy



III. Discussion

A new view consider DMD as the neuromuscular pathology with damage brain, skeletal muscles, heart - three general factors of movement. Intensive movements crease overload physical stress which hearts membranes. Nature produced cover membranes from early vertebrates million years ago. Complicated regulation this cover made the gene the longest human genome. Suppose role this cover membranes play System Dystrophines (DSystem).

Two factors: damage D-System and apoptosisbase a new hypothesis.

D System unites all complexes D placing everywhere. Existence System D help to understand present D at optical and acoustic analyzators, which signals can to increase or stop movement. Like symphonic orchestra, where each instrument has own party, different isomers dystrophines complexes have own party, but together they express one idea, one melody, one general aim - cover membranes during physical stress. Only all family dystrophines do this task, like only orchestra may express idea compositor. How System save membranes is unclear: limit time for intensive movements, for example high speed for short distance or another measures.

Contact D System withmembranes the most interesting, especially through D-complexes.

Complexes D are well studying in the skeletal muscles, especially DGC. Suppose it stabilizes the sarcolemma; takes a part as scaffold neuromuscular junctions; connects the cytoskeleton to the extracellular matrix. The popular scheme shows connection Dystrophin associated complex with proteins (DPC) trough beta-dystroglucan-laminin with sarcolemma. Disassociation complex lead to disrubtion of cell signaling, loss connection with contractile elements (22-30).

In brain isomers D syntrophin, dystroglucan, dystrobrevin are in glial, blood-brain barrier, cells Purkinje, astrocytes, hyppocamp, vascular cells.

All three clinical symptoms characterized by absent the full-lengh isomers D.(31 -37).

The couple-D and proteins, did not analysed: who determinate general role, who has contact with membrane? May be D only staffold for signal and transport, contact with membrane have different proteins, possible the cause in bad contact or damage signal ?

Nobody consider connection D complexes with phospholipids membranes in spite of the fact that damage lipid metabolism determinate the typical appearance patient with pseudo hypertrophiesmany skeletal muscles. Patients blood shows hyperlipidemia, hypercholesterol, increasing correlation fatty acids/ glucerol.(20,41).

The work with the dipepdites is not finished, its meaning needs in studying especially the dipeptide carnosin (beta alanyl-L-gistidin). Carnosin hasthe high concentration in the skeletal muscles, has close connect with synapsis, its early disappearance make think of its role in pathogenesis, especially comparison with other forms myopathies.

The great interest call the conflict between the intensive breaking metabolism at the patients and absent reaction organism. The blood circulation overcrowded proteins, lipids, membranes ,channels are breaking, the work heart is destroy, but don't call complaints; creasing impression "remedy" in blood like narcotic.

Damage D-System destroy metabolism and homeostasis which lead to apoptosis - programmed cells death from cells immune system.

Apoptosis– is a form of programmed cell death or cell "suicide" which observed in multicellular organism. Unlike necrosis apoptosis produces cell fragments called apoptic bodies that fagocytes are able to engulf and remove before contents of the cell. Apoptosis begins the nucleus of the cell begins to shrink. After it plasma membrane blebs and folds around different organells and move away from one another. Immuno histochemical studies describe the signs apoptosis in the patients; DNA fragmentation, caspases activation, cytochrome c release, mRNA decay; in skeletal muscles the typical changes: cells decreased, round of, condensation chromatin (38-42). Take away half of the mass of skeletal muscles during 1-2 years can do only apoptosis. Shock apoptosis on immature brain patient excites the deep retardation delay intellectual development,. cognitive difficulties are revealed during learning. (17,42). Some authors connect cognitive troubles with pathology definite D-complex.

Hypoxia play the general role in the pathogenesis increasing destroy metabolism , but it origin unclear, possible apoptosis and hypoxia appear simultaneously. There are some factors delay or increase apoptosis, but they not be analysed. Possible think that Becker form has not apoptosis.

A new conception of pathogenesis connect the onset of the disease with destroy work System, rapid course with apoptosis, the total dystrophy with serious damage metabolism. The presented facts show how much information of pathological process we have at late period and little of early period; how much is known about skeletal muscles and how little of brain.(43-48).

Using non-mammalian model, especially drosophila melanogaster, show connection D with movements. Suppose D-System is a part of the complex locomotor system. A new view offer the first attempt explain role D-complexes in organism.

The title Duchenne Muscular Dystrophy necessary be replaced Duchenne"s Disease or Duchenne Dystropathia (DD).

IV. Results

The existence D - System basis on 1 – the highest activity CK which shows simultaneusly damage many membranes-damage system. 2-full-lengh isomers D distinguish heart, skeletal muscles, brain as the general components System. 3 D- System is the ancient from early vertebrates, its perfect made the gene the longest in man genome.

Damage D-System begins pathologic process of the disease. Destroy metabolism and the specific features age patients excite apoptosis Apoptosis – the general factor rapid course of the disease, loss walking, destruction skeletal muscles, brain, heart, D-System. two events are in preclinical period of the disease.

V. Conclusion

System D unit all complexes dystrophines - the first attempt to explain the role D complexes in organism. The role System D-defence membranes during physical stress. Destroy System D lead to destruction membranes, metabolism, appearing

apoptosis – the general factor rapid course of the disease. The onset of the disease and apoptosis have place in preclinical stage

Resume

Duchenne Muscular Dystrophy - neuromuscular disease- has three clinical symptoms: damage skeletal muscles, heart and brain – threecomponents of movements. Intensive forming walk accompany the physical stress, which damage work of membranes.. The activity CK - 23 000 ME point to damage mass membranes on large territory- damage system. Such System D units the whole dystrophines for work as functional system., thanks signal communication complexes. Destroy System excite damage membranes, metabolism. This factor and specific features age patients call apoptosis, which is the general destructive factor leading organism to fatal end. Two factors determinate course the classic type DD: damage System D and apoptosis in preclinical stage.

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References Références Referencias

1. Kunkel L. et al. Analysis of deletions in DNA from patients with Becker and Duchenn Muscular Dystrophy. Nature 1986
2. Hoffmann E.,Brown R, Kunkel L. Dystrophin: protein product of Duchenne muscular dystrophy. Cell, 1987, 51, 919-928.
3. KoenigM. Kunkel L/ detailed analysis of the rapid domain of dystrophin reveals four potential hinge segmsnts that may confer flexibility J/Biol.Chem. 1990,265,8. 4560-4566.
4. Rumeir E. Winder S. Hubert J. Dystrophin.2012 FEBS Lett.586, 2717-2719.
5. Blake D., Weir A. Newey S. Davies K. Function and genetics of dystrophin and dystrophin-related proteins in muscles. Physiological Rewiews 2002, 82, 2291-3
6. LidovH., Byers T., Kunkel L., The distribution of dystrophin in the murine central nervous system cortical neurons. Nature.1990, 348, 72-8.
7. Banks. G., Gregorevic P., Allen J., Finn E. Chamberlain J. Functional capacity of dystrophins carrying deletions in the N-terminal actin-binding domain. Human Molecular Genetics 2007,16 (17), 2105-211323.
8. Niels B., Takedo S., Yokota T. Nonmechanicalrols of Dystrophin and associated proteins in exercise neuromuscular junctions and brain BrainSci, 2015. 5. 275- 298.
9. Sojos V., Curto M., Reali C., Gremo F., Developmentally regulated expression and localization of dystrophin and utropin in the human fetal brain. Mech Ageing Dev. 2002, 123, 5, 455-62.
10. Koenig X.,Ebner J.,Hilber K., Voltage-dependent sarcolemmalione channels abnormalities J.Mol.Sci. 2018,19,11,3296
11. VanPutten M., van Pul, Hulsker M., Low dystrophin levels in heart can delay heart failure in mdx mice.J, Mol. Cellul. Cardiology 2014, 1.
12. Hendriksen R., Schipper S., Hoogland G., Dystrophin distribution and expression in human. 2016. Cell Neuroscience, 2, 00174.
13. Waite A., BlakeD., Brown S., The dystrophin – glucoprotein complex in brain development and disease. Neurosciences 2012,35,8, 497-9.
14. CulliganK., GloverL., Dowling P. Brain dystrophin-glucoprotein complex persistent expression of B-dystroglucan impaired oligolregulation of Dp71 and up-regulation of utropin in animal models. Cell Biology 2991.
15. DarrasB., Kunkel L., "Dystrophinopathies" in Neuro-muscular Disorder of Infancy. 2011 chapter 107, 684-697.
16. Tracey I., Dunn J., Radda G., Brain metabolism is abnormal in the mdx model of DMD., Brain., 1996., 119., 1039-44.9.
17. Wicksell R., Kihlgren M et al. Specific cognitive deficits are common in children with Duchenne Muscular Dystrophy. Dev. Med. Child Neurol. 2004,46,154.
18. Sekiguochi M.,Zushide.,K.,Yoshida M. A deficit of brain dystrophin impairs specific amygdala GABAergic transmission and enhances defensive behavior in mice. Brain 2009,132,1, 1.
19. Banks.G.,GregorevicP.,Allen J.,Finn E.Chamberlain J. Functional capacity of dystrophins carrying deletions in the N-terminal actin-binding domain. Human Molecular Genetics 2007,16 (17),2105-211.
20. Grinio L.,. Duchenne Myodystrophy. Russ. monografy pub. НГМА,1998,1-100
21. Grinio L., Pathogenesis of Duchenne Muscular Dystrophy. Russ. J. Neurol. Psych. 2019, 119, 3, 79-81.
22. Morikawa Y., Heallen T., Leach J., Xiao Y., Morton J. Dyst-glucoprotein complex sequesters to inhibid-cardiomy ocyte proliferation. Nature 2017.13, 547, 227-231.
23. Soyos V., Curto M., Reali C., Grenio F. Developmentally regulated expression and localization of dystrophin and utropin in the human fetal brain. Mech. Ageing Dev. 2002, 123, 5, 455-62.
24. Milad. N., White Z., Tehrani A., Rossi F., Increased plasma lipid levels in the mdx model. Nature 2017.13, 547, 227-231.
25. Goodnough c., Gao Y,. Qutaish Q., Lack dystrophin results in abnormal cerebral diffusion and perfusion in vivo., Neuromage 2014,192,2,809-816

26. Gambardelle A. Dystrophin distribution and expression in human and experimental temporal lobe. Epilepsy Research University Catanzago., 2019.153. 49-58.
27. Haenggi T., Fritschy J., Role of Dystrophin and utropin for assembly and function of the dystrophinglucoprotein complex in non-muscle tissue. Cell. Mol. Life Sciences 2006, 69, 14,161441.
28. Culligan K., Beta-Dystrobrevin and Dystrophin in brain neurons. Cell Biology 2001, 2, 2-10.
29. Goldstein J,. McNally E. Mechanism of musle weakness in muscular dystrophy Archive 2010,136,1-129.
30. Morikawa Y., HeallenT., Leach J. Xiao Y. Martin J. Dyst-glucoprotein Complex sequesters Yap to in cardiomyocytes. Nature 2017, 13, 547, 227-231.
31. Anderson J., Head S., Morley J., Long-term depression is reduced in cerebellarar Purkinje cells of dystrophin-deficient mdx mice. Brain. Research 2009.19, 1-2,289 -29223.
32. Gambardelle A. Dystrophin distribution and expression in human and experimental temporal lobe. Epilepsy Research University Catanzago., 2019.153. 49-58.
33. Ujhara Y. Estimation of fukutin reveals cellular and molecular pathomechanims in muscular dystrophy. Nature 2020,10,1038-41.
34. Vican S., Piccini G., Mercuri E,. Alfieri P..Implacit learning deficit in children with Duchenne Muscular Dystrophy: evidence for a cerebellar cognitive impairtment Nature 2018 10, 82.
35. Pins A., Spitali P., Circulating biomarkers for Duchenne Muscular Dystrophy. J. Neuromuscular Disord 2015, 2, 49 -58.
36. Allen D., Whitehead N,. Frochner S., Absence of Dystrophindisrubtion skeletal muscle signaling: roles of Ca2,reactive oxygen species, nitric oxide in the development of muscular dystrophy. Physical Rew. 2016,96,253-305.
37. Guiraud S., Davies K. Regenerative biomarkers for Duchenne Muscular Dystrophy. Neural. Regen Res. 2019,14,8,1317-1320.
38. Grounds M., Ferrill J., Al-Mehdani B., Duong M. Biomarkers for Duchenne Muscular Dystrophy: myonecrosis, inflammation, and oxidative stress. Dis.Model. Mech. 2020, 2,13,12-42.
39. Savitz S., Daniel B., Rosenbaum M., Apoptosis in neurological siseases., Neurosurgery 1998,42, 555-72.
40. Arends M. Wyllie A., Apoptosis, Mechanism and role in pathology 11 Int.Rev.Exp.Pathol.1991, 32, 223-2.
41. Tang H. Cell survival DNA damage and oncogenic transformation after atransiet and reversible response. Mol.Biol.Cell 2012,23, 29, 40-52.
42. Borras G. Programmed cell death in plants and animals. Biotechnologia Aplicada.2006, 23, 1.
43. Soyos V., Curto M., Reali C., Grenio F. Developmentally regulated expression and localization of dystrophin and utropin in the human fetal brain. Mech. Ageing Dev. 2002,123,5, 455-62.
44. Milad. N.,White Z.,Tehrani A., Rossi F., Increased plasma lipid levels in the mdx model. Nature 2017.13, 547,227-231.
45. Goodnough c., Gao Y,. Qutaish Q., Lack dystrophin results in abnormal cerebral diffusion and perfusion in vivo., Neuromage 2014,192,2,809-816.
46. Gambardelle A. Dystrophin distribution and expression in human and experimental temporal lobe. Epilepsy Research University Catanzago., 2019.153. 49-58.
47. Haenggi T.,Fritschy J., Role of Dystrophin and utropin for assembly and function of the dystrophinglucoprotein complex in non-muscle tissue. Cell. Mol. Life Sciences 2006, 69, 14, 161-441.
48. Culligan K.,Beta-Dystrobrevin and Dystrophin in brain neurons. Cell Biology 2001, 2, 2-9.

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Isolation, Selection and Antimicrobial Activity of Actinomycetes from Mangrove Soil of Can Gio Forest, Hochiminh City, Vietnam

By Nguyen Tuan Hai & Cao Ngoc Diep

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Abstract- The Can Gio Mangrove is a Biosphere Reserve of UNESCO since 2000 and it is also a well-known example of "mangrove afforestation and reforestation area''. A total of 63 actinomycetes were isolated from 25 samples of 9 different sites in mangrove forest soil Can Gio, HoChiMinh city, Vietnam. Almost their colonies have round-shaped; milky, white clear and yellow, entire or loabate margin; diameter size of these colonies varied from 0.2 to 3.0 mm. Twenty-nine of 63 tested isolates could produce antimicrobial active metabolites inhibiting at least one of the tested pathogens and 9 isolates were selected for 16S rDNA sequencing. The result showed that genus *Streptomyces* (8 isolates) and other genera including 1 isolates were Gram-negative bacteria (*Stenotrophomonas*). The antimicrobial activity and the amplifying genes coding for polyketide synthetase (PKS) and nonribosomal peptide synthetase (NRPS) showed that 8 strains had broad-spectrum antimicrobial activity, mainly against gram-positive bacteria as *Bacillus cereus* and *Staphylococcus aureus*.

Keywords: *actinobacteria, antimicrobial activity, bacteria references, can gio, mangrove forest soil.*

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Keywords: actinobacteria, antimicrobial activity, bacteria references, can gio, mangrove forest soil.

