INTRODUCTION

Diatoms are eukaryotic microscopic single-celled autotrophic organisms with an intricately and ornately shaped cell wall composed of silica called ‘frustules’. Diatoms are ubiquitously distributed in aqueous habitats, both freshwater and marine environments, as well as adapted to living in soils and on moist surfaces. Cells are either solitary or united to form colonies or chains, which may be either linked by siliceous structures or by mucilage. Individual diatom ranges in size from ~2 μ to 2 mm, whereas some chains can be several millimeters in length.

Traditionally, diatoms are placed under the class Bacillariophyceae and have over 250 genera with perhaps as many as 100,000 species (Norton et al. 1996; Van den Hoek et al. 1997). The prime requirements for the growth of diatoms include light, CO₂, water and inorganic salts, thereby making it easy to culture both in fresh or marine water without any expensive supplements, as like other photosynthetic organisms (Lopez et al. 2005). Diatoms can occur in large amounts and forms, and generate their sculptured siliceous cell wall by silica biomineralization.

Depending upon the symmetry of frustules, diatoms are generally classified into two major types. Centric diatoms are radially symmetrical and in general circular in shape, whereas pennate diatoms are bilaterally symmetrical and elongated. The former tends to be mostly planktonic which are found in all open water masses, whereas the latter are benthic, growing on sediments or attached to various substrates or as epilithon on rocks. Historically, centric and pennate diatoms have been classified into two classes or orders. However, some other workers have recognized three classes of diatoms, viz., Coscinodiscophyceae (centric diatoms), Fragilariophyceae (araphid pennate diatoms) and Bacillariophyceae (raphid pennate diatoms). This classification gave equal ranking to the raphid pennate diatoms with a double slit opening i.e., raphe in the cell wall for movement and the araphid pennate diatoms without this slit (Round et al. 1990). Although, most of the classification and identification of diatoms have been based on the structure and markings on the cell wall as revealed under the light microscope (LM), but recent
developments through electron microscopy have resulted in many nomenclatural changes and taxonomy of the group has been repeatedly revised in recent years.

Diatoms exhibit a remarkable variety of geometric shapes, usually being circular or boat-shaped, but sometimes may be triangular, square, elliptical or straight rod-like. The silica cell wall of diatoms is highly patterned with an array of pores, processes, spines and ribs that are used extensively for identification purposes. The individual pores on the valve surface vary according to their size and spacing, some are so small that their presence can only be inferred from linear patterns (striae), whereas others are large and can be easily resolved with light microscope. The patterning observed on most valves of diatom is due to the result of regular small perforations through the silica. These specific patterns and structural diversity of the frustule helps in taxonomic classification of diatoms and forms a base for their identification. Out of the immense diversity of forms, Hendey (1964) has classified shape-groups into seven types, viz., linear, cuneiform, cymbiform, carinoid, discoid, gonoid and solenoid.

Diatoms are considered to be the most important group of eukaryotic phytoplankton which colonizes the oceans to a depth where only photosynthetically available radiation can reach, accounting for approximately 40% of the total marine primary productivity. They are the only organisms known to possess genetic ability to mineralize amorphous silica into complex structures. Diatoms serve as important members of the planktonic food chain and are of extreme importance for the biogeochemical cycling of minerals such as silica. When the cells of diatoms die, the remnant silica cell wall deposit at the floor of the ocean, forming fossils. These fossilized cell walls are called diatomaceous earth (DE) or kieselguhr or diatomite. Diatomaceous earth is an abundant, extremely cheap material, which has been extensively used in a wide range of applications, such as, heat and sound insulation, gel filtration, absorption, wine filtration, as catalyst in various chemical reactions, sensor components and in the production of beer, dynamites etc (Ongerth and Hutton, 1997; Scala and Bowler, 2001; Stoermer and Smol, 2004; Cai et al. 2005). Diatomite was also used as reinforcement elements in polymeric composites (Tasdemirci et al. 2008). Diatoms are also important from the point of cell biological and phylogenetic
studies. Recently, the potentiality of diatoms in diverse fields of engineering and medical sciences have been proposed like pinpoint drug delivery, metal film membranes and processing of nano powder silica (Wee et al. 2005). Using bottom-up self-assembly process, diatoms fabricate the inorganic materials into complex hierarchical patterns. Diatoms generate intricate nanostructures which provide materials ideal for biotechnological exploitation and has been widely used in applications like biosensing, optics, biophotonics, filtration, microfluidics and drug delivery (Drum and Gordon, 2003; Fuhrmann et al. 2004; Rosi et al. 2004; Hamm, 2005).

