Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 9, Issue: 2, Year: 2022 **PP: 12-22**
DOI: https://doi.org/10.5281/zenodo.7932904 **Peer Reviewed Journal**

Foldscope As A Tool For Analysis Of Gut Microbiota Of The Mud Eel, *Monopterus cuchia.*

***Jayshree Deka, *Ann Borah and **Banasri Mech**

***Cell and Molecular Biology Laboratory, Department of Zoology, Gauhati University, Guwahati, Assam**

****Assistant Professor, Department of Zoology, Gauhati University, Assam**

Article History

Received: 28/07/2022
Accepted: 15/09/2022

Article ID: RRBB/151

Corresponding Author:
E-Mail: banasrimech@gauhati.ac.in

Abstract:

Foldscope is a very low- cost origami based microscope, that can be used as an educational cum research tool. The foldscope provides for a easily accessible, frugal with on the site usage by the reasearchers and field scientist. In the present study, the affordable Foldscope was used to study the microbial diversity living in the gut of the mud eel, *Monopterus cuchia*. A total of 20 bacterial isolates were identified by Gram staining and different biochemical tests. All of the bacterial isolates were found to be aerobic. Bacterial isolates G7, G16 and G17 were cocci while rest are bacilli. Among them except G11, all bacterial isolates were found to be Gram positive.The gut microbiome consists of microorganisms that populate the gastro-intestinal tract of the host. The gut microbiome plays various important roles such as resistance against the pathogens, maintaining the epithelial layer of the intestine, in metabolising the dietary compounds, influences the immune system and as well as influencing the behaviour via the gut-brain axis. Therefore, studying the microbial population provides important insight into the host and the surrounding it lives in and the interaction between the two.

Keywords: Foldscope, gut microbiota, *Monopterus cuchia*

Introduction

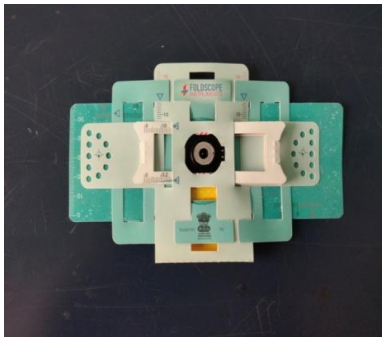
Microscopes are omnipresent laboratory instruments used to observe organism that are otherwise not visible to the naked eyes. They are the main source of visual connection between the ever large macro-world and the fascinating micro- world. Conventional microscope includes simple and compound microscope, dissecting microscope, fluorescence microscope, inverted microscope, phase- contrast microscope, scanning electron microscope, transmission electron microscope to name

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 9, Issue: 2, Year: 2022 **PP: 12-22**
DOI: https://doi.org/10.5281/zenodo.7932904 **Peer Reviewed Journal**

just a few. Microscopes are generally used to visualise microorganism such as bacteria, but also depending on the type of microscope can be used to visualise insects, plants, animal tissues samples etc. However one cannot deny the cost and tediousness of handling and processing of tissues, sample preparation, expertise in handling techniques required for the conventionally used microscope. Now- a- days the world is moving more towards a frugal way of living, and the same goes for research in life science (1). Researchers and field scientist generally prefer and will opt for a more cost-effective, easy to handle, and easy to carry instrument. Folding in science has become the new trend as seen from folding of the ever microscopic DNA to create beautiful 3 D shapes (2), to folding as big as seen in space telescopes (3). Even possibility of robots folding is not far- fetched (4, 5). One common goal of folding is to make things more accessible, easy to handle and at a cheaper price.

