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Mapping Biodiversity of Indigenous Freshwater Chlorophytes.

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ABSTRACT

Algae are the most ancient, versatile and robust forms of organisms adapted to varied habitat throughout the world. They hold enormous potential as neutraceuticals, cosmoceuticals, pharmaceuticals, renewable energy sources, carbon dioxide sequesters, phycoremediators etc. The tropical climatic conditions prevailing in India favors natural biodiversity of algae over a wide range, that needs to be explored for their environmental and industrial applications. The study includes a systematic survey of collection and identification of various freshwater algae from different zones of Indian freshwater bodies; beginning with the state of Maharashtra and will consequently cover other regions of the nation. The procured data has been presented in the form of a database, created by extracting and integrating various online resources to provide the true and reliable data. It is implemented by SQL server relational database through user friendly web interfaces using HTML, asp.net and vb.net. Flexible search options are provided for data extraction and exploration. To summarize, algal species, their natural availability, flourishing season, optimum temperature, rainfall, soil type, physiology in relation to their ecology ; with all in one place along with the necessary option of cross-referencing; this database ensures to be an utile for researchers to carry out further work in phycology.

Keywords: Algae, Diversity, Habitats, Database

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INTRODUCTION

Algae range from single-celled organisms to multi-cellular organisms, some with a fairly complex differentiated form as in case of macroalgae. Algae have been traditionally regarded as simple plants. The absence of vascular system in algae differentiates them from higher plants. Reproduction in algae exhibits over a wide range from simple vegetative, asexual cell division to complex forms of sexual reproduction. Microalgae can be autotrophic (photoautotroph or chemoautotroph), heterotrophic (photoheterotroph or chemoheterotroph) or mixotrophic [1]. The three most prominent groups of algae are the brown algae, the red algae, and the green algae, amongst which some of the most complex forms belong to green algae. This lineage eventually led to the higher land plants. Algae are some of the most robust organisms on earth and are able to grow in a wide range of climatic conditions. Due to the prevalence of varied natural environmental condition in India, the subcontinent is a rich source for the growth and diversity of algae [2]. The taxon flourishing in freshwater bodies are freshwater algae, while those distributed in the sea, oceans and inland saltwater bodies are marine algae. Over 40, 000 algal species around the world are already identified and with many more yet to be discovered [3]. Microalgae are classified as (1) Chlorophyceae (Green algae) (2) Cryptophyceae (3) Phaeophyceae (Brown algae) (4) Rhodophyceae (Red algae) (5) Xanthophyceae (Yellow-green algae) (6) Dinophyceae (7) Bacillariophyceae (Diatoms) (8) Chloromonadineae (9) Eugleniae (10) Chrysophyceae and (11) Myxophyceae, based on the number and mode of attachment of flagella in the motile cells, thallus structure, chemical nature of pigments, reserve food materials, method of reproduction and variation in the life cycles [4].

Algae have numerous advantages: i) it can provide with surplus supply of renewable, carbon neutral biofuels [1] and can produce considerably higher rates of biomass as well as lipids per hectare as compared to the food crops being cultivated for oil extraction ii) Some oleaginous algal strains, e.g., *Botryococcus braunii* are known to produce up to 80 % lipids of its dry weight of biomass. Many unicellular algae have rapid doubling rate [5] and can grow throughout the year [6] iii) They can be cultivated in any aquatic medium ranging from sea water to brackish water, non arable land, in artificial photo bioreactors and iv) in return they do not compete with the food crops in agriculture sector [7]. Microalgae, termed as third generation biofuel feedstock assures economic and environmental sustainability.

The importance of ecological physiology in designing bioreactor for large scale production of biodiesel from algal feedstock has already been illustrated in the literature [8].

This research aimed to screen and study biodiversity of freshwater microalgae thriving in nature in their native forms. It involved collection, isolation, purification, identification and cultivation of strains under laboratory conditions. The current study intended to design a simple generalized biological database by compiling the raw data accumulated in this survey into the form of a systematic data bank with the help of bioinformatics tools.

