Published with Open Access at Journal BiNET
Vol. 26, Issue 02: 2211-2216
Journal of Bioscience and Agriculture Research
Journal Home: www.journalbinet.com/jbar-journal.html

Isolation, identification and characterization of bacteria from Lawachara National Park, Moulovibazar, Bangladesh

Rajnee Hasan, M. N. H. Rony, Mohammad Saiful Alam Sarker, Md. Zablul Tareq and Rasel Ahmed

Basic and Applied Research on Jute Project, Bangladesh Jute Research Institute, Dhaka, Bangladesh

✉ For any information: rajnee.bmbdu@gmail.com (Hasan, R)
Article received: 19.10.2020; Revised: 20.12.2020; First published online: 30 December 2020

ABSTRACT

Bacteria are essential elements of natural environments. As bacteria are the key critical components of food webs and nutrient cycles, they contribute to ecosystem functioning via mutualists and pathogens for larger species. The present study has provided substantial grounds to confirm that microbial communities present in natural environments are much more diverse. Here, we tend to study a singular environment Lawachara National Park. Total 125 bacterial strains were isolated using serial dilution method. Thirteen unique colonies were selected, cultured and characterized by gram staining and biochemical tests. Based on morphological, biochemical, 16S rDNA gene sequencing and phylogeny analysis revealed that the isolates were identified as Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Bacillus subtilis, Bacillus thuringensis, Serratia marcescens, Aeromonas hydrophila, Enterobacter cowanii, Acidobacterium capsulatum, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis. This study serves as a baseline survey of bacterial diversity in the Lawachara National Park.

Key Words*: Bacterial diversity, Biochemical test, 16S rDNA gene sequencing and Phylogeny*

Cite Article: Hasan, R., Rony, M. N. H., Sarker, M. S. A., Tareq, M. Z. and Ahmed, R. (2020). Isolation, identification and characterization of bacteria from Lawachara National Park, Moulovibazar, Bangladesh. Journal of Bioscience and Agriculture Research, 26(02), 2211-2216.
Crossref: https://doi.org/10.18801/jbar.260220.270

I. Introduction

Microorganisms serve as an essential part of biodiversity in nature and are an integral part of terrestrial ecosystems. They produce some compounds that are beneficial to soil health and plant growth, which plays a crucial role in nutritional chains integral to the biological balance in the living organism's life (Kummerer, 2004). Lawachara National Park is an important nature reserve in Bangladesh. This Park is an undisturbed natural forest within the West Bhanugach Reserved Forest Biodiversity hotspot zone. This region contains topical diverse ecological niches with leading hotspots that provide distinctive niches for the evolution of novel microorganisms. At the same time, this ecologically vital region has been poorly explored in terms of natural microflora (Myers et al., 2000).

Published with open access at journalbinet.com
EISSN: 2312-7945, ©2020 The Authors, Research paper

Bacterial communities respond to environmental disturbances and therefore, the following of those communities might function as indicators of ecosystem health in conservation concern areas (Farrell et al., 2019). Additionally, many bacteria have been identified which produce different types of chemicals that are being exploited in biotechnology industries. Most of the soil bacteria remain unidentified. Both academic and industrial scientists accomplished that Soil bacteria are a potential source to biologically active metabolites and commercially important novel products according to academic and industrial scientists. Many valuable chemicals like-ethanol, acetone, enzymes, perfumes and antibiotics have been producing from bacterial sources (Begum et al., 2017).

The role of the bacteria must be considered during the management of park conservation and analyzing long-run stability of the ecosystem. Consequences of the interactive role of microorganisms on environmental and geochemical factors may lead to ecosystem changes with significant consequences for nutrient cycling and availability. Preservation of the vast diversity of microbial life in Lawachara Parkland is a must to make the balance between equilibrium and stability of these environments and ecosystems. In our present study, we have collected the environmental samples (Soil and water sample) from Lawachara National Park to survey the bacterial diversity through bacterial identification and characterization.

II. Materials and Methods

Collection of samples

Different soil and water samples were collected from Lawachara National Park, Moulovibazar at 20 May, 2017, in pre-sterilized Zip-lock plastic bag and bottles respectively. To avoid any physical or chemical changes in the sample, they were transferred to the laboratory immediately and stored at 4°C

Isolation of Bacterial strains

1g of each soil sample or 1ml of each water sample was introduced into a falcon tube containing 9ml of distilled water and diluted up to 10^{-5}. 1ml of each sample was spread on the nutrient agar plate and incubated at 37°C for 24 hours. Morphologically different colonies were isolated, purified and stored at −80°C on nutrient broth containing glycerol for further studies.