Foldscope as an idea came to light by Dr. Manu Prakash and his team at Stanford University to design an inexpensive foldable microscope that can be accessed by anyone in the world by overcoming the financial barrier (6, 7). Foldscope is designed by combining the beautiful art of origami (8, 9, 10), with the principles of optics to create a flat microscope at a very cost- effective manner and in bulk (6). Such is the versality of the foldscope that it can be very easily made by bare hands by anyone from a child to adult in just under10 minutes with a cost of just a dollar. All it takes to design a foldscope is scissors, forceps, tapes, foldscope paper components, ball lens, button- cell battery, LED, a switch, magnetic coupler and single sheet of plastic coated polymeric filters making it water-resistant(11, 12). It provides a magnification of 2000x, weights less then 8.8 g, does not need any sort of external power, sturdy enough to survive a fall from a 3- story building or being stepped on by a human and fits very easily in purses, pockets etc. Foldscope comes with an accessory toolkit that has all of the above mentioned components (13). The LED provides illumination powered by a watch battery connected to the switch used to turn it off or on and attached to the microscope itself (14). One most interesting exciting feature and of the foldscope is that instead of the eyepiece the lens can also be attached to the sheet which can ultimately be connected to the camera of a smartphone (15). With foldscope both dark filed and light field as well as fluorescence imaging can also be visualised (6).



©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 9, Issue: 2, Year: 2022 **PP: 12-22**
DOI: https://doi.org/10.5281/zenodo.7932904 **Peer Reviewed Journal**

a)Front side view of the assembled functioning foldscope..

b)Back side view of the Foldscope

The gastrointestinal tract of organism harbours millions of microorganism and they together forms the gut microbiota of that organism. All the microorganisms that live together in harmony and co- exist with their host is referred to as gut microbiota or microflora (16, 17, 18, 19). Human microbiota is estimated to contain a whopping 10^{14} bacterial cells a number that is ten times greater then the amount of cells that make up the body itself (20, 21, 22). Although the microorganism colonizes virtually every part of the body of a living organism such as nails, skin, respiratory tract, genitourinary tract, GI tract (19, 23, 24, 25), the most heavily colonized organ is the GI tract out of all having an estimated 70% of total microorganism present in the whole body(22). The GI tract micorbiome consists of a number of complex microorganisms which can be either aerobic, anaerobic or even obligatory aerobic bacteria(26).The gastrointestinal tract microbiota can also be classified as either autochthonus thatcolonize on the mucosal surface only or allochthonus which are mostly free living.The autochthonus bacteria are considered to be more important than allochthonus bacteria. Fishes, are a class of aquatic organism, and therefore composition their gut microbiota will very likely be influenced by the surrounding aquatic environment they live in(27).Though they are very complex, the gut microbial community plays a very pivotal role in a fish's nutrition, strengthening, host metabolism, physiology and increasing immunity (26, 27).Some fish species also have been reported to obtain many of their intestinal enzymes from the microbiota inhabiting their gut (27). Probiotic bacteria found in the gut of host fish resist the growth of pathogenic bacteria by successfully colonizing the gut where it produces antimicrobials or compete for space and nutrients with opportunistic pathogens. Though universal, it is seen that microflora in different fishes vary depending on nutrition, intestinal microenvironment, age, geographical location, environmental factors, stress (28, 29).

The swamp eel, is a native genus of the Asian countries and are widely distributed throughout Asia. They live only in freshwater habitats. *M. cuchia*, is very tasteful, nutritionally rich and medicinally valuable fish species. The fish species is also taditionally preferred because it has been reported that the comsumption of this fish can cure anaemia. The mud eel reside in freshwater muddy environment and is fossorial in lifestyle. However if the habitat it lives in becomes unsuitable due to for example increase in pollution of water or mud, the eel crawls away in a snake-like fashion to more suitable area. Thus it is sensitive to its environment and therefore, *Monopterus cuchia* can be used for environmental monitoring to check water and soil quality. During dry seasons, when there is absence of food and water, this Asian swamp eel can survive longer periods of drought by burrowing in moist muddy earth. The perspective of the present study was to isolate and identify the gut and skin microflora of swamp eel with the help of morphological and biochemical

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 9, Issue: 2, Year: 2022 **PP: 12-22**
DOI: https://doi.org/10.5281/zenodo.7932904 **Peer Reviewed Journal**

characterization. And the observation of the microorganism was done in the Foldscope.