I. Introduction

Mangroves are saline resistant forest ecosystems found in tropical and sub – tropical intertidal regions around the world (Ottoni et al., 2015). Mangrove soils have a unique character for the growth of various kinds of microorganisms that play an important role in degrading the soil. Several factors that affect mangrove ecosystems cause microorganisms to adapt by producing unique metabolites, namely primary and secondary metabolites (Thatoi et al., 2013). Mangrove soils provide a unique ecological niche for the growth of diversified microorganisms which find use in recycling environmental nutrients and production of exclusive secondary metabolites of pharmaceutical importance. The total microbial community of tropical mangrove forest comprise 91% of bacteria and fungi, 7% of algae and 2% of protozoa. (Pallaa et al., 2018).

Actinomycetes are group of aerobic, branched, unicellular Grampositive bacteria with high percentage of G+C (70%) in their geneticmaterial. This bacterium has many important roles in various industries because of its ability to produce a number of diverse metabolite compounds. These metabolite compounds have benefits such as, antibiotics, antifungal, antiviral, anticancer, enzymes, immune suppressants and other compounds that are beneficial in industry (Xu et al., 2014). Actinomycetes particularly *Streptomyces* are well known as major sources of secondary metabolites particularly antibiotics (Berdy, 2005).

Many researchers discovered novel actinobacteria from poorly explored mangrove habitats such as isolation of *Asanoa iriomotensis* (Tamura and Sakane, 2005), *Nonomuraea maheshkhaliensis* (Ara et al., 2008) and *Streptomyces xiamenensis* (Xu et al., 2009).Aquatic actinomysetes have been shown to have an important role in the discovery of several new bioactive compounds such as research conducted by Huang et al. (Huang et al., 2008) on rifamycin from *Micromonospora*, Fehling et al. (Feling et al., 2003) found salinosporamide-A as an anticancer metabolite of the *Salinispora* strain, Marinomisin from *Marinophilus* sp. and much more. According to Anzai et al. (2008) out of 22,500 biologically active compounds, 45% are derived from actinomycetes.

Actinobacterial diversity from these ecosystems has been studied worldwide for their unique biochemical processes. The present study includes isolation, morphological characterization and identification of rhizospheric actinobacteria using biochemical and molecular biology techniques (Brinda and Mathew, 2012; Page, 1997). Molecular biology techniques like 16S rRNA techniques are an important tool in final identification of bacteria sequencing this gene, and provide genus and species identification for isolates that do not fit any recognized biochemical profiles. It gives acceptable identification which otherwise according to conventional system of taxonomy is not possible (Malik et al., 2008). Zhao et al. (2011) recognized that antimicrobial activity and amplifying genes coding for PKS-I, PKS-II and NRPS from endophytic actinomycetes isolated from medicinal plants in Panxi plateau

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performedas valuable reservoirs of novel bioactive compounds therefore the method has been studied the distribution of PKS and NRPS biosynthetic systemsin a collection of wild-type actinomycetes isolated fromtropical soil samples (Ayuso et al., 2005).

This research was conducted to isolate and identify actinomycetes from Can Gio Mangrove ecosystem, HoChiMinh city, Vietnam. Actinomycetes in this ecosystem are thought to have the potential to produce primary and secondary metabolites. Actinomycetes will produce primary and seconday metabolites in extreme conditions like the Can Gio mangrove ecosytem, which is polluted by various types of waste from rivers passing through big city as HoChiMinh city. The results of isolation from this study can be used for further research such as screening for primary and secondary metabolite produced by actinomyces.

The Can Gio Mangrove (approximately 35,000 ha, extending from 10_220-10_440N and 106_460-107_010E (Tuan and Kuenzer,2012)) is located in the south of Vietnam, at the downstream part of the Sai Gon and Dong Nai Rivers watershed and in the SouthChina Sea coastal zone (Figure 1).Very little information is available about beneficial bacterial diversity (Thanh Nho et al., 2018) and their activity in mangrove soil of Vietnam. Therefore, the aims of this study were (i) to isolate and select together with identify actinobacterial isolates having good resistance to human pathogenic bacteria (ii) to obtain their characterization as analysing genes coding for PKS-I, PKS-II, and NRPS actinobacteria as well as to explore the potential use of these newly actinobacteria as a novel source of bioactives, CMC degradation and phosphate solubilization (iii) to identifying by 16S rDNA genes techniques and sequencing.

II. Materials and Methods

a) Isolation

i. *Collect of plant samples and rhizosphere soil*

Plant soil samples were collected carefully from many species of mangroves viz. *Bruguiera sexagula, Ceriops decandra, Sonnertia, Avicennia, Rhirophora*…., from the 3 years-old plants in plantation site, Tam Thon Hiep – village, (soil pH = 4.22, salinity 10‰; Thanh An site, (soil pH= 6.18, salinity 7‰;An Thoi Đông village (soil pH= 4.16, salinity 8‰). (Lat. 10°68' 04" N; Long. 107° 02' 64" E) (Figure 1).

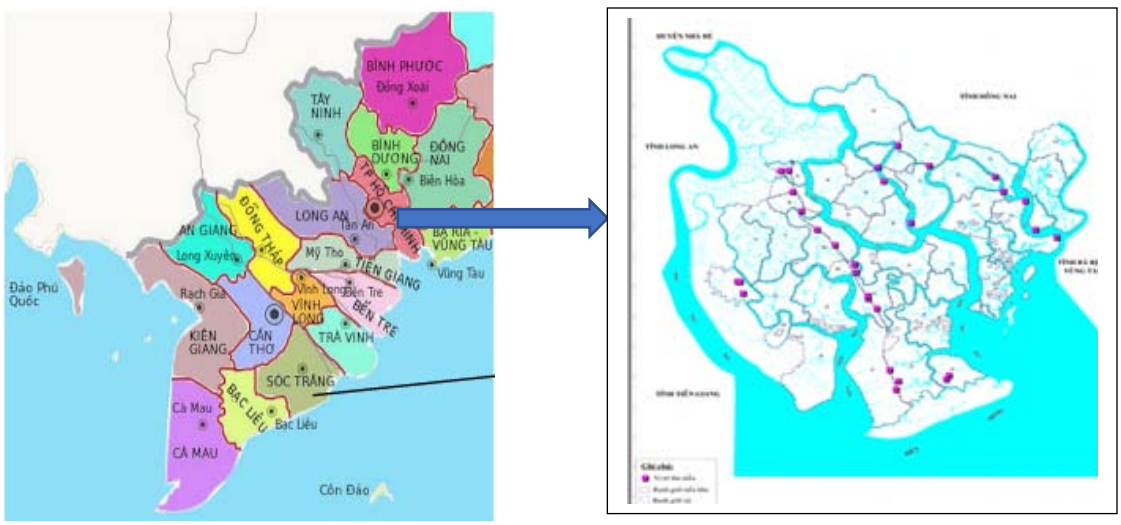


Figure 1: HoChiMinh city and Can Gio district with sample collection sites in Can Gio mangrove forests of HCMC, Vietnam (red dot: sites to collect samples)

ii. *Bacterial isolation*

The samples were collected on in December, 2019. For isolation of bacterial rhizosphere, samples were collected during the low tide. Soil samples were collected by using a sterile spatula and stored in sterile polythene bags, and then were stored in ice-box (5°C) and transportation to Can Tho University as soon as possible; soil samples were stored in refrigerator (-10°C) in Microbiology Lab. until isolation. The soil samples were removed adherent particles and were superficially disinfected according to Araújo et al.(2014).

A known weight of soil (1 g) was aseptically weighed and transferred to a stoppered (150 mL) sterile conical flask containing 99 mL of sterile saline (0.9%) diluent. The sediment-diluent mixture was agitated by means of mechanical shaking for about 45 minutes. After the above time, the supernatant was collected and streaked on the Starch Casein Agar medium (Mohseni et al., 2013) was used for the isolation of actinobacteria. It was supplemented with Aginalxic (0.5 mg/L) and Nystatin (0.5 mg/L) to inhibit fungi and Gram-negative bacteria. The inoculated plates were incubated at 28°C

for 3–6 weeks. The colonies bearing distinct morphological characteristics were picked up and transferred to freshly prepared media until pure cultures were obtained.

b) Morphological Characterization

The morphological characterization of the bacterial colonies were carried out according to on the basis of their shape, size, colour, margin, elevation on the media and Gram staining method was also performed to decide the further determinative protocol. All isolates were tested on CSA media with higher NaCl concentrations (4.50% NaCl).

c) Screening assays for antibacterial activity

The bioactivity of bacterial isolates was examined (Gobi et al., 2012; Manikandan et al., 2014). The pathogenic bacteria including *Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Vibrio haemoparalyticus* were provided by College of Aquaculture and Fisheries, Biotechnology Research and Development Institute (Can Tho University) and Can Tho Center for Technology, Standard, Quality (Department of Science and Technology, Can Tho City). The liquid cultures were grown with shaking at 150 rpm for 7–14 days depending on their growth rate at 30°C. The broth was centrifuged in 50 mL falcon tubes at 4,193xg for 15 min at room temperature (28-32°C); Megafuge 1.0R, Heraeus) and the supernatant was stored at 4°C. The bacterial organisms were plated in Mueller Hinton Agar medium. Antimicrobial extract was added to the wells, then the plates were incubated at 30°C for 2 – 3 weeks for diffusion of antimicrobial extract and observed for the zones of inhibition at 21th day after incubation.

d) The Agar well diffusion method

The active isolates were cultured by the method given in the previous step. The supernatants were used for testing extracellular antimicrobial activity by the agar well diffusion method. By using a sterile cork borer, the wells were punctured in appropriate agar medium previously seeded with one of the test organisms. One hundred microlitre of the culture supernatants were added to each well. The plates were then incubated at 4°C for at least 2 h to allow the diffusion of crude extracts followed by incubation for 2-3weeks at 37°C for bacteria. The diameters of inhibition zones were monitored and measured (Rinaudo, 1992).

e) 16S rDNA Gene Amplification and Sequencing

The amplification of 16S rDNA by PCR was carried out using the universal primers 27F (Weisburg et al., 1991) and 1492R (Reysenbach et al., 1992). The 50 μL reaction mixture consisted of 2.5 U Taq Polymerase (Fermentas), 50 μM of each deooxynucleotide triphosphate, 500 nM of each primer (Fermentas) and 20 ng DNA. The thermocycling profile was carried out with an initial denaturation at 95°C (5 min) followed by 30 cycles of denaturation at 95°C (30 s), annealing at 55°C (30 s), extension at 72°C (90 s) and a final extension at 72°C (10 min) in C1000 Thermal Cycler (Bio-Rad). Aliquots (10 μL) of PCR products were electrophoresed and visualized in 1% agarose gels using standard electrophoresis procedures.

The following actinobacteria-specific primers were used for the amplification of actinobacterial 16S rRNA gene fragment (Stach et al., 2003). Cycling conditions were as follows: initial denaturation at 95°C for 4 min, 30 cycles of 95°C for 45 s, 68°C for 45 s, and 72°C for 1 min, and a final extension of 5 min at 72°C. S-C-Act-0235-a-S-20(5′-CGCGGCCTATCAGCTTGTTG 3′), and S-C-Act-0878-a-A-19 (5′-CCGTACTCCCCAGGC GGGG-3′)

f) Sequence Analysis

The 16S rRNA gene sequences were compared with those from the type strains available in NCBI (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). For the phylogenetic analysis, multiple sequence alignment was performed using CLUSTALX, version 1.81. Phylogenetic tree was constructed using Mega 7.0 (Kumar et al., 2018). The consistency of the trees was verified by bootstrapping (1000 replicates) for Maximum Likelihood method.

Detection and Analysis of PKS-I, PKS-II, and NRPS

PCR primers and amplifications were carried out and analysed (Ayuso et al., 2005; Zhao et al., 2011).

g) Statistical analysis

The experimental results were analysed as a two-way ANOVA with the isolates and with levels of diameters of inhibition zones. All analyses were conducted using the programme MSTATC, Minitab 16. The data were considered significantly different at $p < 0.01$. Duncan test at $P = 0.01$ was used to differentiate between average value statistically.

III. Results and Discussion

a) Isolation

A total of 63 isolates of actinomycetes was purified from 25soil samples collected at 9 sites of CanGio mangrove forest. Almost their colonies have round-shaped; milky, white clear and yellow, entire or loabate margin; diameter size of these colonies varied from 0.2 to 3.0 mm and all of them have Gram-positive. (Figure 2).

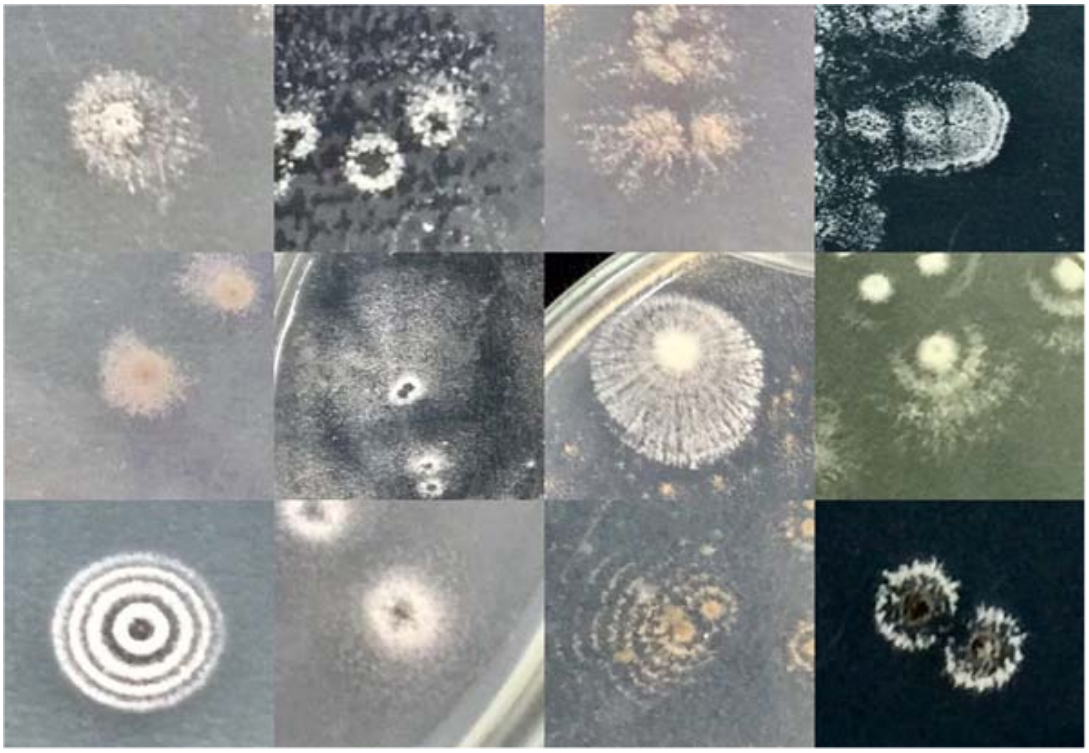
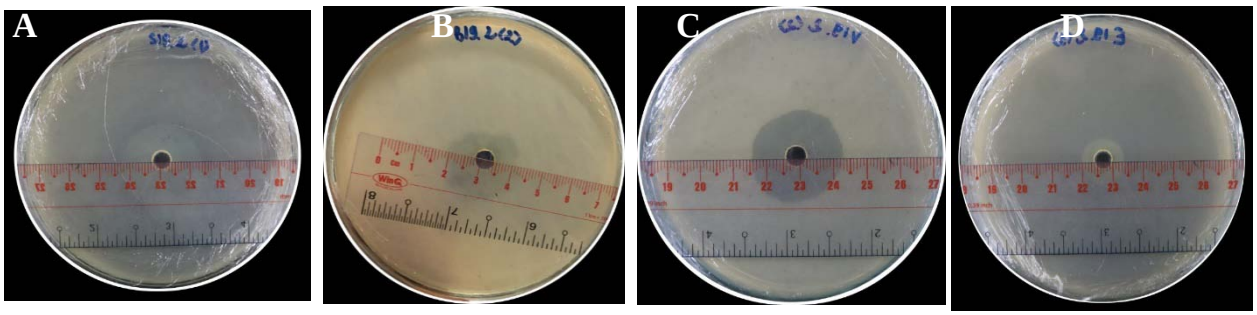