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MATERIALS AND METHODS

Collection, Isolation and Maintenance of Cultures

Around fourty genera belonging to different classes of algae were identified and studied in the survey conducted from two districts namely, Pune District (Mulsi Taluka and Maval Taluka) and Raigad District (Alibagh Taluka) during late summers to early monsoon. Microalgae samples were collected manually in sterile transparent polyethylene bags/ bottles by hand or with the help of sieving nets from various water bodies like water streams, river side, wells, pits, ponds, paddy fields, lakes etc. and studied for their characteristic features under the microscope.

They were identified based on their morphology, taxonomy and mode of reproduction [9, 10, 11]. In most of the cases, filamentous algae were found associated with unicellular algae, (existing as mixed cultures), protozoa and algal grazers whereas in few cases they existed as mono cultures. The collected algal cultures were cultivated in the original water from their natural habitat, in enriched media and in synthetic media. The widely used media included Bold Basal Media, BG-11 media, Bristol media, C media, Kuhn's media, Fog's Media and D11 media [12]. The cultures were maintained in appropriate media specific to the strains under optimum light and temperature conditions. The strains were stored in 5 % formalin and preserved by cryopreservation technique with glycerol as a cryoprotectant.

Methodology of Development of Database

The relational database was developed, using SQL server as backend. The website is powered by Microsoft HTTP Server, HTML, asp.net and Vb-Script based web interfaces have been developed to execute the SQL queries dynamically. The application layer between the web interface and the backend relational tables has been implemented by using vb.net.

Data Access and Generation: The database interfaces includes, Home (general information), Advanced Search (a combined multiple search), Basic Search (by organism, pH, and Location), Classification (systematic classification of organism) and External Links.

Defining Research Design

The database generated is based on inferential research which opens the key to experimental and simulative research. At the same time, the survey is also an example of descriptive research which leads to longitudinal research in the future. Sampling design is based on the principle of stratified sampling along with area sampling where the complete national geographical area of India can be divided into strata's, i.e., states; states into districts; districts to collecting sites. Survey methods adopted are comparative and co-relational.

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RESULTS AND DISCUSSION

Freshwater algae sample were collected, isolated and identified from various locations in Maharashtra. Forty genera of algae collected from thirty six habitats belonging to two districts namely, Pune District (Mulsi Taluka and Maval Taluka) and Raigad District (Alibagh Taluka). Sampling sites like Ganesh Talav and Padamji Paper Mills did not attribute to the distribution of algae because of the presence of chemically treated water. In Mulsi Taluka, algal density was high, but the diversity of algal flora was low. On the other hand, Maval Taluka and Alibagh Taluka reflected low density and high diversity of freshwater microalgae.