Materials and methods:

a). Collection of the fish samples: Fishes were collected from the local fish landing centre at Pandu Port, Guwahati, Assam, India. The fishes were then immediately placed individually in the pre- sterilized (Sterilized with 70% alcholol prior) plastic containers, sealed and kept in portable ice chest and transported to the laboratory for further bacteriological analysis.

b). Sample preparation for isolation and culture of gut flora:

Prior to performing the isolation experiment, the entire body surface of the fish was sterilized with 70% alcohol. All the specimens were dissected lengthwise under sterile conditions to remove the whole gut from mouth to anus for extracting its contents with scissors which was again disinfected in 70% ethyl alcohol.

From each fish about 1 gm of gut content was taken. The gut content was homogenized and homogenate was made in 0.9% NaCl which was then centrifuged (10:1 ; Volume:Weight) (30). 0.1 ml of the supernatant was used to spread on the Luria Bertani agar media culture plates in duplicates and incubated for 18- 24 hours at 37^0 C. Following incubation, the total number of colony forming unit or CFU was determined and the representative colonies were sub- cultured for identification. Bacteria was isolated by a random collection of colonies from the agar plates. The colonies were purified by repeated sub-culture. Each time during culture one control culture was kept and incubated together with the bacterial culture plates to check if there is any contamination. For the identification of the type of bacteria, Gram staining was done.

c. Morphological characterization:

Morphological characterization was done by searching for different shape, size, colour, elevation, margin, opacity, consistency and Gram staining from the bacterial colonies obtained from primary culture inside the Laminar flow hood. The study was done using a foldscope.

Results:

Total 20 bacterial isolates were found from culture-dependent study of the swamp eel, *Monopterus cuchia.* The pure culture was done by looking for their different colony sizes, shapes, margins of colonies, elevation, transparency, color etc. All of the bacterial isolates were found to be aerobic. Bacterial isolates G7, G16 and G17 were cocci while rest are bacilli. Among them except G11, all bacterial isolates were found to be Gram positive. The different morphological characteristics of the bacterial colonies along with Gram staining results are listed on table 1.

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

Research & Reviews in Biotechnology & Biosciences
Website: www.biotechjournal.in
Volume: 9, Issue: 2, Year: 2022
DOI: https://doi.org/10.5281/zenodo.7932904
ISSN No: 2321-8681
Research article
PP: 12-22
Peer Reviewed Journal

Table 1: Morphological and Gram staining characteristics of Gut microbial isolates

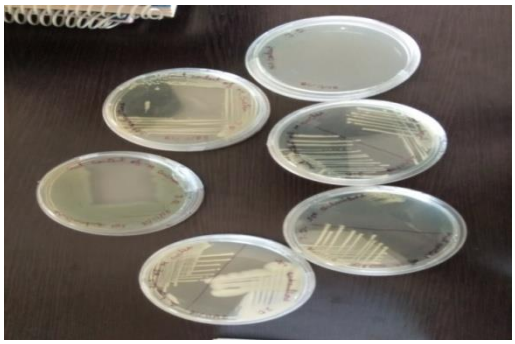
Bacterial isolates	Color of colony	State	Margin of colony	Gram stain result	Shape
G1	White	Mucoid	Uneven	Positive	Rod
G2	White	Mucoid	Uneven	Positive	Rod
G3	Off white	Mucoid	Uneven	Positive	Rod
G4	Off white	Mucoid	Uneven	Positive	Rod
G5	Off white	Mucoid	Uneven	Positive	Rod
G6	Off white	Mucoid	Uneven	Positive	Rod
G7	Off white	Mucoid	Even	Positive	Cocci
G9	White	Mucoid	Uneven	Positive	Rod
G11	Off white	Mucoid	Uneven	Negative	Rod
G12	Off white	Mucoid	Uneven	Positive	Rod
G13	Off white	Mucoid	Uneven	Positive	Rod
G14	Off white	Mucoid	Uneven	Positive	Rod
G15	Off white	Mucoid	Uneven	Positive	Rod
G16	Off white	Mucoid	Uneven	Positive	Cocci
G17	Off white	Mucoid	Even	Positive	Cocci
G18	White	Mucoid	Even	Positive	Rod
G19	White	Mucoid	Uneven	Positive	Rod
G20	White	Dry	Even	Positive	Rod