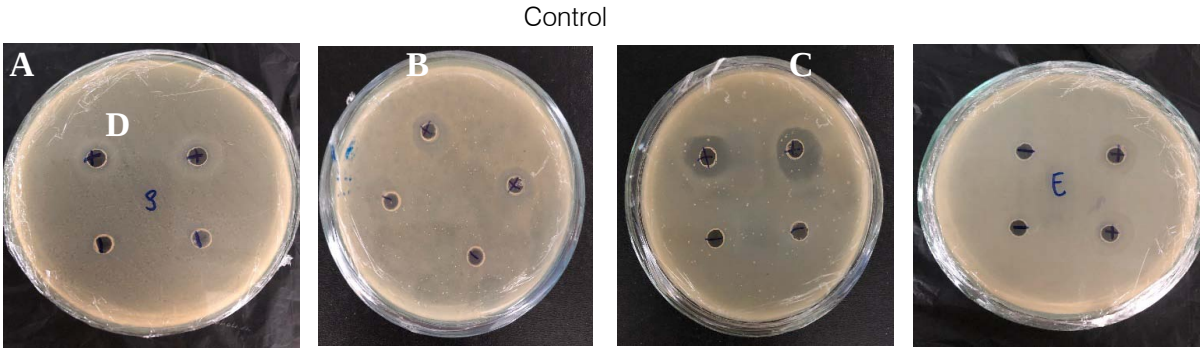
Figure 2: The different shapes and sizes of colonies of actinobacteria on SCA agar medium

Twenty-nine of 75 tested isolates could produce antimicrobial active metabolites inhibiting at least one of the test pathogens (Figure 3). Over fifty percent isolates were capable of inhibiting the growth of Gram-positives, 23 isolates were actively against Gram-positive bacteria and 26 isolates showed activity against Gram-negative bacteria and 5 isolates were actively against following pathogens including *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Vibrio haemoparalyticus* (Table 1).

Streptomyces parvulus ANTHOIDONG3.1 tested with 4 human pathogenic bacteria



A: Staphylococcus areus B: Bacillus cereus D: Escherichia coli
C: Vibrio parahaemolyticus



A: Staphylococcus areus (Streptomycin)

B: Bacillus cereus (Streptomycin)

C: Vibrio parahaemolyticus (Tetracyclin)

D: Escherichia coli (Tetracyclin)

Figure 3: Antimicrobial activity of ANTHOIDONG 3.1 isolate against Staphylococcus areus (A),Bacillus cereus (B), V. parahaemolyticus(C)and Escherichia coli (D) by observation of halo zone around colony (inhibition of ring)

Table 1: Antimicrobial activity of isolated actinobacteria from Can Gio mangrove forest soil (HoChiMinhCity)

No.	Actinobacterial name	B	E	V	S
01	TAMTHONHIEP 2.1	ND	ND	ND	ND
02	TAMTHONHIEP 4.1	ND	ND	ND	ND
03	TAMTHONHIEP 5.1	ND	ND	ND	ND
04	THANHAN 5.1	ND	ND	ND	ND
05	THANHAN 3.2	ND	ND	ND	ND
06	THANHAN 3.3	ND	ND	ND	ND
07	THANHAN 3.4	ND	ND	ND	ND
08	THANHAN 4.1	ND	ND	ND	ND
09	THANHAN 4.2	ND	ND	ND	ND
10	THANHAN 4.3	ND	ND	ND	ND
11	THANHAN 4.4	16	ND	ND	16
12	THANHAN 4.5	ND	ND	ND	10
13	THANHAN 5.1	ND	ND	ND	ND
14	LYNHON 6.1	ND	ND	ND	ND
15	LYNHON 6.2	ND	ND	ND	ND
16	LYNHON 7.1	ND	ND	ND	ND
17	LYNHON 7.2	ND	ND	ND	ND
18	LYNHON 8.1	ND	ND	ND	ND
19	LYNHON 8.2	ND	ND	ND	ND
20	ANTHOIDONG 1.1	ND	ND	ND	ND
21	ANTHOIDONG 1.2	ND	ND	ND	ND
22	ANTHOIDONG 1.3	ND	ND	ND	ND
23	ANTHOIDONG 1.4	ND	ND	ND	ND
24	ANTHOIDONG 1.5	ND	ND	ND	ND
25	ANTHOIDONG 1.6	ND	ND	ND	ND
26	ANTHOIDONG 1.7	ND	ND	ND	ND
27	ANTHOIDONG 3.1	10.7	07.7	17.3	21.7
28	ANTHOIDONG 3.2	05.7	ND	ND	ND
29	ANTHOIDONG 3.3	ND	ND	ND	ND
30	ANTHOIDONG 3.4	ND	05	ND	05
31	ANTHOIDONG 3.5	ND	ND	ND	ND
32	ANTHOIDONG 3.6	ND	ND	ND	ND
33	ANTHOIDONG 3.7	ND	ND	ND	ND
34	ANTHOIDONG 3.8	ND	ND	ND	ND
35	ANTHOIDONG 3.9	ND	ND	ND	ND

36	ANTHOIDONG 4.1	12.3	11.7	21.7	15.7
37	ANTHOIDONG 6.1	19.7	ND	ND	ND
38	ANTHOIDONG 6.2	ND	ND	ND	ND
39	ANTHOIDONG 7.1	27.7	05.6	17.7	35.1
40	ANTHOIDONG 7.2	ND	ND	ND	ND
41	LONGHOA 1.1	ND	ND	ND	ND
42	LONGHOA 2.1	ND	ND	ND	ND
43	LONGHOA 2.2	ND	ND	ND	ND
44	LONGHOA 4.1	ND	ND	ND	ND
45	LONGHOA 4.2	11.3	ND	ND	ND
46	LONGHOA 4.3	ND	ND	ND	ND
47	LONGHOA 4.4	ND	ND	ND	ND
48	LONGHOA 5.1	ND	ND	ND	ND
49	LONGHOA 5.2	ND	ND	ND	ND
50	CANTHANH 1.1	ND	ND	ND	ND
51	CANTHANH 1.2	ND	ND	ND	ND
52	CANTHANH 2.1	ND	ND	ND	ND
53	ANTHOIDONG 8.1	ND	ND	ND	ND
54	ANTHOIDONG 9.1	ND	ND	ND	ND
55	ANTHOIDONG 9.2	ND	ND	ND	ND
56	ANTHOIDONG 10.1	ND	ND	ND	ND
57	ANTHOIDONG 10.2	ND	ND	ND	ND
58	ANTHOIDONG 10.3	ND	ND	ND	ND
59	ANTHOIDONG 11.1	07.3	ND	ND	ND
60	ANTHOIDONG 12.1	ND	ND	ND	ND
61	ANTHOIDONG 13.1	ND	ND	ND	ND
62	ANTHOIDONG 13.2	ND	ND	ND	ND
63	ANTHOIDONG 13.3	ND	ND	ND	ND
	control	08.7 streptomycin	09.0 tetracyclin	13.7 tetraclin	12 streptomycin

B: Bacillus cereus, E: Escherichia coli, V: Vibrio parahaematolicua S: Staphylococcus aureus

D = diameter of inhibition zone of isolates, d1 = diameter of inhibition zone, d2 = diameter of well, ND: not detected

A total of 9 isolates was chosen for identification due to their highly antimicrobial activity. The fragments of 1050-1225 bp 16S rRNA were obtained from PCR and sequencing. Homology searches of 16S rRNA gene sequence of selected strains in GenBank by BLAST (Table 2) revealed that they had similarity to sequences of genus *Streptomyces* (8 isolates) and other genera including 1 isolates was Gram-negative bacteria (*Stenotrophomonas*) [not suitable for this study].

Table 2: Phylogenetic affiliation of 8 isolates on the basis of 16S rRNA gene sequences by using BLAST programme in the GenBank database based on sequences similarity

Taxonomic group and strain	Closest species relative	Similarity (%)
LONGHOA 4.2	MH472998 *Streptomyces africanus* strain E3SQ	97.7
	MK368443 *Streptomyces* sp. strain MUM203J	97.6
THANH AN 4	HM594286 *Streptomyces tendae* strain M23	97.4
	MN339840 *Streptomyces* sp. strain MGB 2769	97.4
ANTHOIDONG 3.2	U841673 *Streptomyces tanashiensis* strain HBUM174095	97.7
	EU593580 *Streptomyces tanashiensis* strain 173004	97.6
ANTHOIDONG 3.1	MW217198 *Streptomyces pavulus* strain DSD2596	98.8
	MW217191 *Streptomyces* sp. strain DSD1692	98.8
ANTHOIDONG 4.1	KP235209 *Streptomyces celluloflavus*strain D4-17	99.6
	MN116554 *Kitasatospora* sp. strain SKW16	99.6
ANTHOIDONG 7.1	HQ607433 *Streptomyces albogriseolus* strain 1168	99.4
	MK281547 *Streptomyces albogriseolus* strain SCAU-101	98.6
ANTHOIDONG 6.1	U841673 *Streptomyces tanashiensis* strain HBUM174095	97.8
	MT505707 *Streptomyces aegyptia* strain 7	97.8
ANTHOIDONG 11.1	MT072138 *Streptomyces laurentii* strain QT214	98.7
	LC497896 *Streptomyces* sp. 9R005	98.7

A Maximum Likelihood phylogenetic tree (Figure 4) of these isolates described the two clusters. Cluster A had 6 strains including 2 smaller clusters as cluster A1 with 4 strains divided to 2 smaller: cluster A11 composed of *Streptomyces tendae* THANHAN 4 and *Streptomyces tanashiensis* ANTHOIDONG 3.2 and cluster A12 with 2 strains: *Streptomyces aegyptia* ANTHOIDONG 6.1 and *Streptomyces laurentii* ANTHOIDONG 11.1 while cluster A2 with 2 strains were *Streptomyces albogriseolus* ANTHOIDONG 7.1 and *Streptomyces africanus* LONGHOA 4.2. Cluster B had 2 strains: *Streptomyces celluloflavus* ANTHOIDONG 4.1 and *Streptomyces pavulus* ANTHOIDONG3.1.

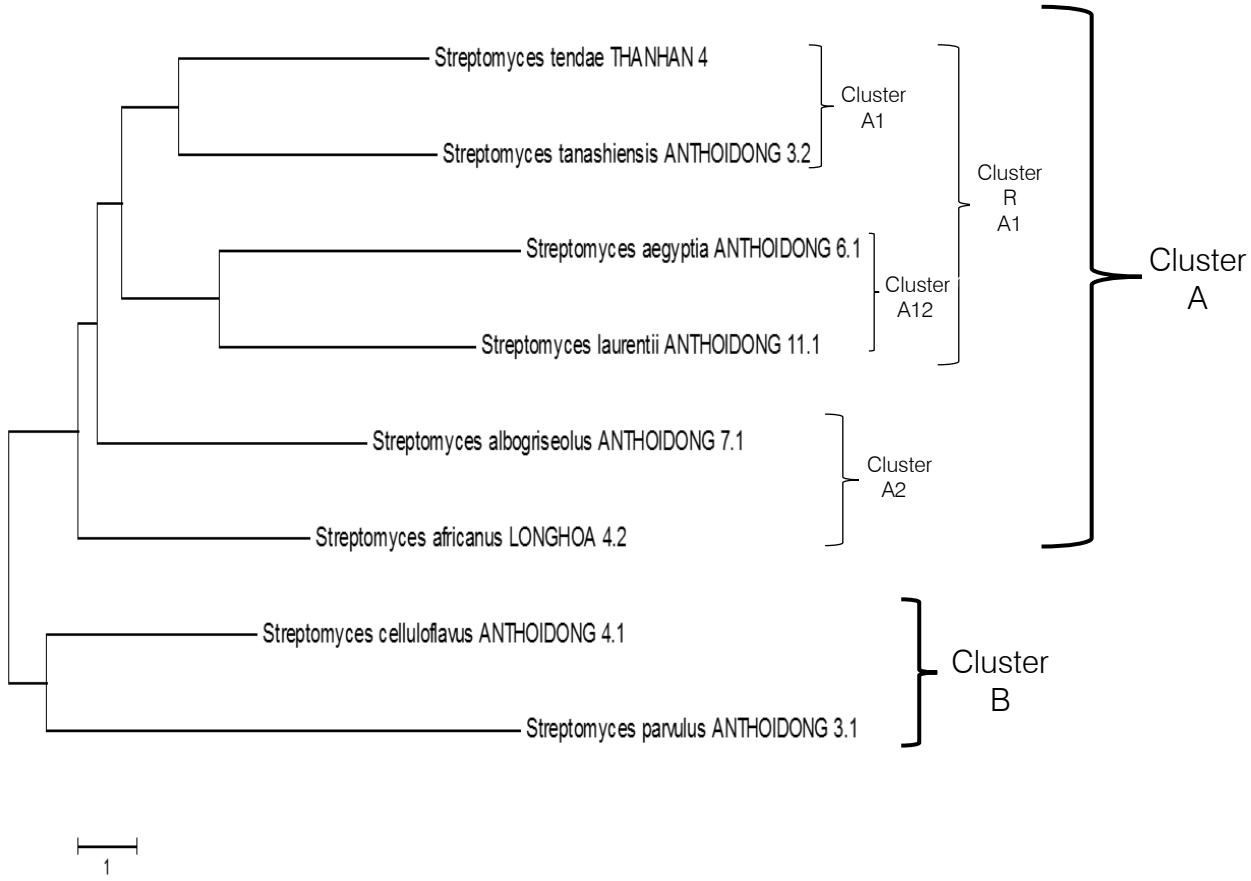


Figure 4: The Maximum Likelihood phylogenetic tree of partial 16S rRNA gene sequences of actinobacteria isolated from sediments of Can Gio mangrove forest and closely related type strains. Numbers in the figure refers to percentage bootstrap values which were calculated for 1000 replicates. Bar, 0.02 was per nucleotide position

Our results identified 8 strains of genus *Streptomyces* and all of them had the ability of antimicrobial activity, our results also conformed with the results of Qiu et al., (1994), when they deteremined that genus *Streptomyces* is the most resources of antibiotic production in comparison to other microbes.

The species of genus *Streptomyces*, *Streptomycescelluloflavus* ANTHOIDONG 4.1(Figure 5A), their colonies had red white color, circular shape, many rays around colony on the ISP medium. The spores have many spines on the surface of spore and the cells connected to the string under the electric microscope X 8500 (Figure 5B).