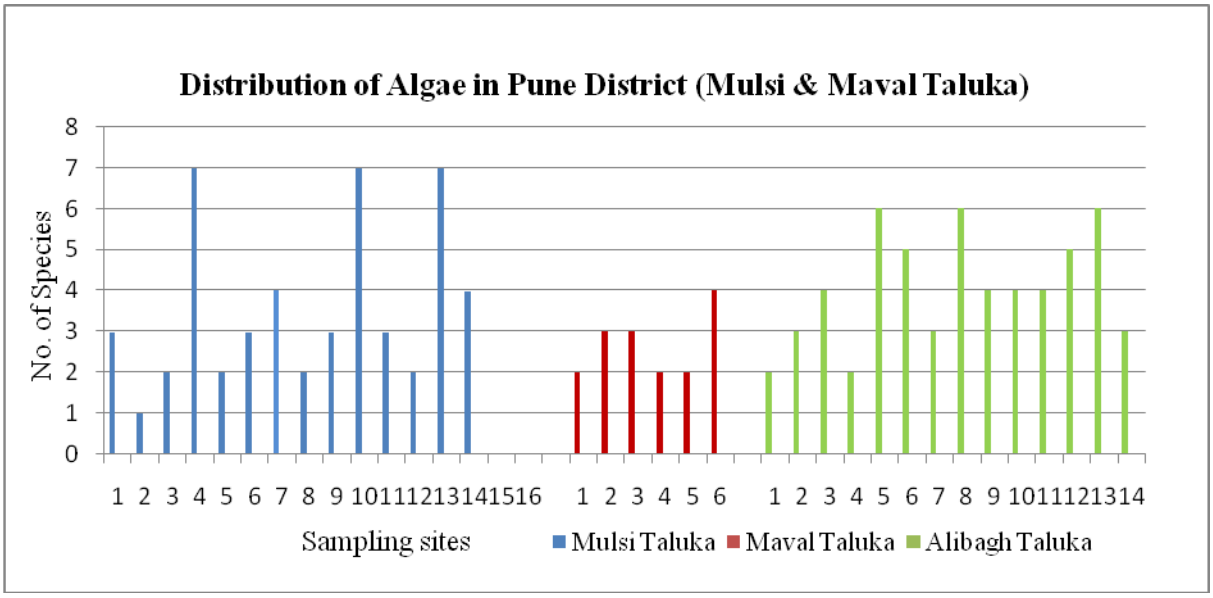

Figure 1: Distribution of Algae in Pune (Mulsi & Maval Taluka) and Raighad districts (Alibagh Taluka) Mulsi Taluka: 1. Akurdi Tunnel 2. Algae from *Dracaena sanderiana* 3. Aundh Bridge 4. Ravet Bridge 5. Sangawade Village 6. Morya Gosavi Mandir 7. Kiwale Village 8. Panshet Lake 9. Artificial Pond at Tathawade 10. Mamurde Village 11. Dapodi Bridge 12. Aquarium 13. Bird Valley 14. Pond at Tathawade 15. Ganesh Talav 16. Padamji Paper Mills. Maval Taluka: 1. Lonavala Waterfalls 2. Lonavala Lake 3. Bhushi Dam 4. Malavli Village 5. Khandala 6. Kamshet Village.
Alibagh Taluka: 1. Paddy field, Chari Village 2. Paddy field, Pale Village 3. Vashvi Village 4. Paddy field, Khidki Village 5. Bhuneshwar Lake 6. Pale Well 7. Vadavli Village, Well 8. Katalpada Lake 9. Medhakhar Well 10. Pezari Lake 11. Poinad Lake 12. Shregoan Bridge 13. Hemnagar Pond 14. Chorgundi Artificial Tank

Contamination of natural water reservoirs by industrial and municipal wastes results in increased conductivity which facilitates the growth of limited flora of microalgae e.g. *Spirogyra*, *Nitzchia*, *Euglena* etc. (called as dirty water algae) while it hampers the growth of rare and endangered algal species. Non pollutant sampling zones generally exhibited comparatively lower conductivity, contributing to species richness (Fig 1). The study indicated maximum occurrence and distribution of *Chlorella* sp. followed by the species of *Spirogyra*, *Euglena*, *Scenedesmus*, *Oscillatorium* and *Diatoms*.

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Species richness, abundance was measured in terms of frequency and relative frequency.

Frequency and Relative frequency was calculated by the formula [13],

$$\text{Species Frequency, } F = \frac{\text{Total Number of Samples in which Species occurred}}{\text{Total Number of Samples studied}} \times 100$$

$$\text{Relative Frequency, } RF = \frac{\text{Frequency of a particular species}}{\text{Total Frequency of all the Species}} \times 100$$