Among all the bacterial isolates, G1, G5, G6, G7, G9, G11, G12, G13, G15, G16, G17, G18, G19 and G20 showed starch hydrolyzing characteristic. The Gram positive result obtained from Gram staining was further confirmed by growing the bacteria on selective media for Gram positive bacteria, i.e., Mannitol salt agar. Except bacterial isolate G11, none of the bacterial isolates showed growth on MacConkey agar and Eosine Methylene Blue agar which are selective culture media only for Gram negative bacteria. None of these isolates have shown gelatin hydrolysis and lipid utilizing property. Each bacterial isolate showed positive test result tolerance result to Bile test. Among all the bacterial isolates only bacterial isolate G1 has shown citrate utilization characteristic and only G11 showed catalase negative result.

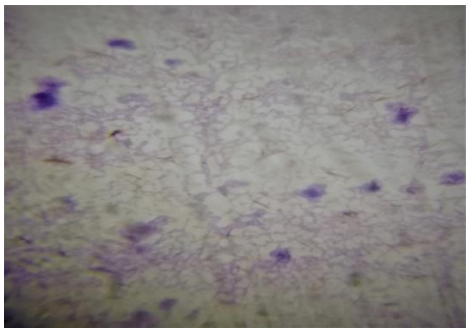
The most major relevant colored pictures of images taken under the Foldscope are attached below:

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

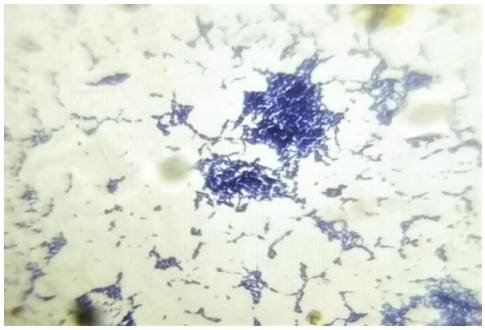
Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 9, Issue: 2, Year: 2022 **PP: 12-22**
DOI: https://doi.org/10.5281/zenodo.7932904 **Peer Reviewed Journal**



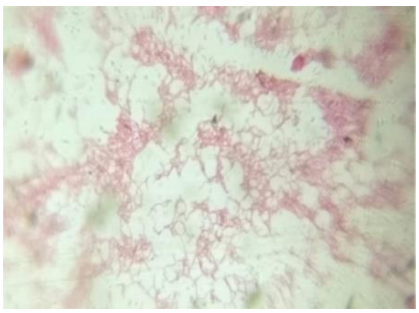
a) Bacterial sub-culture plates.



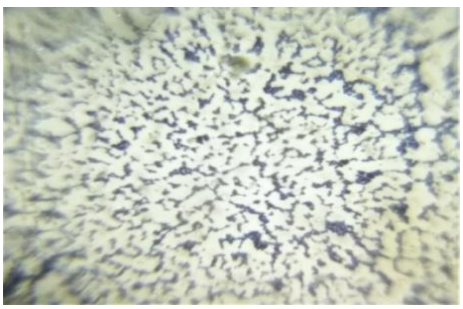
b)Gram positive *Oceanobacillus* as observed under the Foldscope.



c) Gram positive *Bacillus* observed under foldscope.