Morphological and biochemical characterization of Bacteria

The bacterial isolates were viewed under a standard microscope for morphological characterization, including gram stain, shape, sore forming etc. Additionally, isolates were biochemically analyzed for the activities of oxidase, catalase, Voges Proskauer (VP) test, Methyl red test, motility, nitrate reductase test, urea hydrolysis test, indole production and citrate utilization. The tests were used to identify the isolates according to the tests were used to identify the isolates according to Bergey's Manual of Systematic Bacteriology (Holt et al., 1994).

Molecular characterization of Bacteria

Isolation of genomic DNA

A method from Sambrook and Russel (Sambrook and Russel, 2001) was used for genomic DNA isolation followed by treating with RNAsae. The quality of DNA in terms of quantity and purity was evaluated using a NanoDrop 2000 spectrophotometer (NanoDrop, Thermo Scientific). 1% agarose gel electrophoresis was used to assess the integrity of DNA.

Amplification of 16S rDNA and PCR product purification

16S rRNA gene amplification was performed in GeneAmp PCR System 9700 (Applied Biosystems, Life Technologies, USA) with reaction mixture volume of 50 µl containing 250 ng DNA, 10x PCR buffer with 20 mM $MgSO_4$ and 10 mM dNTP, 1.25 U Taq DNA Polymerase (Invitrogen, Life Technologies, USA), 10 µM primer of 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492 R (5′-GGTTACCTTGTTACGACTT-3′). PCR reaction was performed as 5min denaturation at 94°C, then 35 cycles of 30sec at 94°C, 30sec at 55°C and 1 min at 68°C and final extension at 68°C for 7min. The amplified PCR products were loaded onto 1% agarose gel electrophoresis. 1kb plus DNA ladders were used as size markers. The gels were viewed under UV light and photographed using Bio-Rad gel documentation system. Amplified PCR products of 16S rRNA genes were eluted from agarose gel using Qiagen QuickSpin PCR purification columns (Qiagen, Catalog No. 28706).

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2020 The Authors, Research paper

Sequencing of 16S rDNA fragment, assembly and BLAST search

The purified PCR products were sequenced with ABI 3730XL DNA Analyzer (Applied Bio systems, Life Technologies, USA) using Big Dye V3.1 sequencing chemistry and 27F (5′-GAGTTTGATCMTGGCTCAG-3′), 533R (5′-TTACCGCGGCTGCTGGCAC-3′), 981R (5′-GGGTTGCGCTCGTTGCGGG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used as primers. The sequences for each primer of the same isolate were assembled with CAP3, an online server (http://doua.prabi.fr/software/cap3). The assembled sequences were compared in Gen Bank database available in National Centre for Biotechnology Information (NCBI) to get maximum identity.

Phylogenetic analysis

The 16S rDNA sequences of isolates were aligned with the Clustal Omega and phylogenetic trees were inferred using the maximum-likelihood method. The software MEGA, version X, was used to construct tree (Kumar et al., 2018). Bootstrap analysis (500 replicates) was used to test the topology.

III. Results and Discussion

Isolation of bacterial strains

In the present study, we identify and characterize bacteria isolated from Lawachara National park. One hundred and twenty-five colonies were primarily obtained from Nutrient agar plate. Among them, 13 isolates were selected based on their colony morphology and biochemical test (Table 01)

Morphological and biochemical characterization of Bacteria

Most of the isolates were found to produce cream colour colonies with a dry or moist surface. Majority of them were rod shaped and non-spore former. However, some colonies with different colour and shape were also observed (Figure 01). The colony morphology of isolates, as found herein, is comparable with the previous report by Hasan et al. (2020). Table 01 listed the colony morphology of bacterial isolates. It was found that 42% of isolates were gram positive while 58% were gram negative.

Figure 01. Different colony morphology of the isolates - shape, colour, margin, evaluation, size, opacity and appearance of the isolates.