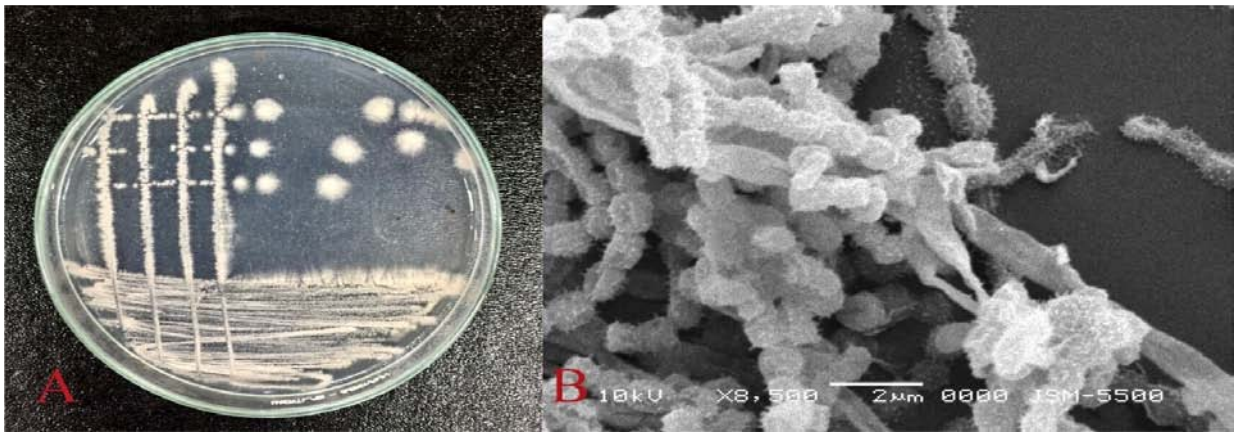


Figure 5: Colonies of *Streptomycescelluloflavus* ANTHOIDONG 4.1 strain(A) and cells and spores *S.celluloflavus* ANTHOIDONG 4.1 strainunder SEM (B)

Streptomyces celluloflavus first isolated at Japan soil, *Streptomyces celluloflavus*synthesized aureothricin and they have the ability of degrading-cellulose (Munray *et al*., 2008). In this study, *Streptomyces celluloflavus* has the ability of antibacterial activity to human pathogenic bacteria as *Vibrio parahaemolyticus*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* and this strain is the strongest phosphate solubilization and CMC degradation.

The second colonies of *Streptomyces aegyptia* ANTHOIDONG 6.1strain have white color, circular shape, many rays around colony on the ISP medium (Figure 6A). Observation on SEM, *Streptomyces aegyptia*have smooth surface of spores, and connected to the string (Figure 6B and 6C).

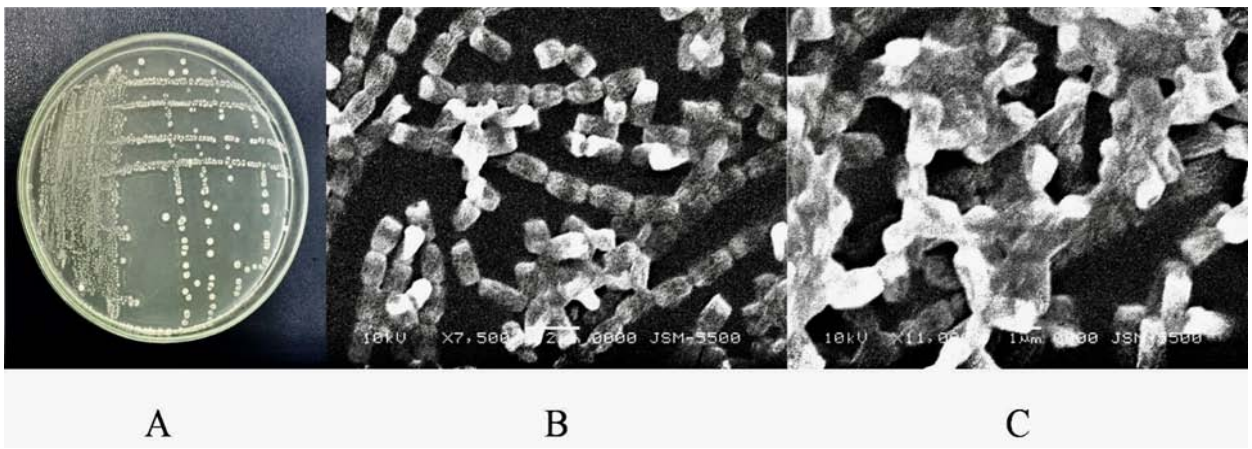


Figure 6: Colonies of *Streptomyces aegyptia* ANTHOIDONG 6.1 (A) shapes and spores of *S. aegyptia* ANTHOIDONG 6.1 under SEM (B and C)

Streptomyces aegyptia isolated from soil of Dakahliyah province, Egypt, they have the high ability of cellulose degration (El-Naggar *et al*., 2011). Consequently, they produced cholesterol oxidase, anticancer in vitro, antimuscle infection, breast cancer and apoptosis in vivo (El-Naggar *et al*., 2018). *Streptomyces aegyptia* synthezied the nano-Ag seeds against bacteria as nanofactory friendly with the environment (Osama *et al*., 2014). In this study, *Streptomyces aegyptia* ANTHOIDONG 6.1 have the ability of against *Bacillus cereus.*

The third strain, *Streptomyces laurentii* ANTHOIDONG 11.1 strain had colony with concentric circle surrounded by rays. Observation on SEM, *Streptomyces laurentii* ANTHOIDONG 11.1 have smooth surface of spores, and connected to the string (Figure 7A).

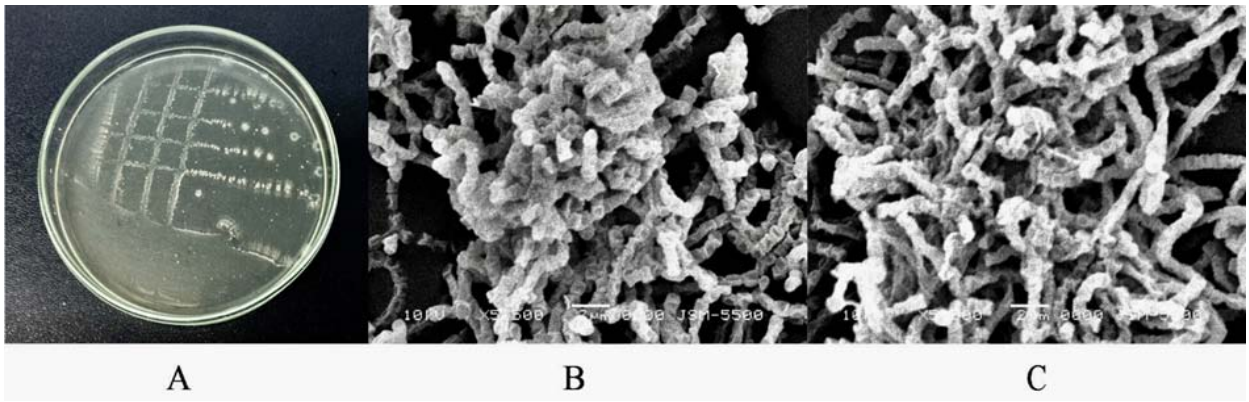


Figure 7: Colonies of Streptomyces laurentii ANTHOIDONG 11.1 (A) shapes and spores of S. laurentii ANTHOIDONG 11.1 under SEM (B and C)

Streptomyces laurentii isolated from soil and they have the ability of Thiostrepton production (Trejo-Astreda *et al*., 1998). In this study, *Streptomyces laurentii* ANTHOIDONG 11.1 strain had the ability of agaisnt *Bacillus cereus*.

With the fourth strain, *Streptomyces tanashiensis* ANTHOIDONG 3.2 had characteristic of colonies as white color, circular shape, curled margin, pulvinate elevation, average diameter 6 mm; cells had circular, connected to short spring, no motile under observation of microscope; strait spores chain, smooth of surface of spore under SEM (Figure 8A, B, C).

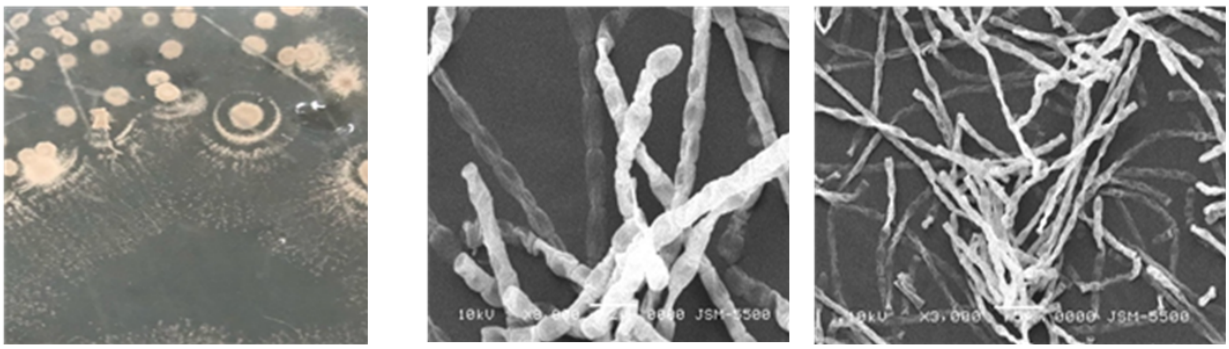


Figure 8: Colonies of *Streptomyces tanashiensis* ANTHOIDONG 3.2 (A) shapes and spores of *S. tanashiensis* ANTHOIDONG 3.2 under SEM (B and C)

Streptomyces tanashiensis isolated from mangrove forest soil Maowei, China, it had the ability of antibacterial activity to *Bacillus cereus*(Lu *et al.*, 2019). According to Johnson and Dietz (1968),*Streptomyces tanashiensis* synthesized and secrete out to luteomycin (antifungal), mithramycin (anticancer), phosphoramidon (inhibition of enzyme thermolysin from *Bacillus* spp.) and kalafungin (antifunfal, anti-protozoa, and anti-gram-positive).The resaerch'Singh *et al.* (2009), showed that *Streptomyces tanashiensis* had the ability of against gram-posive bacteria and gram-negative bacteria together with it resistances to *Candida albicans* and *Fusarium moniliform*.

In the fifth strain, *Streptomyces albogriseolus* ANTHOIDONG 7.1 strain had characteristic of colonies as circular shape, one concentric ring, curled margin, pulvinate elevation, diameter of colony 5 mm; cells had circular and motile under light microscope; spiral spore chain, spiny surface of spore under observation of SEM (Figure 9).

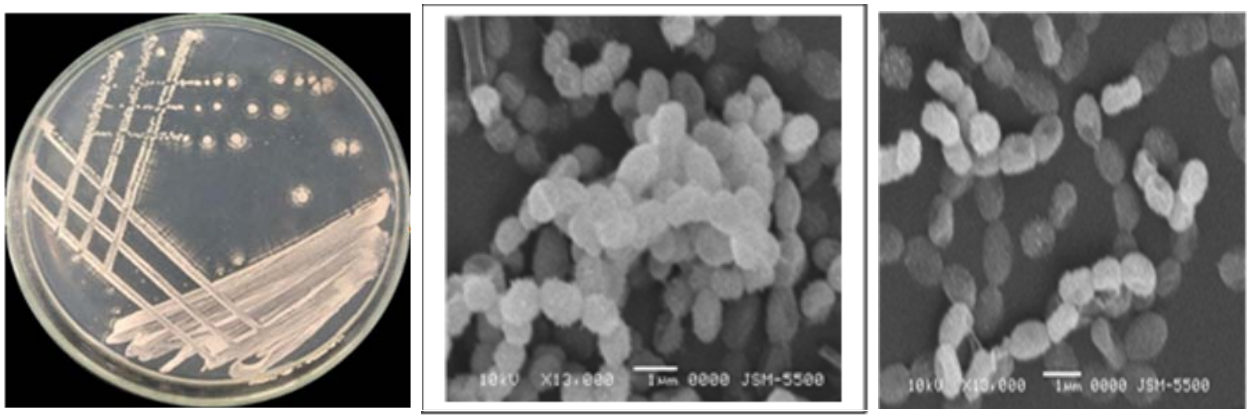


Figure 9: Colonies of *Streptomyces albogriseolus* ANTHOIDONG 7.1 (A) shapes and spores of *Streptomyces albogriseolus* ANTHOIDONG 7.1 under SEM (B and C)

Streptomyces albogriseolus isolated from mangrove forest soil of Hainan, China. They had the ability of Macrocyclic Lactones production, this compound against *Staphylococcus aureus* and *Escherichia coli*(Xu *et al.*, 2014). Qattan and Khattab (2019), *Streptomyces albogriseolus* had the ability of neomycin production, antibacterial compound, in the stress condition. Besides, Shao *et al.* (2019) determined *Streptomyces albogriseolus* using of polyethylene as carbon resource.

Streptomyces parvulus ANTHOIDONG3.1 strain had colonies had brown dark, circular shape and 1 long ray, curlved margin, pulvinate elevation, diameter of colony 5 mm; cells had coccus, motile wken observation under light microscope, spores had long chain; *Streptomyces parvulus* had spiral spore chain, warty on surface of spore under SEM. (Figure 10A, B,C).

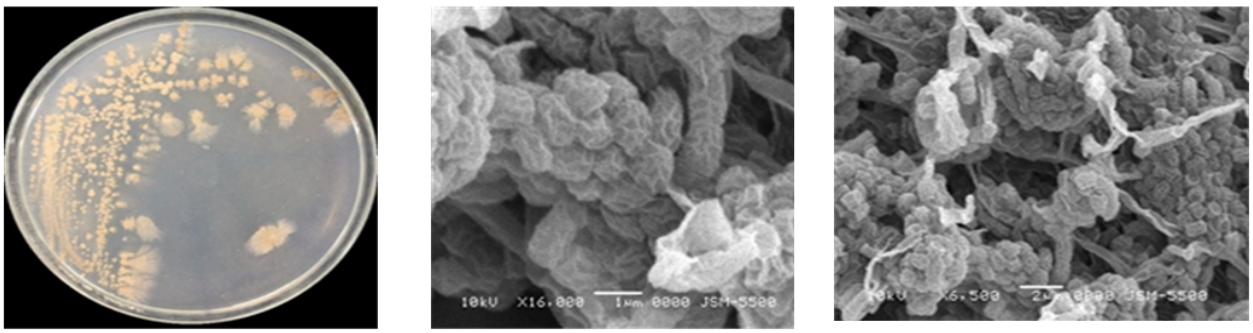


Figure 10: Colonies of *Streptomyces parvulus* ANTHOIDONG 3.1 strain (A) shapes and spores of *Streptomyces parvulus* ANTHOIDONG 3.1 strain under SEM (B and C)

Streptomyces parvulus isolated from mangrove forest soil Visakhapatnam, Bengal by Prakasham *et al.* (2014), they had the ability of antibacterial activity as polypeptide (Actinomycin D) to agaisnt with 14 kinds of human pathogenic bacteria. *Streptomyces parvulus* had the ability of resistance to *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus fecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaricus* and *Escherichia coli* when test of antimicrobial activity by diffusion-well agar method(Usha et al. 2010),

The seventh strain, *Streptomyces africanus* LONGHOA 4.2 strain had white colonies color, circular shape, concentric circle on ISP medium. Under SEM (7,500 X), spores of *Streptomyces africanus* LONGHOA 4.2 strain had smooth surface, rods connected to string (Figure 11 A, B, C)

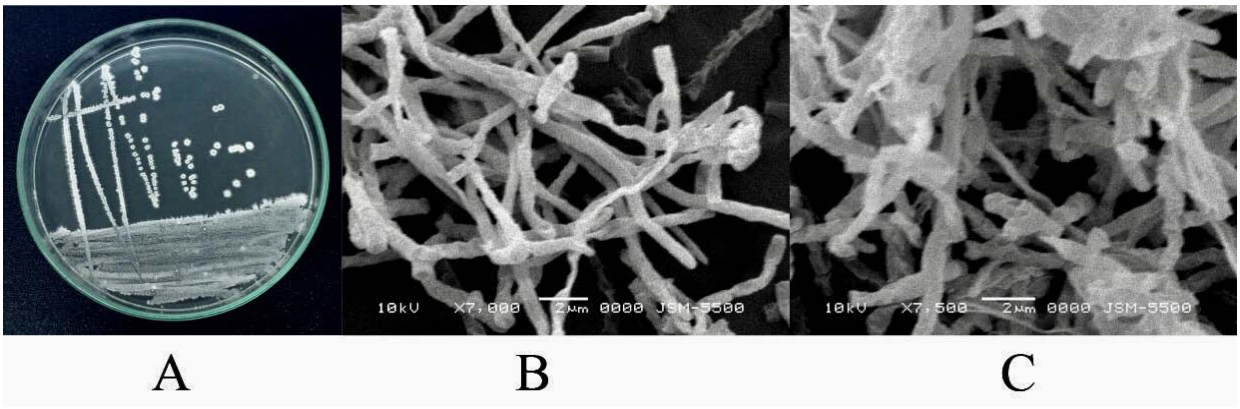


Figure 11: Colonies of *Streptomyces africanus*LONGHOA 4.2 strain (A) shapes and spores of *Streptomyces africanus* LONGHOA 4.2 strain under SEM (B and C)

Streptomyces africanus isolated from soil at Cape Town, South Africa and they had low antibacterial activity to *Enterococcus faecium* (Paul *et al*., 2004). In this study, *Streptomycesafricanus* LONGHOA 4.2 strain had good antibacterial activity to *Bacillus cereus*.