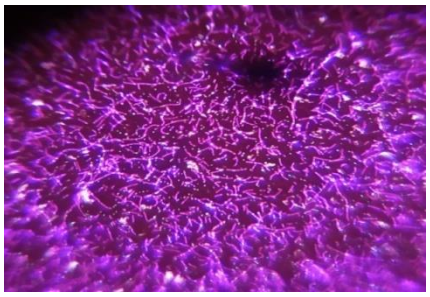
c).Gram negative bacteria isolated from the gut observed under Foldscope.



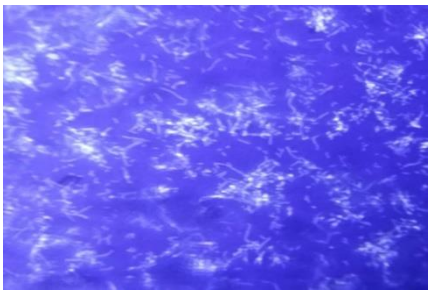
e)Gram positive cocci bacteria isolated from gut observed under Foldscope.

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

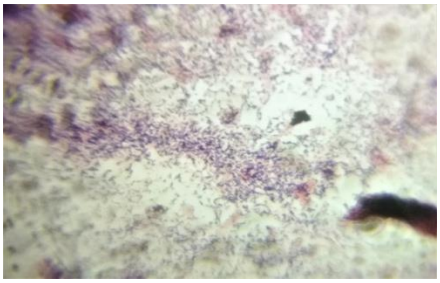
Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 9, Issue: 2, Year: 2022 **PP: 12-22**
DOI: https://doi.org/10.5281/zenodo.7932904 **Peer Reviewed Journal**



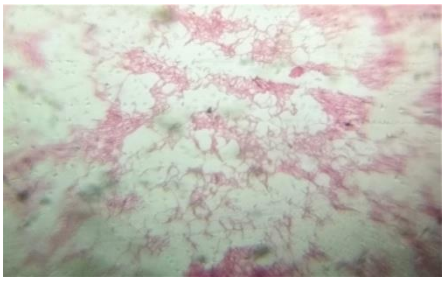
g)Capsule staining of gut bacteria observed observed under the Foldscope.



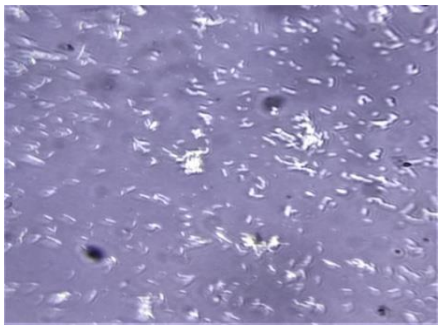
i)Negative staining of gut bacteria observed under the Foldscope



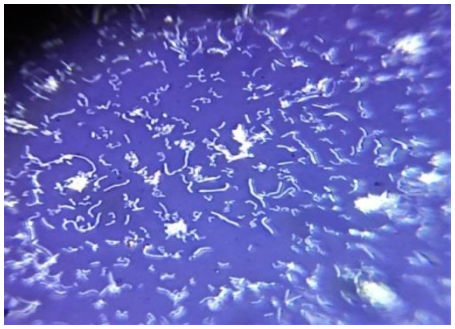
k)Gram positive bacteria isolated from gut observed under the Foldscope



l)Gram negative bacteria isolated from gut observed under the Foldscope



m)Negative staining of bacteria isolated from isolated from the gut as observed under the Foldscope.



n)Negative staining of filamentous bacteria the gut of observed under the Foldscope

Discussion:

Foldscope is a simple, easy to handle, easy to carry instrument that provides a fast tool to identify and in the present study it was used to study the gut microbiota and the integumentary system of the mud eel, *Monopterus cuchia*. The fish gut microbiota varies from species to species and in