Table 01. Colony morphology of bacterial isolates

ID	Gram Stain	Colour on NA plate	Shape	Spore	Motility
LC-1	+	Creamy	Rod	Yes	Non-motile
LC-2	+	Yellow	Coccus	No	Non-motile
LC-3	+	Gray-yellow	Rod	Yes	Motile
LC-4	–	Cream	Rod	No	Motile
LC-5	–	orange	Rod	No	Motile
LC-6	–	Creamy	Rod	No	Motile
LC-7	–	Greyish white	Rod	No	Non-motile
LC-8	–	White	Rod	No	Motile
LC-9	–	Greenish	Rod	No	Motile
LC-10	+	Creamy	Rod	Yes	Motile
LC-11	–	Greyish	Rod	No	Motile
LC-12	+	bright yellow	Coccus	No	Non-motile
LC-13	–	Red	Rod	No	Motile

Published with open access at journalbinet.com
EISSN: 2312-7945, ©2020 The Authors, Research paper

Following the colony morphology of bacterial isolates, they were further characterized biochemically. Table 02 depicted the results of biochemical tests carried out following Bergey's Manual of Determinative Bacteriology to identify each microorganism., All 125 of the isolates were identified to their genus using this methodology. Among them, a total of 13 unique isolates were identified. It was observed that all the isolates were catalase positive and nitrate reductase positive (except LC-12). Based on biochemical characterization, the isolates LC 1,3,10 and 12 were identified as *Bacillus sp.*, and LC-2 as *Staphylococcus sp*. Whereas, LC- 4, 5, 6, 7, 8, 9 and 11 were identified as *Proteus sp, Acidobacterium sp, Enterobacter sp, Klebsiellas p, Escherichia coli, Pseudomonas sp, Aeromonas sp* and *Serratia sp.* The species of this identified genus could be confirmed by analysis of 16s rRNA gene sequences.

Table 02. Biochemical characterization of bacterial isolates

ID	Oxidase	Catalase	nitrate reductase	Citrate	VP	Indole test	Methyl red test	Urease	Tentatively identified bacterial genus
LC-1	–	+	+	+	+	–	–	–	Bacillus
LC-2	–	+	+	+	+	+	+	+	Staphylococcus
LC-3	–	+	+	+	+	–	–	+	Bacillus
LC-4	–	+	+	+	–	–	+	+	Proteus
LC-5	–	+	+	+	–	–	–	–	Acidobacterium
LC-6	–	+	+	+	+	–	–	–	Enterobacter
LC-7	–	+	+	+	+	–	–	+	Klebsiella
LC-8	–	+	+	–	–	+	+	–	Escherichia coli
LC-9	+	+	+	+	–	–	–	–	Pseudomonas
LC-10	+	+	+	+	+	+	–	+	Bacillus
LC-11	+	+	+	+	+	+	+	–	Aeromonas
LC-12	+	+	–	–	–	–	–	–	Micrococcus
LC-13	–	+	+	+	+	–	–	+	Serratia

Molecular characterization of Bacteria

Comparative analysis of the sequences with already available database showed that the strains were almost close to the genus members, which were identified earlier through biochemical tests. The highest sequence similarities of bacteria as follows: LC1, *Bacillus subtilis* (100% similarity to *Bacillus subtilis* strain LBY, accession number LT745969.1); LC2, *Staphylococcus aureus* (100% similarity to *Staphylococcus aureus* SS251, accession number MT628674.1); LC3, *Bacillus cereus* (99.8% similarity to *Bacillus cereus* strain Cu48, accession numberKY085984.1); LC4, *Proteus mirabilis* (99.93% similarity to *Proteus mirabilis* BTCB21, accession number MN956898.1); LC5, *Acidobacterium capsulatum* (100% similarity to *Acidobacterium capsulatum* 20 0D9, accession number AM086241.1); LC6, *Enterobacter cowanii* (99.56% similarity to *Enterobacter cowanii* 10DI2-2, accession number KY451837.1); LC7, *Klebsiella pneumonia* (99.37% similarity to *Klebsiella pneumonia* ZK 89, accession number MT764346.1); LC8, *Escherichia coli* (99.69% similarity to *Escherichia coli* EB32 accession number MT509620.1); LC9, *Pseudomonas aeruginosa* (99.93% similarity to *Pseudomonas aeruginosa* ZB1, accession number EU236261.1); LC10, *Bacillus thuringensis* (98.95% similarity to *Bacillus thuringensis* strain ASK13, accession number KC527056.1); LC11, *Aeromonas hydrophila* (99.72% similarity to *Aeromonas hydrophila* strain M29, accession number AF099021.1); LC12, *Micrococcus luteus* (98.96% similarity to *Micrococcus luteus* strain SCH0405, accession number AY881238.1) and LC13, *Serratia marcescens*(99.48% similarity to *Serratia marcescens* strain S1, accession number KT992358.1). Table 03 listed the species of identified bacteria obtained in this study. Chitra et al., (2014) had stated that from the agriculture soil microorganisms like *E. coli, Micrococcus sp, Escherichia sp and Staphylococcus sp* had been isolated. Again, most of the bacteria such as *Bacillus cereus, B. subtilis, S. aureus* and *P. aeruginosa* isolated in this study have been reported by other researchers (Amir & Pineau, 1998).