The final strain, *Streptomyces tendae* THANHAN 4 with the colonies had dark milk color, circular shape, curved margin, pulvinate elevation, diameter from 1.5 to 3 mm; cells had rods shape and motility. In this study, *Streptomyces africanus* LONGHOA 4.2 strain only had the ability of resistance to gram-positive bacteria as *Bacillus cereus* and *Staphylococus aureus* at average level.

In order to test the other ability of *Streptomyces* isolates, 40 isolates were chosen to survey the ability of CMC (cellulose) degradation and phoshate solubilization. The isolates were grown on ISP medium in 15 days and supernatants were dropped into the wells on ISP added CMC agar (for degraded CMC test) and NBRIP (for phosphate solubilization test) and incubation in 21 days at room temperature. The results from Table 3 and Table 4 showed that 12/40 isolates had CMC degradation ability among two strains *Streptomycescelluloflavus* ANTHOIDONG 4.1 and *Streptomyces africanus* LONGHOA 4.2 had good cellulase synthesis ability and 12/40 isolates solubilzed insolving phosphate among *Streptomyces parvulus* ANTHOIDONG 3.1, *Streptomyces tanashiensis* ANTHOIDONG 3.2 and *Streptomyces albogriseolus* ANTHOIDONG 7.1 solubilozed phosphate. Therefore, five in twelve strains were sequenced and identified as species of genus *Streptomyces*. Actinobacteriafrom rhizosphere have the direct promotion of plant growth as nitrogen fixation, solubilization of Phosphorus and Potassium, IAA production and the indirect promotion of plant growth as Production of Antibiotics, Production of Lytic Enzymes, Production of Siderophores…(Yadav et al., 2018). Besides antibacterial compounds producing by *Streptomyces*species, our results also received 5/8 strains with capable of dissolving cellulose (CMC) and insoluble phosphate.

Table 3: The ability of CMC degradation of 12/40 actinobacterial isolates

No.	Isolate	Diameter of well (mm)	No.	Isolate	Diameter of well (mm)
01	LYNHON 6.1	24.33 bc	07	ANTHOIDONG 33	19.33 d
02	LYNHON 7.2	09.33 d	08	ANTHOIDONG 5.1	27.67 b
03	LYNHON 8.1	11.33 d	09	LONGHOA 4.1	5.45 cd
04	ANTHOIDONG 4.1	40.00 a	10	LONGHOA 4.2	06.22 c
05	LONGHOA 4.3	30.67 b	11	CANTHANH 1.1	03.93 d
06	LONGHOA 5.1	31.00 b	12	ANTHOIDONG 31	12.52 b

Means within a column followed by the same letter/s are not significantly different at $p < 0.01$

Table 4: The ability of phosphate solubilization of 12/40 actinobacterial isolates

No.	Isolate	Diameter of well (mm)	No.	Isolate	Diameter of well (mm)
01	LYNHON 7.1	05.67 cd	07	ANTHOIDONG 3.4	02.03 d
02	LYNHON 7.2	05.01 d	08	ANTHOIDONG 3.5	05.67 cd
03	LYNHON 8.2	04.67 d	09	ANTHOIDONG 6.1	06.67 c
04	ANTHOIDONG 3.1	04.33 d	10	ANTHOIDONG 6.2	03.01 d
05	ANTHOIDONG 3.2	10.67 b	11	ANTHOIDONG 7.1	02.45 d
06	ANTHOIDONG 3.3	12.67 b	12	ANTHOIDONG 7.2	19.03 a

Means within a column followed by the same letter/s are not significantly different at p < 0.01

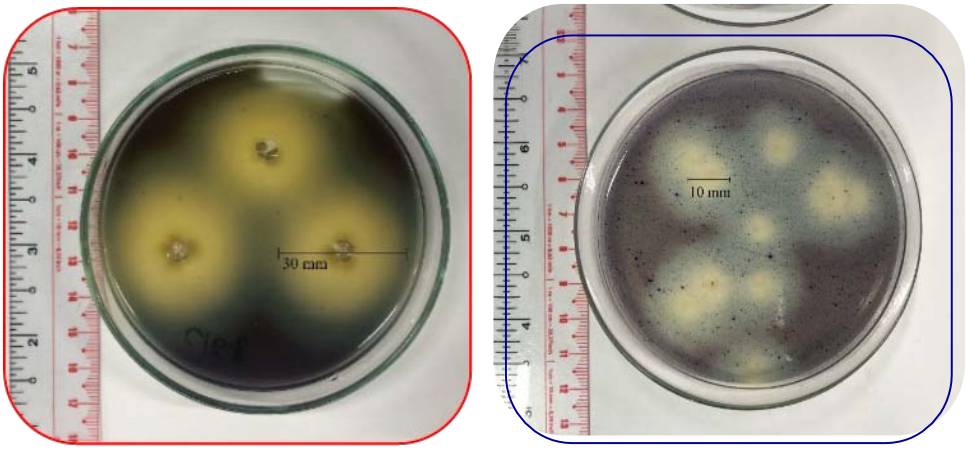
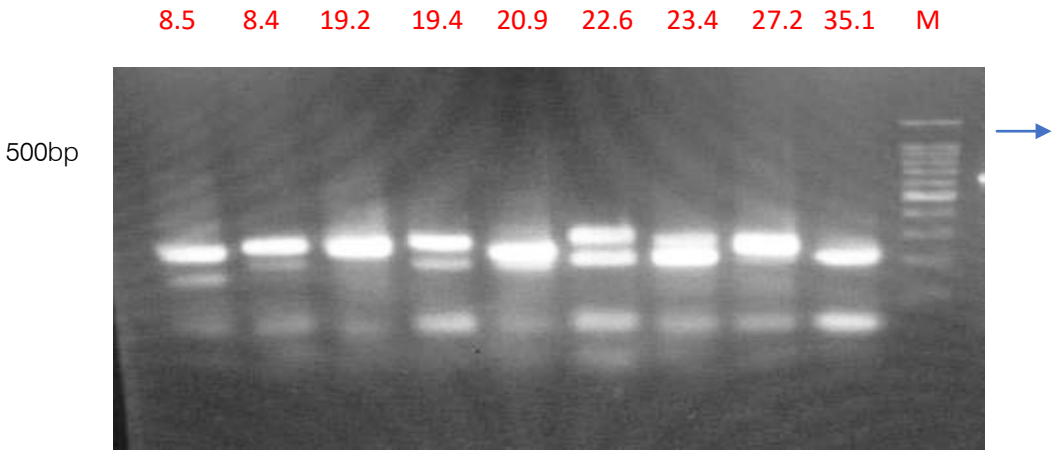


Figure 12: CMC degradation of two strains of *Streptomyces* on NBRIP plus CMC

The biosynthetic potential of 8 strains was investigated by the detection of polyketidesynthetase (PKS) and non-ribosomal polyketidesynthetase (NRPS) genes, the hall marks of secondary metabolites production. Results showed that many isolates were positive for PKS-I (2/8), without PKS-II (0), and NRPS (8/8) genes, indicating that mangrove Actinobacteria have significant biosynthetic potential. Our results highlighted that mangrove environment represented a rich reservoir for isolation of Actinobacteria, which are potential sources for discovery of antimicrobial secondary metabolites. Eight strains against Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) which were indicated with NPKS genes (8/8) while four strains resistant to Gram-negative bacteria (*E. coli* and *Vibrio parahaemolyticus*)which indicated with PKS-I genes (3/8) (3 strains: *Streptomycescelluloflavus* ANTHOIDONG 4.1, *Streptomyces albogriseolus* ANTHOI -DONG 7.1 and *Streptomyces parvulus* ANTHOIDONG 3.1) (Figure 13 and Table 5).



Note: 8.5: THANHAN 4.5, 8.4: THANHAN 4.4, 19.2: ANTHOIDONG 3.1, 19.4: ANTHOIDONG 3.2; 20.9: ANTHOIDONG 4.1; 22.6: ANTHOIDONG 6.1; 23.6; ANTHOIDONG 7.1; 27.2: LONGHOA 4.2; 35.1: ANTHOIDONG 11.

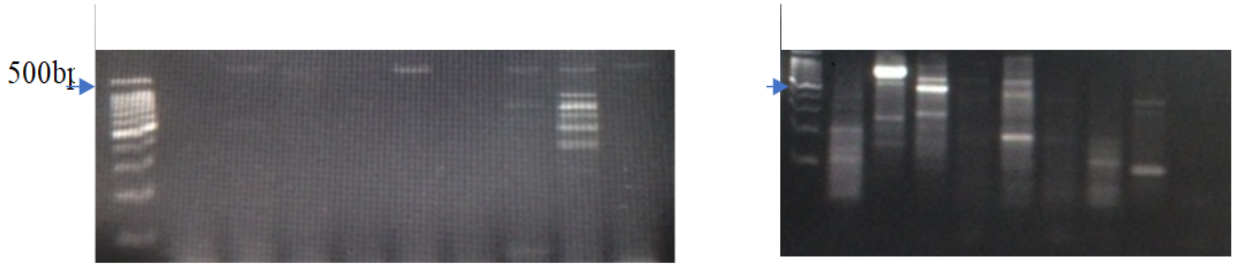


Figure 13: Agarose gel electrophoresis of PCR products from DNA isolated from representative actinomycete strains: (A) Selective amplification of 700–800 bp fragments using primers A3F/A7R specific for NRPS adenylation sequences; (B) Selective amplification of the 1200–1400 bp fragments using K1F/K2R and K1F/M6R; (C) Specific primers for PKS-I ketosynthase and methyl-malonyl-CoA transferase sequences

Table 5: Distribution of NRPS, PKS-I, and PKS-II in 8 *Streptomyces* species or strains

No	Taxa	NPRS (A3F/A7R)	PKS-I		PKS-II (KSα/ KSβ)
			(K1F/K2R)	(K1F/M6R)	
01	*Streptomyces tendae* THANHAN 4	+			
02	*Streptomyces tanashiensis* ANTHOIDONG 3.2	+	+		
03	*Streptomyces pavulus* ANTHOIDONG 3.1	+	+		
04	*Streptomyces celluloflavus* ANTHOIDONG 4.1	+		+	
05	*Streptomyces aegyptia* ANTHOIDONG 6.1	+			
06	*Streptomyces africanus* LONGHOA 4.2	+			
07	*Streptomyces albogriseolus* ANTHOIDONG 7.1	+		+	
08	*Streptomyces laurentii*ANTHOIDONG 11.1	+			

The high rate of detection of PKS-I and NPKS genes in the isolates tested was mostly *Streptomyces*, providing strong evidence for the high potential of *Streptomyces* to produce high number of biologically active metabolites; the fact complies with other researches. Therefore, the molecular screening of Actinobacteria isolates for genes encoding biosynthesis of bioactive compounds is still an effective and valuable approach for pre-selecting isolates for useful secondary metabolites production (Ginolhac et al., 2004, Qui et al., 2009; Courtois et al., 2003; Hornung et al., 2007; Mets¨a-Ketel¨a et al., 1999; Schneemann, et al., 2010).

Can Gio mangrove forest located on the east coast of HoChiMinh city Vietnam is mostly unexplored the microbe resources; especially actinobacteria communnities. Therefore, this location is anticipated to be able to provide a rich source of Actinobacteria, the prolific producers of antimicrobial secondary metabolites. To date, no studies have reported the diversity and antimicrobial activities of Actinobacteria from Can Gio mangrove environment. For this reason, there is a high possibility to identify novel Actinobacteria and discover valuable antimicrobial secondary metabolites.

IV. Conclusion

Sixty-three actinomycetes were isolated from 25 soil samples of 9 different sites in mangrove forest in Can Gio, HoChiMinh city, Vietnam; Selected 29/63 isolates could produce antimicrobial active metabolites inhibiting at least one of the test pathogens and 8 best isolates were chosen to identify by 16S rRNA technique and sequencing. Eight strains belonged to genus *Streptomyces* among 5/8 strain had ability of dissolving cellulose (CMC) and insoluble phosphate, especially three strains: *Streptomycescelluloflavus* ANTHOIDONG 4.1,*Streptomyces albogriseolus* ANTHOIDONG 7.1 and *Streptomyces parvulus* ANTHOIDONG 3.1 had the ability of antibacterial activity with 4 human pathogenic bacteria.

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References Références Referencias

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403-410.
2. Anzai K, Ohno M, Nakashima T, Kuwahara N, Suzuki R, Tamura T, et al. 2008. Taxonomic distribution of *Streptomyces* species capable of

producing bioactive compounds among strains preserved at NITE/NBRC. Appl Microbiol Biotechnol; 80: 287–95.