Majority of the collected eukaryotic algae belonged to Chlorophyta division with a few of them belonging to Charophyta, Cyanobacteria, Euglenoids and Desmids. Among the studied algae, *Spirogyra* sp. consisted of *S.singularis*, *S.crassa*, *S.colligata*, *S.hyalina* and *S.ellipsospora*. *Oedogonium* sp. constituted of *O.cardiacum* and *O. australe*. *Cladophora* sp. existed as *C.glomerata*. Several species of *Oscillatorium* were identified such as *O. subbrevis*, *O.anguina*, *O.granulata*, *O.curviceps*. *Euglena* sp. (*E.proxima*, *E.gracilis)* was found associated with majority of filamentous and unicellular microalgae. *Scenedesmus* sp. existed as *S.abundans*, *S.abundans var. brevicauda*, *S.bijuga* and *S.quadricauda*. *Sirocladium* sp. was identified to be *S.maharashtrense* and *Zygnema* sp. as *Z.terrestre* and *Z.orientalis*. *Sirocladium* is amongst the rare species typical to our country that was been identified. *Oedegonium* consists of high lipid content where as spirogyra is found to have sufficient carbohydrate content along with average oil contents. *Cladophora*, *Cosmarium*, *Neutrium*, *Closterium*, have high metal absorption capacities. *Chlorella* exhibited high protein content.

The algal biodiversity is too large to be explored and there exists a greater probability of discovering new ecologically and environmentally important algal genera. Barman.et.al [14] has screened twenty one algae taxa from sundarban mangroves to discover a high lipid containing strain for biodiesel production. A total of thirty one species were identified by Makandar and Bhatnagar [15] to study diversity of algae in arid zones of Rajasthan. In both the survey studies, the majority taxons identified constituted of cyanobacteria which are known for their toxins production. But their commercial exploitation for biofuel production is not yet completed. Cyanobacteria are the most commonly found taxons at all the habitats since they can bear extreme conditions of temperature and pH. The current study covers all the available freshwater regions thus increasing the probability of isolating higly productive strains.

Features of the Database

Forty species belonging to 5 different divisions and 9 classes from 24 families were listed in the database (available at the site, http://algaedb.dpu.edu.in). The database constitutes and provides the following information:

Home Page: It provides information to search algae based on its species name, pH and location. Gallery provides images of the entire algal genus along with pictures of algal grazers.

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Google Map marks the site of geographical distribution of algae in the surveyed regions. Home gives the general introduction about algae. Pictures of the sites of collection of sample are also been provided. Useful links provides links to other sites and web pages to provide more information related to particular algae (Fig 2).