Phylogenetic analysis

The 16s rDNA sequence analysis and estimation of phylogenetic relationships (Figure 02) assigned All 13 strains were assigned into two predominant clades based on 16s rDNA sequence analysis and estimation of phylogenetic relationships (Figure 02). One clade contained all gram negative bacteria- *Staphylococcus aureus, Micrococcus luteus, Bacillus anthracis, Bacillus subtilis* and *Bacillus thuringensis* whereas, another one had all gram positive bacteria- *Serratia marcescens, Aeromonas hydrophila,*

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2020 The Authors, Research paper

Enterobacter cowanii, Acidobacterium capsulatum, Klebsiella pneumonia, Pseudomonas aeruginosa and *Proteus mirabilis*. The comparative sequence analysis revealed a rich spectrum of bacterial diversity and the *Bacillus spp*. was dominant. This study showed that Lawachara National Park was the habitat of different types of bacteria.

Table 03. Identified bacterial species using sequencing of a 16S rRNA gene based on similarity to NCBI database

ID	Similar to	Accession number	% Identity
LC-1	*Bacillus subtilis* strain LBY	LT745969.1	100
LC-2	*Staphylococcus aureus* strain SS251	MT628674.1	100
LC-3	*Bacillus cereus* strain Cu48	KY085984.1	99.8
LC-4	*Proteus mirabilis* strain BTCB21	MN956898.1	99.93
LC-5	*Acidobacterium capsulatum* strain 20 0D9	AM086241.1	100
LC-6	*Enterobacter cowanii* strain 10DI2-2	KY451837.1	99.56
LC-7	*Klebsiella pneumonia* strain ZK89	MT764346.1	99.37
LC-8	*Escherichia coli* strain EB32	MT509620.1	99.69
LC-9	*Pseudomonas aeruginosa* strain ZB1	EU236261.1	99.93
LC-10	*Bacillus thuringensis* strain ASK13	KC527056.1	98.95
LC-11	*Aeromonas hydrophila* strain M29	AF099021.1	99.72
LC-12	*Micrococcus luteus* strain SCH0405	AY881238.1	98.96
LC-13	*Serratia marcescens* strain S1	KT992358.1	99.48

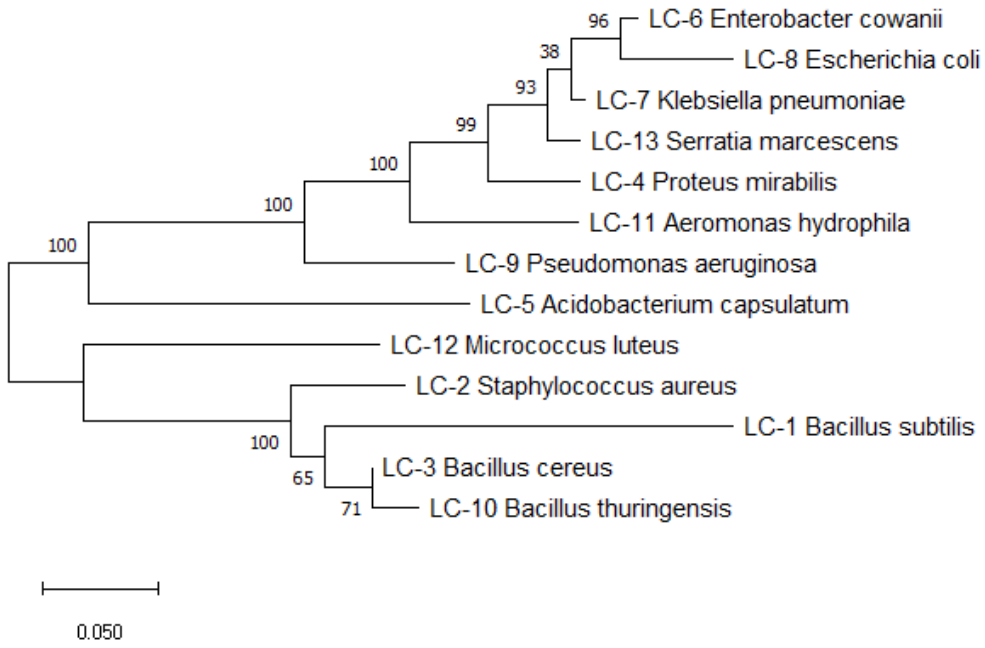

Figure 02. Maximum-likelihood phylogenetic tree construction using 16SrRNA gene sequences by Mega X.