3. Ara, I, Matsumoto, A, Abdul Bakir, M, Kudo, T, Omura, S, Takahashi, Y. 2008. *Actinomadura maheshkhaliensis* sp. nov., a novel actinomycete isolated from mangrove rhizosphere soil of Maheshkhali, Bangladesh. J. Gen. Appl. Microbiol. 54: 335−342. http://dx.doi.org/10.2323/jgam.54.335.
4. Araújo, MH de Faria.Ottoboni Dias, AN, Oliveira, LA.Pereira, CS. Kuffner, BHB. Sachs. D. Silva, D. 2018. Microstructural and mechanical analysis of 316L SS/ β-TCP composites produced though the powder metallurgy route. Int. Eng. J., Ouro Preto; 71(4), 605-611. http://dx.doi.org/10.1590/0370-4467 2017710175 REM
5. Ayuso-Sacido, A. Genilloud, O. 2005. New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: detection and distribution of these biosynthetic gene sequences in major taxonomic groups. Microbial Ecology, 49(1): 10–24.
6. Ayuso, A. Clark, D. González, I, Salazar, O, Anderson, A, Genilloud, O. 2005. A novel actinomycete strain de-replication approach based on the diversity of polyketide synthase and nonribosomal peptide synthetase biosynthetic pathways. Appl. Microbiol. Biot. 67: 759-806
7. Berdy, J. 2005. Bioactive microbial metabolites. J. Antibiot. (Tokyo) 58: 1−26. http://dx.doi.org/10.1038 /ja.2005.1.
8. Brinda, A. Mathew, A. 2012. Molecular Characterization and Identification of Unknown Bacteria from Waste Water. Indian Journal of Innovations and Developments, 1: 87-91.
9. Courtois, S. Cappellano, CM. Ball, M. et al. 2003. Recombinant environmental libraries provide access to microbial diversity for drug discovery from natural products, Applied and Environmental Microbiology, 69(1):49–55.
10. Dharmaraj, S. 2010. Marine *Streptomyces* as a novel source of bioactive substances. World J. Microb. Biot. 26: 2123-2139.
11. El-Naggar, NE. Sherief, AA. Hamza, SS. 2011. *Streptomyces aegyptia* sp. nov., a novel cellulolytic streptomycete isolated from soil in Egypt. Afr J Microbiol Res; 29: 5308–15.
12. El-Naggar NE, Mohamedin, A. Hamza, SS. Sherief, Abdel-Dayem. 2018. Extracellular Biofabrication, Characterization, and Antimicrobial Efficacy of Silver Nanoparticles Loaded on Cotton Fabrics Using Newly Isolated *Streptomyces* sp. SSHH-1E. Journal of Nanomaterials; Article ID 3257359, 17 pages; Hindawi Publishing Corporation http://dx.doi.org/ 10.1155/2016/3257359
13. Feling, RH, Buchanan, GO, Mincer, TJ, Kauffman, CA, Jensen, PR, Fenical, W. 2003. Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*. Angew Chem Int Ed Engl.; 42: 355–7.
14. Galindo AB. 2004. *Lactobacillus plantarum* 44A as a live feed supperlement for freshwater fish. PhD thesis, Wageningen University, The Netherlands.
15. Ginolhac, A. Jarrin, C. Gillet, B. et al. 2004. Phylogenetic analysis of polyketide synthase I domains from soil metagenomic libraries allows selection of promising clones. Applied and Environmental Microbiology. 70(9): 5522–5527.
16. Gopi, M. Kumaran, S. Kumar, TTA. Deivasigamnani, B. Alagappan, K. Prasad, SG. 2012. Antibacterial potential of sponge endosymbiont marine *Enterobacter* sp at Kavaratti Island, Lakshadweep archipelago. Asian Pac. J. Trop. Med. 5: 142-146
17. Kumar, S. Stecher, G. Li, M. Knyaz, C. Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol. 35: 1547-1549
18. Hornung, A. Bertazzo, A. Dziarnowski, A. et al. 2007. A genomic screening approach to the structure-guided identification of drug candidates from natural sources. Chem Bio Chem, 8(7): 757–766.
19. Huang, H. Lv, J. Hu, Y. Fang, Z. Zhang, K. Bao, S. 2008. *Micromonospora rifamycinica* sp. nov, a novel actinomycete from mangrove sediment. Int J Syst Evol Microbiol; 58:17−20.
20. Johnson, LE. Dietz, A. 1968. Kalafungin, a new antibiotic produced by *Streptomyces tanashiensis* Strain Kala. Appl Microbiol. 16(12): 1815-1821.
21. Lu, QP. Ye, J. Huang, Y. Liu, D. Liu, L. Dong, K. Razumova, EA. Osterman, IA. Sergiev, PV. Dontsova, OA. Jia, S. Huang, D. Sun, C. 2019. Exploitation of Potentially New Antibiotics from Mangrove Actinobacteria in Maowei Sea by Combination of Multiple Discovery Strategies. Antibiotics (Basel). 8(4): 236.
22. Malik, S. Beer, M. Megharaj, M, Naidu, RR. 2008. The Use of Molecular Techniques to Characterize the Microbial Communities in Contaminated Soil and Water." Environment International, 34: 265-276. http://dx.doi.org/10.1016/j.envint.2007.09.001.
23. Manikandan S, Ganesapandian S, Sangeetha N, Kumaraguru AK. 2014. Antimicrobial activity of marine sponges associated some marine bacterial metabolites against multi drug resistance human pathogens. Res. J. Microbiol. 9: 25-33
24. Mets¨a-Ketel¨a, M. Salo, V. Halo, L.et al. 1999. An efficient approach for screening minimal PKS genes from *Streptomyces*. FEMS Microbiology Letters, 180(1): 1–6.

25. Mohseni, M. Norouzi, H. Hamedi, J. Roohi, A. 2013. Screening of antibacterial producing actinomycetes from sediments of the Caspian sea. Int. J. Mol. Cell Med. 2: 64-71
26. McMurray, S. Blum, J. Pawlik, J. 2008. Redwood of the reef: growth and age of the giant barrel sponge *Xestospongia muta* in the Florida Keys. Marine Biology. 155:159-171.
27. Osama, S. Ayesha, S. Mohammad, FI. 2014. Effects of papaya leaves on thrombocyte counts in dengue: A case report. J. Pakist Med Assoc. 64:364–366.
28. Ottoni, CA, Amini, MS., Alam, I., Alzubaidy, H., Mokhtar, N., Archer, JAC, Bajic, VB. 2015. Soil and rhizosphere associated fungi in gray Mangroves (*Avicennia marina*) from the Red Sea–a metagenomic approach. Genom. Prot. Bioinform. 13: 310–320. http://dx.doi.org/10.1016/j.gpb.2015.07.002.
29. Pace, NR. 1997. A Molecular View of Microbial Diversity and the Biosphere. Science, 272: 734-740. http://dx.doi.org/10.1126/science.276.5313.734.
30. Pallaa, MS, Guntuku, GS, Muthyala, MKK, Pingalia, S., Sahub, PK. 2018. Isolation and molecular characterization of antifungal metabolite producing actinomycete from mangrove soil. Beni-Suef University Journal of Basic and Applied Sciences; 7: 250–256.https://doi.org/10.1016/j.bjbas.2018.02.006
31. Paul, R. Dwight, R. Fenical, W. 1991. Distribution of Actinomycetes in Near- Shore Tropical Marine Sediments, Applied and Environmental Microbiology.57(3): 1102-1108.
32. Prakasham, RS. Kumar, BS. Kumar, YS. Kumar, KP. 2014. Production and characterization of protein encapsulated silver nanoparticles by marine isolate Streptomyces parvulus SSNP11. Ind. J. Microbiol. 54: 329–336. doi: 10.1007/s12088-014-0452-1
33. Qattan, SYA. Khattab, AA. 2012. Molecular Characterization *of Streptomyces albogriseolus* Excellent Mutants for Neomycin Production. Journal of Pure and Applied Microbiology. 13(3): 1489-1498.
34. Qin, S. Li, J. Chen, H.-H. et al. 2009. Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuang banna China. Applied and Environmental Microbiology, 75(19): 6176–6186.
35. Reysenbach AL, Giver LJ, Wickham GS, Pace NR. 1992. Differential amplification of rRNA genes by polymerase chain reaction. Appl. Environ. Microb. 58: 3417-3418.
36. Schneemann, I. Nagel, K. Kajahn, I. Labes, A. Wiese, J. Imhoff, JF. 2010. Comprehensive investigation of marine actinobacteria associated with the sponge *Halichondria panicea*. Applied and Environmental Microbiology, 76(11): 3702–3714.
37. Shao, H. Chen, M. Fei, X. Zhang, R. Zhong, Y. Ni, W. Tao, X. He, XY. Zhang, E. Yong, B. Tan, X. 2019. Complete Genome Sequence and Characterization of a Polyethylene Biodegradation Strain, *Streptomyces albogriseolus* LBX-2. Microorganisms. 7(10): 379.
38. Singh, LS, Mazumder, S. Bora, TC. 2009. Optimisation of process parameters for growth and bioactive metabolite produced by a salt- tolerant and alkaliphilic actinomycete, *Streptomyces tanashiensis* strain A2D. J. Med. Mycol. 19: 225-233.
39. Stach, JEM, Maldonado, LA, Ward, AC, Goodfellow, M. Bull, AT. 20003. New primers for the class Actinobacteria: application to marine and terrestrial environments. Environ. Microbiol. 5: 828-841
40. Tamura, T. Sakane, T. 2005. *Asanoa iriomotensis* sp. nov., isolated from mangrove soil. Int. J. Syst. Evol. Microbiol. 55: 725–727. http://dx.doi.org/10.1099/ijs.0.02982-0.
41. Thatoi, H, Behera, BC, Mishra, RR, Dutta, SK. 2013. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. Ann. Microbiol. 63: 1 –19. http://dx.doi.org/10.1007/s13213-012-0442
42. Trejo-Estrada, S. Paszczynski, A. Crawford, D. 1998. Antibiotics and enzymes produced by the biocontrol agent *Streptomyces violaceusniger* YCED-9. J. Ind. Microbiol. Biotechnol. 21: 81–90. doi: 10.1038/sj.jim.2900549
43. Tuan, VQ. Kuenzer, C. 2012. Can Gio Mangrove Biosphere Reserve Evaluation 2012: Current Status, Dynamics and Ecosystem Services Ha Noi. IUCN, Viet Nam, p. 102.
44. Usha, KM. Sudhakar, P. Sreenivasulu, K, Vijayalakshmi, M. 2011. Optimization of Culturing Conditions for Improved Production of Bioactive Metabolites by *Pseudonocardia* sp. VUK-10. Mycobiology, 39: 174-181.
45. Xu, J. Wang, Y. Xie, SJ. Xu, J. Xiao, J. Ruan, JS. 2009. *Streptomyces xiamenensis* sp. nov., isolated from mangrove sediment. Int. J. Syst. Evol. Microbiol. 59: 472–476. http://dx.doi.org/10.1099/ijs.0.000497-0.
46. Xu, DB, Ye, WW, Han, Y, Deng, ZX. Hong, K. 2014. Natural Products from Mangrove *Actinomycetes*. Mar Drugs. 12(5): 2590–2613.
47. Yadav, AN. Priyanka, V. Sunil K., Vinod K. Manish K. Thankappan, C. KS. Bhim PS, Anil KS. Harcharan S.D. 2018. Actinobacteria from Rhizosphere: Molecular Diversity, Distributions, and Potential Biotechnological Applications; New and Future Developments in Microbial Biotechnology and Bioengineering; chapter 2: 13-50 Elsevier B.V. All rights reserved.
48. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173: 697-703.

49. Zhao, K, Penttinen, P. Guan, T. Xiao, J. Chen, Q. Xu, J. Lindstrom, K. Zhang, L. Zhang, X. Strobel, GA. 2011.The diversity and anti-microbial activity of endophytic actinomycetes isolated from medicinal plants in Panxi Plateau, China. Curr. Microbiol. 62: 182-190.

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Phyto-Melatonin and Immunity: A Review

By Dr. Somenath Ghosh

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Abstract- The basic structure of melatonin is evolutionarily conserved. Hence, it was speculated that melatonin may be present in different animals (from unicellular to multi-cellular) and even in plants. Melatonin in plans is generally regarded as phyto-melatonin. Like the role of melatonin in animals, phyto-melatonin can perform a number of functions like attenuation of apoptosis, prevention of free radical generation, protection against UV irradiation etc. But, unlike phyto-estrogen, the role of phyto-melatonin in animals is totally an unexplored area. Hence, aim of the present study was to note the role of phyto-melatonin in maintenance of general health and immunity of goats. To fulfil the aim, we supplemented the goats with phyto-melatonin rich diet i.e. corn (*Zea mays*) which is having 1.4 ng/gm of dry weight of tissue and they are also edible to goats. We noted significantly high level of body weight, haematological (AST, ALT level, total RBC count and %Hb), immunological (TLC, %LC, %SR of PBMCs), metabolic (plasma glucose, cholesterol, HDL, LDL, protein levels and HDL: LDL ration), free radical (SOD, catalase, GPx levels), hormonal (estrogen, melatonin), cytokine (IL-6 and TNF-α) levels and significantly low level of MDA.

Keywords: *cytokine, goat, immunity, melatonin, phyto-melatonin, supplementation.*

GJSFR-C Classification: *FOR Code: 069999*

PHYTOMELATONINANDIMMUNITYAREVIEW

Strictly as per the compliance and regulations of:

Phyto-Melatonin and Immunity: A Review

Dr. Somenath Ghosh

Abstract- The basic structure of melatonin is evolutionarily conserved. Hence, it was speculated that melatonin may be present in different animals (from unicellular to multi-cellular) and even in plants. Melatonin in plans is generally regarded as phyto-melatonin. Like the role of melatonin in animals, phyto-melatonin can perform a number of functions like attenuation of apoptosis, prevention of free radical generation, protection against UV irradiation etc. But, unlike phyto-estrogen, the role of phyto-melatonin in animals is totally an unexplored area. Hence, aim of the present study was to note the role of phyto-melatonin in maintenance of general health and immunity of goats. To fulfil the aim, we supplemented the goats with phyto-melatonin rich diet i.e. corn (*Zea mays*) which is having 1.4 ng/gm of dry weight of tissue and they are also edible to goats. We noted significantly high level of body weight, haematological (AST, ALT level, total RBC count and %Hb), immunological (TLC, %LC, %SR of PBMCs), metabolic (plasma glucose, cholesterol, HDL, LDL, protein levels and HDL: LDL ration), free radical (SOD, catalase, GPx levels), hormonal (estrogen, melatonin), cytokine (IL-6 and TNF-α) levels and significantly low level of MDA. However, plasma testosterone was unaffected upon phyto-melatonin treatment. Thus, for the first time role of phyto-melatonin as a protective molecule with improving effect on the health and immunity of Indian goat *Capra hircus*is being proposed, as the effect of phyto-melatonin supplementation can be brought back to normal and this dietary supplement might be utilizing the similar pathway as commercial melatonin. There are so many less expensive and readily available sources of phyto-melatonin that requires the proper knowledge of exploitation of these sources for extreme benefit for animals as well as for the human beings in near or far future.

Keywords: cytokine, goat, immunity, melatonin, phyto-melatonin, supplementation.

I. Introduction

The basic structure of melatonin is evolutionarily conserved (Reiter et al., 2007). Hence, it was speculated that melatonin may be present in different animals (from unicellular to multi-cellular) and even in plants. Around nineteenth century the presence of melatonin in plants were evidenced by scientists (Dubbels et al., 1995). Recent studies have suggested that not only the higher plants but also different macro and micro alga (Balzer et al., 1998), red algae (Lorenz and Lu¨ning, 1998), metazoans (Hardeland and Poeggeler, 2003) are also having substantial amount of phyto-melatonin. But, the presence of phyto-melatonin in other plant groups like bryophyta, pteridophyta and most gymno-sperms is under controversy (Reiter et al., 2007). The amount of phyto-melatonin present in plants is also variable due to the extraction and quantification techniques in different tissues of plants (Badria, 2002). It is also evidenced that plants can directly absorb melatonin from soil which has come to the soil upon degradation of different micro-organisms and fungi (Tan et al., 2007; Muller and Hardeland, 1999). However, it is also evidenced by several studies that plants can also synthesize melatonin (Tan et al., 2007) and melatonin can be stored in different body parts of edible plants like fruits (Reiter et al., 2007), dry seed (Badria, 2002) etc. The physiological function of phyto-melatonin in plants is almost similar to those of animals. Phyto-melatonin is responsible for circadian time management (Kola´r and Macha´ckova´, 2001), protection against harsh environment (Posmyk et al., 2008), promotion of vegetative growth (Herna´ndez-Ruiz et al., 2004), attenuation of apoptosis (Lei et al., 2004), scavenging of free radicals (Tan et al., 2007) under normal or physiologically stressed conditions like UV irradiation (Tettamanti et al., 2000).