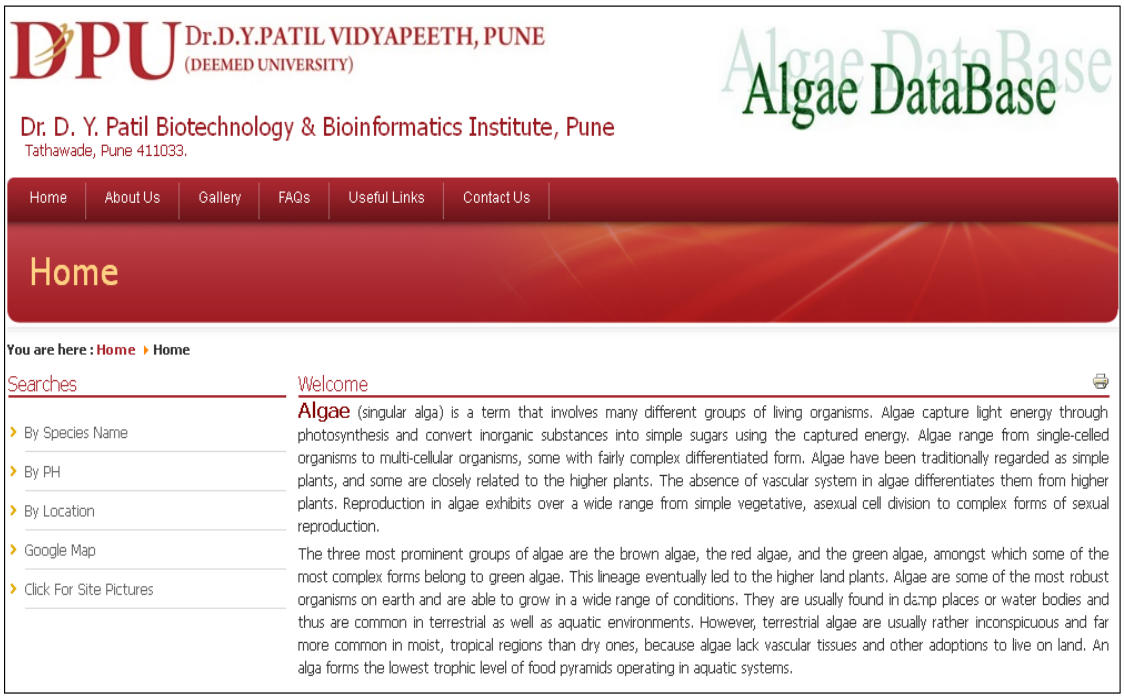

Figure 2: Snapshot of Home Page of Algae Database (available at http://algaedb.dpu.edu.in)

Search Based on Species: Search based on species name provides the details about a particular alga. Studied genus includes *Spirogyra*, *Oedogonium*, *Cladophora*, *Stigeoclonium*, *Oscillatorium*, *Chlorella*, *Scenedesmus*, *Euglena*, *Nitzchia*, *Microcystis*, *Monoraphidium*, *Botryoccocus*, *Tetraselmis*, *Cosmarium*, *Chara*, *Staurastrum*, *Chlorococcum*, *Closterium*, *Characium*, *Selenastrum*, *Anabaena*, *Pediastrum singlex*, *Pediastrum duplex*, *Synedra*, *Chloromonas*, *Crucigenia*, *Zygnema*, *Neutrium*, *Westella*, *Volvox*, *Tabellaria*, *Actinastrum*, *Sirocladium*, *Plagiogrammopsis*, *Aphanothece cyanobacteria*, *Chlamydomonas*, *Rhizoclonium* and *Diatoms*. On selecting the algae of choice, its ecological details can be viewed which includes its Location (the site of collection); flourishing and collecting season, pH and conductivity of the water body; type of water reservoir whether deep, wide, shallow, narrow; intensity of algal growth as algal bloom; soil type of the habitat whether red soil or black soil; mode of reproduction of algae and its association with other algal species. Atmospheric temperature, annual rainfall, latitude and longitude of each habitat is also been provided. A site page provides the access to images of sampling sites.

Search Based on pH: Similarly, an alga can be cited based on the best optimum pH exhibited by its water body. The pH range includes 6.7, 6.8, 6.9, 7, 7.1, 7.2 and 8.3 (Fig 3). The optimum growth of algae is observed at alkaline temperatures. Moreover species like

cyanobacteria; chlorella can bear pH up to 8.3. Search based on selecting Location, groups all the algae existing at that site along with their ecological details in a similar fashion. Water bodies like rivers, ponds, small pits, tunnels, wells, dam, lakes and locations like bridges, waterfalls, bamboo plant, artificial aquarium were covered to highlight the naturally existence strains at all possible areas.