IV. Conclusion

The environment is the residence for various microorganisms, which play a pivotal role in keeping up the biological balance in our planet's life. The present study identified diverse bacterial isolates including Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Bacillus subtilis, Bacillus thuringensis, Serratiamarcescens, Aeromonashydrophila, Enterobacter cowanii, Acidobacterium capsulatum, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis that might help in maintaining biogeochemical cycles as well as sustainable development of the biosphere.

Published with open access at journalbinet.com
EISSN: 2312-7945, ©2020 The Authors, Research paper

VI. References

[1]. Amir, H., and Pineau, R. (1998). Influence of plants and cropping on microbiological characteristics of some New Caledonian ultramafic soils. Soil Research, 36(3), 457. https://doi.org/10.1071/S97063

[2]. Begum, K., Mannan, S. J., Rezwan, R., Rahman, M. M., Rahman, M. S., and Kamal, A. N. (2017). Isolation and Characterization of Bacteria with Biochemical and Pharmacological Importance from Soil Samples of Dhaka City. Dhaka University Journal of Pharmaceutical Sciences, 16(1), 129-136. https://doi.org/10.3329/dujps.v16i1.33390

[3]. Chitra, B., Harshab, P., Sadhana, G., and Soni, R. (2014). Isolation characteriazation of bacterial isolates from agricultural soil at durg district. Indian Journal of Scientific Research, 4(1), 221-226.

[4]. Farrell, M. J., Govender, D., Hajibabaei, M., Bank, M. V., and Davies, T. J. (2019). Bacterial diversity in the waterholes of the Kruger National Park: An eDNA meta bar coding approach. Genome, 62(3), 229-242. https://doi.org/10.1139/gen-2018-0064

[5]. Hasan, R., Aktar, N., Kabir, S. M., Honi, U., Halim, A., Islam, R. and Islam, M. S. (2020). Pectinolytic Bacterial Consortia Reduce Jute Retting Period and Improve Fibre Quality. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-61898-z

[6]. Holt, J., Krieg, N., and Sneath, P. (1994). Bergey's manual of determinative bacteriology (9th ed.) Baltimore: Williams & Wilkins.

[7]. Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution, 35(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

[8]. Kummerer, K. (2004). Resistance in the environment. Journal of Antimicrobial Chemotherapy, 54(2), 311-320. https://doi.org/10.1093/jac/dkh325

[9]. Myers, N., Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853-858. https://doi.org/10.1038/35002501

[10]. Sambrook, J., and Russel, D. W. (2001). Molecular cloning a laboratory manual (3rd ed.). New York: Cold Spring Harbor Laboratory Press.

HOW TO CITE THIS ARTICLE?

Crossref: https://doi.org/10.18801/jbar.260220.270

MLA

Hasan, R. et al. "Isolation, identification and characterization of bacteria from Lawachara National Park, Moulovibazar, Bangladesh". Journal of Bioscience and Agriculture Research, 26(02), (2020): 2211-2216.

APA

Hasan, R., Rony, M. N. H., Sarker, M. S. A., Tareq, M. Z. and Ahmed, R. (2020). Isolation, identification and characterization of bacteria from Lawachara National Park, Moulovibazar, Bangladesh. *Journal of Bioscience and Agriculture Research*, 26(02), 2211-2216.

Chicago

Hasan, R., Rony, M. N. H., Sarker, M. S. A., Tareq, M. Z. and Ahmed, R. "Isolation, identification and characterization of bacteria from Lawachara National Park, Moulovibazar, Bangladesh". Journal of Bioscience and Agriculture Research, 26(02), (2020): 2211-2216.

Harvard

Hasan, R., Rony, M. N. H., Sarker, M. S. A., Tareq, M. Z. and Ahmed, R. 2020. Isolation, identification and characterization of bacteria from Lawachara National Park, Moulovibazar, Bangladesh. Journal of Bioscience and Agriculture Research, 26(02), pp. 2211-2216.

Vancouver

Hasan, R, Rony, MNH, Sarker, MSA, Tareq, MZ and Ahmed, R. Isolation, identification and characterization of bacteria from Lawachara National Park, Moulovibazar, Bangladesh. Journal of Bioscience and Agriculture Research, 2020 December 26(02): 2211-2216.

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2020 The Authors, Research paper