In some countries of Europe, the common maize (*Zea mays*) is offered to cattle for milk enhancement (www.fao.org) and the corn seed is most popular to farmers, breeders and raisers due to their palatability to the cattle. However, in corn the amount of phyto-melatonin is less (1.4 ng/gm of dry weight of tissue) than other phyto-melatonin rich seeds like white mustard seed (189 ng/gm of dry weight of tissue). The role of phyto-melatonin in regulation of different physiological functions in animals is totally lacking. We identified the lacunae of previous studies and therefore, the aim of the present study was to note the role of phyto-melatonin rich diet (i.e. *Zea mays*) in regulation of immunity and physiology of Indian goat *Capra hircus.*

II. Materials and Methods

a) Animals and maintenance

Goats of approximately same age (~1 year) and weight (~20 ± 2 kg) were procured from commercial goat raiser and then were housed in goat shelter under natural conditions of Varanasi ($25^0$18' N, 83^0 01' E, India) in order to maintain a consistency in food and hygiene throughout the year. At the time of procurement, the goats were weighed (Calf Weighing Sling, Munk's Livestock, Kansas, USA) and the age was determined by dentition as described by Fandos et al. (1993). The male and female goats were kept separately to avoid mating or pheromonal effects. The detection of

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heat period was purely based on the visual observations i.e. more vocalization, reddening of vulva and mucorrhea. Goats were fed with usual ration of roughages (dry and green) and concentrate as suggested by Central Institute for Research on Goats, (CIRG), Mathura, Uttar-Pradesh, India. Single goat generally requires 4-5 kg of fodder/day and was fed with usual ration made up of roughages (dry and green) and concentrate. Dry roughages contained crushed barley (*Hordeum vulgare*, 1 part), crushed maize (*Zea mays*, 2 parts), linseed (*Linumusitatissimum*) or mustard seed cake (*Brassica juncea*, 2.25 parts), rice bran (*Oryza sativa*, 2 parts) along with small amount of molasses or a pinch of salt when required. Green roughages contained maize (*Zea mays*), elephant grass (*Pennisetum purpureum), pearl millet (Pennisetum glaucum*), sorghum (*Sorghum* sp.) and oat (*Avena sativa*). The concentrate contained oilseed cakes and soaked gram (*Cicer arietinum*) and water *ad libitum*. They were exposed to 8 hours outdoor for free grazing and 16 hours indoor (during night) conditions. Health of the goats was monitored by noting down the body temperature (normal rectal temperature, 102.5^0F–103^0F) and rumen movement by authorized veterinary doctors. Goats were treated with helminthicide twice per year and 0.5% solution of malathion (acaricidal baths) as described by Chowdhury et al. (2002). The slaughtering of the goats was performed according in the city abattoir to the Slaughter of Animal Act under "Central Provinces Gazette" 1915 and modified in 2002. All the experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional practice within the framework of revised Animal (Specific Procedure) Act of 2007 of Government of India on animal welfare. The study was carried out during three major seasons of a year i. e. summer, monsoon and winter. Thus, the climatic condition during summer months was (April–June, temperature $43.87^0 \pm 1.02^0$ C, percent relative humidity [%RH] 36.74 ± 4.28%, day length, light–dark cycle-13.42 hours:10.18 hours), monsoon months (July–September, temperature $28.68^0 \pm 2.76^0$ C, %RH 87.04 ± 3.50%, day length, light–dark cycle-12 hours:12 hours), and winter months (November–January, temperature $10.76^0 \pm 3.63^0$ C, %RH 64.12 ± 3.05%, day length, light–dark cycle 10.35 hours: 13.25 hours). All of the results were validated with the samples collected from CIRG in a seasonal manner.

i. *Experimental design*

A total number of 12 goats (six males and six females) were selected from the flock for every month of winter season (i.e. n = 6/sex/every month of winter) and were numbered on ears. Thus, for winter, the total numbers of male goats were 18 and the total numbers of female goats were also 18. Hence, for winter the total number of males and females were 36 (18 males + 18 females). The goats were were fed with 250 gm (~350ng melatonin) of maize/animal/day as dietary supplement during the months of winter season. The study was continued for 40 days. After completion of entire experimental period blood of both male and female goats were collected (as described in materials and methods section) and processed for different hematological, biochemical, hormonal, immunological and free radical parameters.

ii. *Measurement of body weight*

The goats were weighed using Calf Weighing Sling of Munk's Livestock, Kansas, USA.

b) Hematological parameters

i. *Estimations of AST and ALT activities in plasma*

Aspartate aminotransferase (AST) also known as glutamate oxaloacetate transaminase (GOT) is a transaminase. The principle of AST estimation is as follows:

Kinetic determination of the aspartate aminotransferase (AST) activity: L-Aspartate + α - Ketoglutarate ----------> Oxaloacetate + L-Glutamate Oxaloacetate + NADH +H^+-->L-Malate + NAD^+. These levels may be estimated as plasma levels and can be regarded as an assay of hepatotoxicity upon hormonal/drug treatment.

Alanine aminotransferase (ALT) also known as glutamate pyruvate transaminase (GPT) is a transaminase. The principle behind the ALT test is as follows:

$$\text{L-Alanine} + \alpha\text{- Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+.$$

ii. *Assessment of Red Blood Cell (RBC) count in blood*

The total blood (as collected from peripheral circulation) was taken in RBC counting tube with RBC fluid i.e. Ringer's solution, mixed well and the entire mixture is placed on Neubaeur Chamber and RBCs were counted.

iii. *Assessment of % haemoglobin (%Hb) blood*

% Hb in blood was estimated with the help of Sahli's hemoglobin meter (Systonic Instruments, India). In principle, hemoglobin is converted to acid haematin by the action of HCL (0.1N). The acid haematin solution is further diluted with HCl until its color matches exactly with that of the permanent standard of the comparator block. The hemoglobin concentration is read directly from the calibration tube. The % hemoglobin was found to be calculated from the standard value as grafted on the standard tube.

c) *Immunological parameters*

i. *Total Leukocyte Count (TLC)*

Blood was taken in a WBC pipette and diluted 20 times in Natt-Herrick diluents and white blood cells counted in Neubauer's counting chamber (Spencer USA) under the microscope. For DLC, a thin blood film was prepared and stained with Leishman's stain and leukocyte subpopulations were counted under oil immersion lens of Nikon microscope (Nikon, E200, Japan; Haldar et al., 2004). Lymphocyte counts (no./mm^3) was determined from total and differential leukocyte count by using the following formula:

$$\text{Lymphocyte count} = \frac{\text{TLC X Lymphocyte percent}}{100}$$

ii. *% Lymphocyte Count*

% Lymphocyte count was performed following the protocol of Haldar et al., (2004) as published elsewhere.

d) *% SR of Peripheral Blood Mononuclear Cells (PBMCs)*

i. *Separation of Peripheral Blood Mononuclear Cells (PBMCs)*

Blood was diluted with PBS (RT) in 1:1 ratio. Three mL of Ficoll (HiSep, Cat. No. LSM 1084) was transferred to a 15 mL sterile centrifuge tube. Ficoll was carefully overlaid with 6 mL of diluted blood and mixing of both was avoided. The centrifuge tube was centrifuged (without brake) at 400 × g for 30 minutes at room temperature. Centrifugation at lower temperature was not performed to avoid the cell clumping and poor recovery. After centrifugation, the sedimentation of erythrocytes, polynuclear leukocytes and band mononuclear lymphocytes above was obtained. Supernatant containing plasma and most of the platelets were aspirated carefully without disturbing the interface band. The opaque interface containing the mononuclear cell band was aspirated with the help of a glass Pasteur pipette and transferred in a sterile 15 mL tube. Ten mL of PBS/appropriate cell culture medium was mixed gently with mononuclear cells. The tube was gently inverted several times only to ensure a proper mixing. The mixture was centrifuged for 10 minutes at 250 × g. The supernatant was discarded. This step was repeated for thrice and finally the cell number was counted and viability of the cells ($\geq$95%) was determined with the help of trypan blue exclusion method.

ii. *Cell harvesting and MTT assay*

Cell harvesting and MTT assay was done following the protocol of Pauly et al., (1973) with few modifications as suggested by Ghosh et al., (2013). Plates were incubated at 37^{0}C with 5% CO_2 in incubator (Heracell, Germany) for 48 h and blastogenic response of thymocytes and splenocytes were measured by using a colorimetric assay based on the reduction of tetrazolium salt (3-(4,5-Dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT, SRL, Mumbai, India) following the protocol of Mosmann, (1983). At 48 h, 200 μL of acidified propanol (0.04M HCl in isopropanol) was added to each well and the optical density (OD) of each well was determined with a micro-plate reader (ELx-800, Biotek Instruments, Winooski VT, USA) equipped with a 570 nm wavelength filter. Mean OD values for each set of triplicate were used in subsequent statistical analysis. Response was calculated as percent stimulation ratio (%SR) representing the ratio of absorbance of mitogen stimulated (challenged with Con A) cultures to basal cultures (without Con-A) for each groups.

$$\%\ \text{Stimulation ratio (\%SR)} = \frac{\text{Optical density of Challenged (Con A)} \times 100}{\text{Optical density of Basal}}$$

e) *Metabolic parameters*

i. *Estimation of plasma glucose*

Glucose was estimated by commercially available glucose estimation kit (Beacon India Pvt. Ltd., Mumbai) following manufacturer's protocol. The plasma was directly used for the assay.

ii. *Estimation of plasma cholesterol*

The cholesterol estimation was done by method of Sackett, (1969). The stock solution of cholesterol was made of 1 mg/mL. Then serial dilutions were made from 0-200μg/mL in chloroform for standard curve. A mixture of acetic anhydride and sulphuric acid (20:1) was added to it and incubated in dark for 30 minutes and the O.D. was measured at 640 nm. For experimental plasma samples the cholesterol was extracted in a mixture of ether: ethanol (3:1). Then it was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken out and evaporated to dryness in boiling water bath. Finally it was reconstituted in 5 mL of chloroform and 1 mL of Acetic Anhydride and sulphuric acid (20:1) mixture was added to it and incubated in dark for 30 minutes and the O.D. was measured at 640 nm (ELx-800, Biotek Instruments, Winooski VT, USA).

iii. *Estimation of plasma protein*

Protein was estimated using commercially available Bradford's reagent following the protocol of Bradford (1976). Plasma was directly used for the protein estimation.

iv. *Estimation of plasma HDL, LDL levels and HDL: LDL ration*

Plasma level of HDL and LDL were measured using a commercial kit (Sigma Aldrich, USA, Cat. No. MAK045) following manufacturer's protocol. The lower and upper limits of detections are 2mg/dL to 300mg/dL.

f) Free Radical parameters

i. *SOD activity in plasma*

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed following the method of Das et al. (2000). 0.5 mL of plasma was added to 1.4 mL of reaction mixture comprised of 50 mM phosphate buffer (pH 7.4), 20 mM L-methionine, 1% (v/v) Triton X- 100, 10 mM hydroxylamine hydrochloride, 50 mM ethylene diaminetetraacetic acid (EDTA) followed by a brief pre-incubation at 37 °C for 5 min. Next, 0.8 mL of riboflavin was added to all samples along with a control containing buffer instead of sample and then exposed to two 20W fluorescent lamps fitted parallel to each other in an aluminium foil coated wooden box. After 10 min of exposure, 1 mL of Greiss reagent was added and absorbance of the colour formed was measured at 543 nm on a spectrophotometer (ELx-800, Biotek Instruments, Winooski VT, USA). One unit of enzyme activity is defined as the amount of SOD inhibiting 50% of nitrite formation under assay conditions.

ii. *Catalase activity in plasma*

Catalase (CAT; EC 1.11.1.6) activity was measured following the procedure of Sinha (1972). This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured calorimetrically. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate calorimetrically after heating the reaction mixture. There is production of green color at the end of the process. 10% homogenate of tissues were prepared in PBS (10 mM; pH 7.0) and then centrifuged at 12,000 × g for 20 min at 4^0 C. Supernatant was taken for enzyme estimation. 5 mL of PBS was added to 4 mL of H_2O_2 (200 mM) and then 1 mL of plasma was added. After 1 min 1 mL of this solution was taken in a tube and 2 mL of $K_2Cr_2O_7$ (5%) solution was added. Then it was boiled for 10 min and absorbance was measured at 570 nm (ELx-800, Biotek Instruments, Winooski VT, USA). The activity of CAT was expressed as amount of H_2O_2 degraded per minute.

iii. *GPx activity in plasma*

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was assayed as described by Mantha et al. (1993). The reaction mixture (1 mL) contained 50 µL plasma, 398 µL of 50 mM phosphate buffer (pH 7.0), 2 µL of 1 mM EDTA, 10 µL of 1 mM sodium azide, 500 µL of 0.5 mM NADPH, 40 µL of 0.2 mM GSH and 1 U glutathione reductase. The reaction mixture was allowed to equilibrate for 1 min at room temperature. After this, the reaction was initiated by addition of 100 mM H_2O_2. The absorbance measured kinetically at 340 nm (ELx-800, Biotek Instruments, Winooski VT, USA) for 3 min. The GPx activity was expressed as nmol of NADPH oxidized to $NADP^+$ per min per mg of protein using an extinction coefficient (6.22 mM/cm) for NADPH.

iv. *MDA level in plasma*

The supernatant was subjected to thiobarbituric acid (TBA) assay by mixing with 8.1% sodium dodecyl sulphate (SDS), 20% acetic acid, 0.8% TBA and then digested it for 1 h at 95 °C (Sharma et al., 2008). The reaction mixture was immediately cooled in running water, vigorously shaken with 2.5 mL of n-butanol and pyridine reagent (15:1) and centrifuged for 10 min at 1500 × g (Ohkawa et al., 1978). The absorbance of the upper phase was measured at 534 nm (ELx-800, Biotek Instruments, Winooski VT, USA). Total thiobarbituric acid reactive substances (TBARS) were expressed as malondialdehyde (MDA; nmol/g tissue weight) taking 1,1,1,1-tetraethoxy propane (TEP) as standard. The standard curve was calibrated using different dilutions of 10 nM TEP. For the activity assay in plasma instead of tissue homogenate, equal volume of plasma was used.

g) Hormonal parameters

i. *Plasma level of testosterone*

An ELISA kit for peripheral testosterone assay was purchased from DiaMetra (Lot No; DKO 002), Italy and was measured according to the manufacturer's protocol. The coefficient of intra and inter assay variation was less than 9% and 15% respectively. The assay was carried out in triplicate.

ii. *Plasma level of estradiol*

The ELISA kit for peripheral estradiol assay was purchased from Biotron Diagnostics Inc., Palm Ave Hemet, CA, USA and was measured according to the manufacturer's protocol. Intra and inter assay variation was less than 5% and 14% respectively. The analytic sensitivity was 10 pg/mL. The assay was carried out in triplicate.

iii. *Plasma level of melatonin*

Peripheral melatonin level was measured in the blood collected at night with the help of a commercial kit (Biosource, Nivelles, Belgium; Cat. No. KIPL3300) according to the manufacturer's protocol. Analytic sensitivity (limit of detection) for melatonin serum was 2 pg/mL. Inter and intra-assay variations were between 9.0% and 15%, respectively. The assay was carried out in triplicate.

h) Cytokine parameters

i. *Plasma level of IL-6*

Sandwich ELISA was performed to quantify the level of IL-6 in plasma collected from the goats according to the manufacturer's instruction (Koma Biotech, Seoul, Korea; Cat. No. K0331230). Lower and upper limits of analytic sensitivities were 16 pg/mL and 1000 pg/mL. All the assays were carried out in triplicate.

ii. *Plasma level of TNF-α*

Sandwich ELISA was performed to quantify the level of TNF-α in plasma collected from the goats according to the manufacturer's instruction (Koma Biotech; Cat. No. K0331186). Lower and upper limits of analytic sensitivities were 16 pg/mL and 2000 pg/mL. All the assays were carried out in triplicate.

i) Statistical analyses

The data were presented as the mean ± standard error of the mean (SEM). All the hematological, biochemical, hormonal, immunological and free radical parameters were analyzed by one way ANOVA followed by Student's unpaired t- test. The mean difference was considered to be statistically significant at the 0.05 level ($p < 0.05$). Statistical analyses were done with Statistical Package of Social Sciences (SPSS), IBM, software version 17.0 in accordance with Bruning and Knitz (1977).