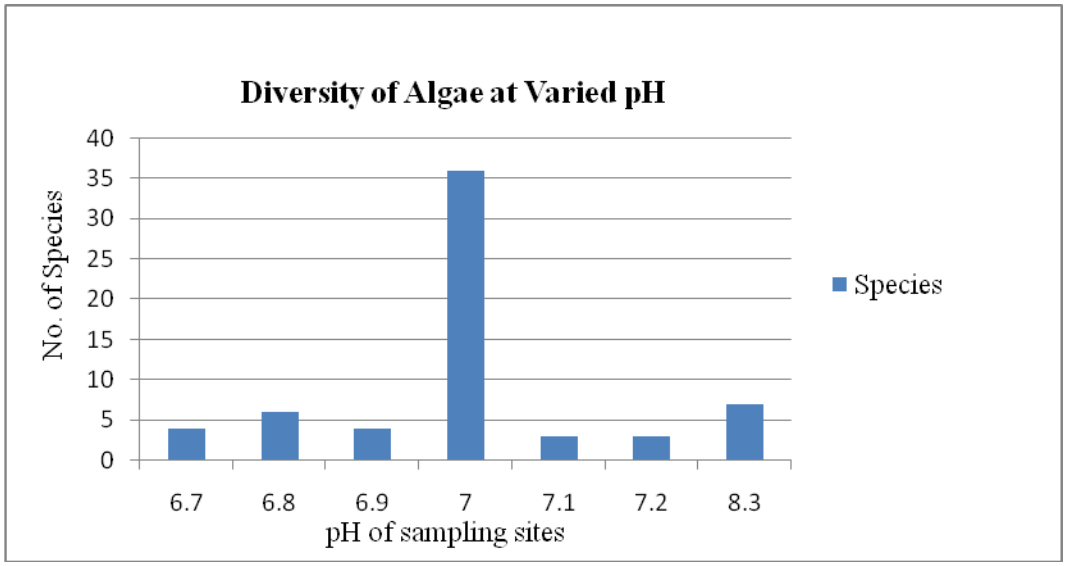

Figure 3: Diversity of Algae at varied pH of the sampling sites

Gallery: Gallery provides a pictorial presentation of all the algae species as viewed under the microscope with their systematic positions. Google map marks the collection site so that it is easy to trace the algae of interest.

The study thus deals with a bioinformatics approach to represent biological data (i.e., screening of algae) in the form of a database to provide results that holds relevance in algal based green technologies.

CONCLUSION

Majority of the collected eukaryotic algae belonged to Chlorophyta division with a few of them belonging to Charophyta, Cyanobacteria, Euglenoids and Desmids. The study indicated maximum occurrence and distribution of *Chlorella* sp. followed by the species of *Spirogyra*, *Euglena*, *Scenedesmus*, *Oscillatorium* and *Diatoms*. Developing such algal databases which account for its multiple usages by the research communities is desirable. As the study progresses, more and more regions of the country would be surveyed to locate and screen for new strains of algae that are yet to be discovered and a repository would be created. Generation of such databases would lead to new insights in the field of algal technologies to isolate and optimize strains for commercial production of biodiesel, bioethanol, vitamins, pigments, fatty acids, proteins, antioxidants etc.

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REFERENCES

[1] Dragone G, Fernandes B, Vicente AA, Teixeira JA. FORMATEX, 2010; 101: 5892-5896.
[2] Maharajh DM , Lalloo R. Indigenous algae: Potential factories for biodiesel production. Science real and relevant: 2nd CSIR Biennial Conference, CSIR *International Convention Centre Pretoria*, 17 & 18 November, 2008; 7.
[3] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M , Posewitz M, Seibert M, Darzins. The Plant J 2008; 54: 621-639.
[4] Fritsch FE. The Structure and Reproduction of the Algae, Cambridge University Press, US, 1945, 2.
[5] Pienkos PT, Darzins AL. Biofuels Bioprod Bioref 2009; 3: 431–440.
[6] Singh J, Gu S. Renewable and Sustainable Energy Reviews 2010; 14: 2596–2610.
[7] Cantrell KB, Ducey T, Kyoung SR, Hunt PG. Biores Technol 2008; 99: 7941–7953.
[8] Smith VH, Sturm BM, DeNoyelles FJ, Billings SA. Trends Ecol Evolution 2010; 25: 301–309.
[9] Prescott GW. Algae of the Western Great Lakes Area, 2nd edition, WM. C Brown Company publishers, USA, 1962; 662-963.
[10] Wehr JD, Sheath RG, Fresh water algae of North America – Ecology and Classification, Academic Press, Elsevier, 2003; 253-751.
[11] Randhawa MS. Zygnemaceae, monograph on algae, ICAR publisher, New Delhi, 1959; 209-436.
[12] Andersen R, Algal Culturing Techniques, 1st Edition, Elsevier, California, 2005; 429-486.
[13] Sharathchandra K, Rajashekhar M. Phykos 2013; 43: 51-66.
[14] Barman N, Satpati, G, Senroy S, Khatoon N, et. al. J Algal Biomass Utln 2012; 3: 42- 49
[15] Makandar M, Bhatnagar A. J. Algal Biomass Utln 2010; 1: 74 -92.