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 9, Issue: 2, Year: 2022 **PP: 12-22**
DOI: https://doi.org/10.5281/zenodo.7932904 **Peer Reviewed Journal**

different environmental conditions. The autochthonus gut bacteria of a fish are known to have significant role in development, physiology and immunity of the host species. Majority of the fish gut microbiota assist the host immunity by occupying maximum spaces in the gut and secreting bacteriocins to resist development of pathogenic bacteria. In this present investigation, the various gut microbiota were identified with their different biochemical characteristics. Among the bacterial isolates, only G1 showed citrate positive result which was inferred by observing the blue coloured slant of Simmon's citrate agar medium. It is a characteristic property of the bacteria of the family Enterobacteriacea. Sugar fermentation patterns of different isolates were studied by the characteristic colour change in the culture medium due to changes in the pH caused by enzyme secretion by the bacteria. The bacterial isolates were able to ferment wide range of carbohydrates. Bile salt tolerance is a significant property of the probiotic bacteria studied so far. The whole gut microbiota of the mud eel showed positive result to the bile salt utilization test. Throughout the years, with advancement in aquaculture practices, there is an increasing need to use probiotic bacteria to resist pathogen growth in the fishes.

Several bacterial strains have been identified and characterised among which bacilli was one of them from the freshwater fishes *Oreochromis mossambicus*, *Oreochromis leucostictus* and *Oreochromis suratensis* (31). These bacilli have antagonistic activity against potent human pathogens like *Klebsiella pneumonia* and *Vibrio cholera.*

During our present investigation, biochemical properties of bacterial isolate G19 has shown strong similarity with that of biochemical properties of *Bacillus cereus*. Previous studies have enumerated that several species of *Bacillus* have antimicrobial property against various Gram positive and negative pathogenic bacteria. Authors have deomstrated that *Bacillus cereus* are capable of tolerating different concentrations of bile salts indicating that this bacterial species has potent role as a probiotic bacteria (32). Moreover, their investigation have shown that *Bacillus cereus* is resistant to ampicillin, amoxicillin and ciprofloxacin. In this study, *Bacillus cereus* isolated and identified from *Labeo calbasu* was found have antimicrobial activity against *Aeromonas hydrophila,* an important fish pathogen that is responsible for epizootic ulcerative syndrome, fin rot, tail rot and hemorrhagic septicemia in Indian major carps (33, 34). Bernard and his co-workers have also tested *Bacillus cereus* JAQ04 strain for potential probiotic activity in red tilapia *Oreochromis* species (35). This study also have established this bacterial strain is safe to be used for tilapia aquaculture. In our present investigation too, the G19 bacterial isolate may also have major role in the mud eel's different behavioral adaptabilities. This require further investigation.

Bacillus strains isolated from soil or channel catfish intestine were proved to be antagonistic against *Edwardsiella ictaluri* and *Aeromonas hydrophila* that are causative agents of enteric septicemia of catfish and motile aeromonas septicemia respectively (36). In the current study also, different types of *Bacillus* species have been identified with the help of biochemical characterization. Many of these bacteria may

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

also play a crucial role in disease resistance and immunity of the host *Monopterus cuchia.*

The colony morphology and biochemical properties of bacterial isolate G1 have shown close resemblance with the morphological and biochemical properties of *Bacillus subtilis.* Authors have stated that administration of *Bacillus subtilis* at a dose of 10^8 CFU/mL in the cultivating water of shrimp (*Litopenaeus vannamei*) for 2 months gives increased growth, increased digestive enzyme activity, good immune response and disease resistance(37). Additionally, In 2012, Seenivasan and his researchers have found that *Bacillus subtilis* can be used as a probiotic at a rate of 3% in the experimental diet provided to the fresh water shrimp *Macrobrachium rosenbergii* culture. The diet provided has enhanced survival, growth, nutritional indices etc (38). Moreover, *Bacillus subtilis* has been proved to have profound affectin increasing the immunity and antioxidant activity. Therefore, after identifying these bacterial isolates upto species level would help us to understand the development, nutrition, physiology and immunity as well as unique behavioral properties in the mud eel *Monopterus cuchia.*

Acknowledgement:

Authors acknowledge Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, and Foldscope Instruments Inc., Prakash Lab (Stanford) for funding the project and providing Foldscope received under the program "Proposals for the use of Foldscope as a research tool"

Conflict of Interest: Authors declares no conflict of interest

Authors Contributions: Each and every author had contributed to the manuscript**. JD:** designed the manuscript, and written the content of manuscript, AB: wet lab, JD: Wet lab.