III. Results

Since the studies are underway (unpublished data); the glimpses of results along with the p-value will be provided where ever necessary.

a) Effect on body weight

Upon phyto-melatonin supplementation we noted significant increase in body weight ($p < 0.05$) in both the sexes.

b) Effect on haematological parameters

Upon phyto-melatonin supplementation there was no significant effect on plasma AST and ALT levels which are markers for hepato-toxicity (Fig. 2A and 2B). However, RBC count and %Hb were significantly high only in female goats upon phyto-melatonin supplementation than males ($p < 0.05$).

c) Effect on immunological parameters

%SR of PBMCs were significantly high ($p < 0.05$) in both the sexes upon phyto-melatonin supplementation. Total Leukocyte Count (TLC) in both the sexes of goats was significantly high upon phyto-melatonin supplementation ($p < 0.01$) but the males presented significantly higher level than females ($p < 0.05$). % Lymphocyte count (%LC) was significantly high in both the sexes upon phyto-melatonin supplementation ($p < 0.05$).

d) Effect on metabolic parameters

Plasma glucose level was significantly high in both the sexes of goats upon phyto-melatonin supplementation ($p < 0.01$) which is significantly higher in females ($p < 0.05$) than males. Further, plasma cholesterol level was significantly high in both the sexes in phyto-melatonin supplemented groups ($p < 0.05$), however plasma protein level did not show any significant variation. Plasma HDL concentration was significantly high in both the sexes upon phyto-melatonin supplementation ($p < 0.05$) which is higher in females ($p < 0.5$) than males. Plasma LDL concentration was significantly low ($p < 0.05$ in males and $p < 0.01$ in females) upon phyto-melatonin supplementation, which further resulted in significantly higher levels of plasma HDL and LDL ration ($p < 0.01$) particularly in females than males ($p < 0.01$).

e) Effect on free radical parameters

SOD level was significantly high in both the sexes upon phyto-melatonin supplementation ($p < 0.05$). Similarly catalase and GPx levels were significantly high in both of the sexes ($p < 0.05$ in male goats for catalase and $p < 0.01$ in males for GPx; $p < 0.01$ in females for both catalase and GPx) upon phyto-melatonin supplementation. The reverse relationship was observed in case of MDA level in plasma which was significantly low ($p < 0.01$) in both the sexes upon phyto-melatonin supplementation.

f) Effect on hormonal parameters

Upon phyto-melatonin supplementation the plasma level of melatonin was significantly high only in males ($p < 0.01$) but the level is unaffected in females. Plasma level of estradiol was significantly high upon phyto-melatonin supplementation ($p < 0.05$) but plasma level of testosterone was unaffected.

g) Effect on cytokine parameters

Upon phyto-melatonin supplementation plasma level of IL-6 was significantly high in both the sexes ($p < 0.05$ in males and $p < 0.01$ in females) while females presented a significantly higher level ($p < 0.05$) than males. Plasma level of TNF-α was significantly high in both the sexes ($p < 0.01$) upon phyto-melatonin supplementation.

IV. Discussions

The effect of phyto-melatonin on regulation of goat health management and immunity is the first attempt in the field of research in ruminant and goat physiology in particular. Corn is not the normal/common food of goats in India and hence, we used the maize as a dietary supplement with the normal food for goats. Further, it was necessary to check if the supplementation had any side effect(s) on the digestive system of goats as this is the major system which maintains the body homeostasis. Therefore, we noted AST and ALT as the markers for liver function test (LFT). This is because, AST catalyses the transfer of the amino group of L-aspartate to a keto-glutarate to give L-glutamate. AST is widely distributed in the body, but the highest levels are found in heart, liver, skeletal muscles and kidneys. ALT catalyzes the transfer of the amino group of L-alanine to a keto-glutarate to produce L-glutamate. The highest levels are found in the liver and kidneys, and in smaller amounts in heart and skeletal muscle. ALT concentration is increased when hepatic

cells are damaged (liver cell necrosis or injury of any cause). Our results suggested that there was no significant variation in the AST, ALT in experimental groups. Further, there were no sex dependent variations in AST and ALT levels suggesting that neither the corns (as a source of phyto-melatonin) nor their metabolites were affecting the body homeostasis in a detrimental manner or having any negative impact on goat health.

Upon supplementation we found there was a significant increase in body mass in the phyto-melatonin treated groups. This might be due to the reason that it provoked basal metabolism (anabolism) of the body and as a result body mass increased. To confirm the same we further studied the different circulatory metabolic parameters. We checked the circulatory level of glucose (a ready source of energy) that was significantly high in phyto-melatonin supplemented groups particularly in females. Cholesterol, the sustainable source of energy was found to be significantly higher in both the sexes of supplemented groups. However, the circulatory level of protein did not show any sex dependent variation in goats. Thus, we may conclude that there might have been an increase in body metabolic processes upon treatment and for that the higher requirement of energy was needed. To balance the same circulatory level of glucose was increased. However, protein, which is only being used as a source of energy under severe pathological or starving condition, was found to be unaffected. Simultaneously and most interestingly, the cholesterol level was also high in the phyto-melatonin treated groups and this higher level of cholesterol might have then deposited to increase body mass. Further, HDL the "Good Cholesterol" was significantly high in both males and females of phyto-melatonin treatment.LDL, the "Bad Cholesterol" was significantly low in both the sexes upon treatment. The ratio of HDL and LDL was high in supplemented groups suggesting a beneficial physiological aspect of phyto-melatonin.

Higher cholesterol in circulation proved to be physiologically beneficial for goats. The higher circulatory level of glucose suggests that body metabolism is high. Increase in peripheral cholesterol level suggests that this high cholesterol might have been used as source of energy for near or far future. This stored energy is channelized to modulate energy demanding process of goat, as during winter two most energy demanding events (reproduction and immune modulation) occur simultaneously. To explore the issue, we studied the haematological, immunological and hormonal parameters. The haematological parameters including total RBC count and %Hb content were significantly high in phyto-melatonin supplemented groups being higher in females. Thus high level of metabolic parameters might be providing higher fitness level to females than males during stressful months of winter.

In the cell mediated immune parameters the Total Leukocyte Count (TLC), % Lymphocyte Count (%LC) and % Stimulation Ratio of Peripheral Blood Mononuclear Cells (%SR of PBMCs) were significantly high in the supplemented groups. Explanation may be that, phyto-melatonin supplementation might have increased the peripheral melatonin level and the increased melatonin level then might have increased the peripheral cell mediated immune parameters which is in agreement with earlier reports of Carrillo-Vico et al., (2013). A similar trend was observed in case of circulatory level of cytokines. We noted significantly high TNF-α and IL-6 levels in both the sexes upon supplementation, thus, like cell mediated immune system, cytokine levels were in parallel with high level of circulatory melatonin and inflammatory status of the body.

We noted a significant increase in circulatory melatonin level particularly in males, upon supplementation of phyto-melatonin but, testosterone level was unaffected while the estrogen level in females was significantly high. Thus, we may conclude that in males the higher level of melatonin might have increased the immune parameters and to cope up with this higher energy demand metabolic parameters were also increased. But, for females, melatonin and the elevated estrogen level increased inflammatory factors or cytokines being in agreement of previous reports in other animals that estrogen might have up regulated inflammatory cytokines (Calippe et al., 2008).Thus, under the influence of both the hormones, the higher immune status of the females were maintained with higher levels of metabolic and haematological parameters than male goats. In male goats melatonin alone was sufficient enough to well manage the immune functions as testosterone is an immune suppressor (Ahmad and Haldar, 2010).

The elevated metabolism of body resulted in generation of high level of Reactive Oxygen Species (ROS). The level of ROS generation can be estimated by the activities of their scavenger enzymes. The main free radical scavenging enzymes in the system are Super oxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidases (GPx). MDA level, a marker for lipid peroxidation was lower in both the sexes of phyto-melatonin supplementation being low in females. We noted the SOD, CAT and GPx in blood of goats upon phyto-melatonin supplementation and found there was a significant increase in SOD, CAT and GPx activities in phyto-melatonin supplementation than control group however sex dependent variation was statistically non-significant. Thus, in both the sexes of phyto-melatonin supplementation group, increased melatonin level maintained its parallelism with increased levels of free radical scavenging enzymes as metabolic activity increased. But, lipid per-oxidation, which generally depicts the level of cellular disintegration (as MDA level

is a universal marker of lipid per-oxidation caused due to cell-membrane disruption; Wong-ekkabut, 2007) was low. Thus, the free radical generation could be the only causal effect of increased metabolism.

V. Conclusion

For the first time role of phyto-melatonin as a protective molecule with improving effect on the health and immunity of Indian goat *Capra hircus* is being proposed, as the effect of phyto-melatonin supplementation can be brought back to normal and this dietary supplement might be utilizing the similar pathway as commercial melatonin. There are so many less expensive and readily available sources of phyto-melatonin that requires the proper knowledge of exploitation of these sources for extreme benefit for animals as well as for the human beings in near or far future.

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Conflict of interest

There is no conflict of interest between the authors including financial.

References Références Referencias

1. Ahmad, R and Haldar, C, Melatonin and androgen receptor expression interplay modulates cell-mediated immunity in tropical rodent *Funambulus pennanti*: an *in-vivo* and *in-vitro* study, Scand J Immunol, 71, 420-430, 2010.
2. Badria, FA, Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants, J Med Food, 5, 153–157, 2002.
3. Balzer, I, Bartolomaeus, B and Ho¨cker, B, Circadian rhythm of melatonin content in Chlorophyceae, Proc Conference News Plant Chronobiol Res Markgrafenheide, 55–56, 1998.
4. Bruning, JL and Knitz, BL, Computational handbook of statistics, Eds. Scott, F and Company, Illinois, 1977.
5. Calippe, B, Douin-Echinard, V and Laffargue, M, Chronic estradiol administration *in vivo*promotes the proinûammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway, J Immunol, 180, 7980-7988, 2008.
6. Carrillo-Vico, A, Patricia, JL, Álvarez-Sánchez, N, Rodríguez-Rodríguez, A, and Guerrero JM, Melatonin: Buffering the Immune System, Int J Mol Sci, 14, 8638-8683, 2013.
7. Chowdhury, SA, Bhuiyan, MSA and Faruk, S, Rearing Black Bengal goat under semi-intensive management 1. Physiological and reproductive performances, Asian-Aust J Anim Sci, 15, 477-484, 2002.
8. Das, K, Samanta, L and Chainy, GBN, A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals, Ind J Biochem Biophys, 37, 201–204, 2000.
9. Dubbels, R, Reiter, RJ, Klenke, E, Goebel, A, Schnakenberg, E and Ehlers, C, Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry, J Pineal Res, 18, 28–31, 1995.
10. Fandos P, Orueta JF and Olanda A, Tooth wear and its relation to kind of food: the repercussion on age criteria in *Capra pyrenaica,* Acta Theriol, 38, 93-102, 1993.
11. Ghosh, S, Singh, AK, Haldar, C, Seasonal modulation of immunity by melatonin and gonadal steroids in a short day breeder goat *Capra hircus*, Theriogenol, 82, 1121-1130.
12. Haldar, C, Rai, S and Singh, R,Melatonin blocks dexamethasone-induced immunosuppression in a seasonally breeding rodent Indian palm squirrel, *Funambulus pennanti*, Steroids, 69, 367-377, 2004.
13. Hardeland, R and Poeggeler, B, Non-vertebrate melatonin, J Pineal Res, 34, 233–324, 2003.
14. Herna´ndez-Ruiz, J, Cano, A and Arnao, MB, Melatonin: a growth-stimulating compound present in lupin tissues, Planta, 220, 140–144, 2004.
15. Kola´r, J and Macha´ckova´ I, Occurrence and possible function of melatonin in plants, A review, Endocytobio Cell Res, 14, 75–84, 2001.
16. Lei, XY, Zhu, RY, Zhang, GY and Dai, YR, Attenuation of cold induced apoptosis by exogenous melatonin in carrot suspension cells: the possible involvement of polyamines, J Pineal Res, 36, 126–131, 2004.
17. Lorenz, M and Lu¨ning, K, Detection of endogenous melatonin in the marine red macroalgae *Porphyra umbilicalis* and *Palmaria palmata* by enzyme-linked immunoassay (ELISA) and effects of melatonin administration on algal growth, Proc Conf Plant Chronobiol Res, Markgrafenheide, 42–43, 1998.
18. Mantha, SV, Prasad, M, Kalra, J and Prasad, K, Anti oxidant enzymes in hypercholesteromia and effect of vitamin E in rabbits, Atherosclerosis, 101, 135–144, 1993.
19. Mueller, U and Hardeland, R, Transient accumulations of exogenous melatonin indicate binding sites in the dinoflagellate *Gonyaulax polyedra*, In: Studies on antioxidants and their metabolites, ed. R. Hardeland, 140–147, Gottingen: Cuvilier, 1999.

20. Ohkawa, H, Ohishi, N and Yagi, K, Reaction of linoleic acid hydroperoxide with thiobarbituric acid, J Lipid Res, 19, 1053–1057, 1978.
21. Pauly, JL, Sokal, JE and Han, T, Whole-blood culture technique for functional studies of lymphocyte reactivity to mitogens, antigens, and homologous lymphocytes, J Lab Clin Med, 82, 500–512, 1973.
22. Posmyk, MM, Kuran, H, Marciniak, K and Janas, KM, Pre-sowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations, J Pineal Res, 45, 24–31, 2008.
23. Reiter, RJ, Tan, DX, Manchester, LC, Simopoulos, AP, Maldonado, MD and Flores, LJ, Melatonin in edible plants (phyto-melatonin): identification, concentrations, bioavailability and proposed functions, World Rev Nutr Diet, 97, 211–230, 2007.
24. Sackett, DL, Clinical epidemiology, Am J Epidemiol, 89, 125- 128, 1969.
25. Sharma, S, Haldar, C and Chaube SK, Effect of exogenous melatonin on X-ray induced cellular toxicity in lymphatic tissue of Indian tropical male squirrel, *Funambulus pennanti*, Int J Radiat Biol, 84, 363-374, 2008.
26. Sinha, AK, Colorimetric assay of catalase, Anal Biochem, 47, 389–394, 1972.
27. Tan, DX, Manchester, LC, Di Mascio, P, Martinez, GR, Prado, FM and Reiter, RJ, Novel rhythms of N1-acetyl-N2- formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phyto-remediation, The FASEB J, 21, 1724–1729, 2007.
28. Tettamanti, C, Cerabolini, B, Gerola, P and Conti, A, Melatonin identification in medicinal plants, Acta Phytotherap, 3, 137–144, 2000.
29. Wong-ekkabut, J, Xu,Z, Triampo, W, Tang, I-M, Tieleman, DP and Monticelli, L, Effect of Lipid Peroxidation on the Properties of Lipid Bilayers: A Molecular Dynamics Study, Biophy J,93,4225–4236, 2007.

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Memberships

Fellows/Associates of Science Frontier Research Council

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Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

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Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

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