Funding Info- DBT Govt of India.

Acknowledgement – Will like to thank **DBT foldscope** for the general support.

References:

1) Whitesides, G. M. (2011). The frugal way. *The Economist*, *17*.

2) Rothemund, P. W. (2006). Folding DNA to create nanoscale shapes and patterns. *Nature*, *440*(7082), 297-302.

3) Hyde, R. A. (1999, March). Large Aperture Fresnel Telescope. In *Proceedings, Ultra Lightweight Space Optics Challenge Workshop, Napa, CA*.

4) Hoover, A. M., & Fearing, R. S. (2008, May). Fast scale prototyping for folded millirobots. In *2008 IEEE International Conference on Robotics and Automation* (pp. 886-892). IEEE.

5) Whitney, J. P., Sreetharan, P. S., Ma, K. Y., & Wood, R. J. (2011). Pop-up book MEMS. *Journal of Micromechanics and Microengineering*, *21*(11), 115021.

6) Cybulski, J. S., Clements, J., & Prakash, M. (2014). Foldscope: origami-based paper microscope. *PloS one*, *9*(6), e98781.

7) Nwaneshiudu, A., Kuschal, C., Sakamoto, F. H., Anderson, R. R., Schwarzenberger, K., & Young, R. C. (2012). Introduction to confocal microscopy. *Journal of Investigative Dermatology*, *132*(12), 1-5.

8) McArthur M, Lang R (2012) Folding paper: The infinite possibilities of Origami. Washington, DC: International Arts & Artists. 96 p.

9) Demaine ED, O'Rourke J (2007) Geometric Folding Algorithms: Linkages, Origami, Polyhedra. Cambridge, MA: Cambridge Univ Press. 472 p.

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

10) Myer, J. H., & Cooke, F. (1969). Optigami—A tool for optical systems design. *Applied Optics*, *8*(2), 260-260.

11) Monique, C. (2014). Paper microscopes will make science more accessible.

12) Almeda, K. S. (2021). Perception on the use of foldscope as an alternativeconventional microscope.

13) Devi, N. S., Singh, T. S., Singh, S. J., Meinam, T., Singh, L. S., Devi, M. S., ... & Singh, T. J. (2019). Under the foldscope: Relatively inexpensive tool for understanding down-to-earth applications in plant sciences. *Journal of Pharmacognosy and Phytochemistry*, *8*(6), 30-34.

14) Mathews, L. (2014). Foldscope is a 50-cent paper microscope that mag-nifies up to 2000 times.

15) Anderson, M. (2018). Foldscope: A paper microscope you can attach to your smartphone-[Resources_Tools and Toys]. *IEEE Spectrum*, *55*(11), 20-20.

16) Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological reviews*.

17) Kunz, C., Kuntz, S., &Rudloff, S. (2009). Intestinal flora. *Breast-Feeding: Early Influences on Later Health*, 67-79.

18) Iapichino, G., Callegari, M. L., Marzorati, S., Cigada, M., Corbella, D., Ferrari, S., & Morelli, L. (2008). Impact of antibiotics on the gut microbiota of critically ill patients. *Journal of medical microbiology*, *57*(8), 1007-1014.

19) Neish, A. S. (2009). Microbes in gastrointestinal health and disease. *Gastroenterology*, *136*(1), 65-80.

20) Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *nature*, *444*(7122), 1027-1031.

21) Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual review of microbiology*, *31*(1), 107-133.

22) Whitman, W. B., Coleman, D. C., &Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences*, *95*(12), 6578-6583.

23) Chiller, K., Selkin, B. A., &Murakawa, G. J. (2001, December). Skin microflora and bacterial infections of the skin. In *Journal of Investigative dermatology Symposium proceedings* (Vol. 6, No. 3, pp. 170-174). Elsevier.

24) Hull, M. W., & Chow, A. W. (2007). Indigenous microflora and innate immunity of the head and neck. *Infectious disease clinics of North America*, *21*(2), 265-282.

25) Swidsinski, A., Loening-Baucke, V., Verstraelen, H., Osowska, S., &Doerffel, Y. (2008). Biostructure of fecal microbiota in healthy subjects and patients with chronic idiopathic diarrhea. *Gastroenterology*, *135*(2), 568-579.

26) Ringø, E., Strøm, E., &Tabachek, J. A. (1995). Intestinal microflora of salmonids: a review. *Aquaculture Research*, *26*(10), 773-789.

27) Cahill, M.M. Bacterial flora of fishes: A review. *MicrobEcol***19**, 21–41 (1990). https://doi.org/10.1007/BF02015051

28) Yang, G., Bao, B., Peatman, E., Li, H., Huang, L., & Ren, D. (2007). Analysis of the composition of the bacterial community in puffer fish Takifuguobscurus. *Aquaculture*, *262*(2-4), 183-191.

29) Kesarcodi-Watson, A., Kaspar, H., Lategan, M. J., & Gibson, L. (2008). Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. *Aquaculture*, *274*(1), 1-14.

30) Das, K. M., &Tripathi, S. D. (1991). Studies on the digestive enzymes of grass carp, Ctenopharyngodonidella (Val.). *Aquaculture*, *92*, 21-32.

31) Sivasubramanian, K., Ravichandran, S., &Kavitha, R. (2012). Isolation and characterization of gut micro biota from some estuarine fishes. *Marine Sci*, *2*(2), 1-6.

32) Kavitha, M., Raja, M., &Perumal, P. (2018). Evaluation of probiotic potential of Bacillus spp. isolated from the digestive tract of

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),

freshwater fish Labeocalbasu (Hamilton, 1822). *Aquaculture Reports*, *11*, 59-69.

33) Pridgeon, J. W., Mu, X., &Klesius, P. H. (2012). Expression profiles of seven channel catfish antimicrobial peptides in response to Edwardsiellaictaluri infection. *Journal of fish diseases*, *35*(3), 227-237.

34) Kozińska, A., Figueras, M. J., Chacon, M. R., &Soler, L. (2002). Phenotypic characteristics and pathogenicity of Aeromonasgenomospecies isolated from common carp (Cyprinuscarpio L.). *Journal of applied microbiology*, *93*(6), 1034-1041.

35) Bernard, A. T. F., Götz, A., Kerwath, S. E., & Wilke, C. G. (2013). Observer bias and detection probability in underwater visual census of fish assemblages measured with independent double-observers. *Journal of Experimental Marine Biology and Ecology*, *443*, 75-84.

36) Ran, C. (2013). *Isolation and Characterization of Bacillus spp. as Potential Probiotics for Channel Catfish, Ictalurus punctatus* (Doctoral dissertation).

37) Zokaeifar, H., Babaei, N., Saad, C. R., Kamarudin, M. S., Sijam, K., &Balcazar, J. L. (2014). Administration of Bacillus subtilis strains in the rearing water enhances the water quality, growth performance, immune response, and resistance against Vibrio harveyi infection in juvenile white shrimp, Litopenaeusvannamei. *Fish & Shellfish Immunology*, *36*(1), 68-74.

38) Seenivasan, C., Bhavan, P. S., Radhakrishnan, S., &Shanthi, R. (2012). Enrichment of Artemianauplii with Lactobacillus sporogenes for enhancing the survival, growth and levels of biochemical constituents in the post-larvae of the freshwater prawn Macrobrachiumrosenbergii. *Turkish Journal of Fisheries and Aquatic Sciences*, *12*(1).

©2022The author(s). Published by National Press Associates. This is an open access article under CC-BY License (https://creativecommons.org/licenses/by/4.0/),