Diatoms absorb silicon from the environment in soluble form as silicic acid, \( \text{Si(OH)}_4 \). Silicic acid is the naturally occurring source of silica in aqueous environments which is transported into the diatom cells via novel membrane localized silica transporters. Diatom silica structure formation occurs during cell division by a fundamentally different mechanism in a specialized moldable membrane-bound vesicle known as the silica deposition vesicle (SDV). Molecular sequence data show that diatoms are heterokont algae. *Thalassiosira pseudonana*, a marine centric diatom was chosen for whole-genome sequencing and the genome of this eukaryotic phytoplankton species was found to be 34 mega base pairs (Armbrust et al. 2004).

The most important characteristic feature of diatoms is their ability to generate a highly patterned external wall called ‘frustule’, which is composed of amorphous silica \( [(\text{SiO}_2)_n(\text{H}_2\text{O})] \). The frustule is constructed of almost two equal overlapping halves, with the smaller fitting into the larger one like a petridish. The larger of the two halves is called the epitheca, and the smaller one is called the hypotheca. Each theca is typically composed of two parts - the main surface and its incurved margins termed valve and overlapping connecting bands respectively. The epivalve and epicingulum comprise the epitheca whereas the hypovalve and hypocingulum comprise the hypotheca. The valve relates either to the top or bottom of the box whereas the two connecting bands represent incurved sides of the lid and the main body. When fitted together, the connecting band of epitheca overlaps with that of hypotheca and by a cementing organic substance, the two bands remain united together in the overlapping region called girdle. Both the valves and the
connecting bands are highly structured, but typically, valves are more ornamented and intricately structured than the bands and when organic material is removed by cleaning, the frustule splits in two. The line connecting the middle of the two valves constitutes the pervalvar axis and the place along which the cell divides at right angles to pervalvar axis is called valvar plane.

The frustule morphology is usually described from two views, when a valve lies in such a position so that the valve side remains uppermost \textit{i.e.}, the valve side is seen only, it is called valve view; when the valve remains uppermost \textit{i.e.}, the girdle side is seen, it is then called girdle view. Valve views usually show greater morphological variability compared to girdle views. The outline of the valve may be circular or oval, or variously angled, linear, lanceolate, rhombic, panduriform or sigmoid. Girdle views tend to be simpler in outline, often appear square or rectangular, sometimes occasionally cuneate and exhibit few differentiating features. The ornamentations are confined only to the valve position of the silica wall. The frustule is usually sculptured with species-specific patterns and structures which consist of rows of pores arranged in a definite pattern which are quite distinctive to individual species.

The complex morphological features of the frustule are being used to delineate the genera and species. The nanopores and slits in the frustule allow the diatom cell to interact with its surrounding environment. The frustule gets its flexibility and toughness from the materials it is composed of, and the main organic components constituting the diatom biosilica are silaffins and polyamine proteins co-precipitated with silicic acid available in the aqueous environment. Uptake of silicic acid by diatoms from the external medium is an active mechanism mediated by silicon transporters (Hildebrand \textit{et al.} 1997). It is believed that silaffins, cationic polypeptides accelerate the polymerization process occurring within the SDV (Kroger \textit{et al.} 2000). Silica deposition to form new valves and cingulum elements occurs within the silica deposition vesicle in the protoplast, being released to the exterior when complete by fusion between the silicalemma and plasmalemma (Pickett-Heaps \textit{et al.} 1990).
Biomineralization is the formation of inorganic materials under the control of a living cell. Diatoms produce their structured cell walls in highly regular porous patterns which range from nano to micrometer scale by biomineralization process. The diatom frustules are variously perforated and heavily ornamented due to regular deposition of siliceous material in different patterns which is laid down by unknown mechanisms. The process of frustule formation is not very clear and mostly based on microscopical observations.

Characterization of porous silica nanostructures of diatom uses diverse analytical tools, viz., scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), atomic force microscopy (AFM) etc. Nanoporous silica sizes of less than 100 nm are considered as excellent materials for a wide range of applications in electronics and IT based industries. Further, nanoporous silica deposited over the frustules of diatoms is geometric, biologically stable, cost-effective and eco-friendly.

The unique frustule morphologies have a lot of optical, mechanical and transport properties that are useful in making highly developed devices such as light harvesting, molecular separation, sensing, photonics and drug delivery systems. Furthermore, the frustule may be exploited for several technical applications such as, biomimicry of silicification routes, direct use of the silica structure, the use of surface chemistry for functionalization, and also as template for structures made from other materials or nanocomposites. The frustule produces complex three-dimensional nano and micro-scale silica structures which may be of great use in a wide range of nanotechnological applications (e.g., masks for lithographic patterning, catalyst supports, gel filtration etc). The ability to alter the frustule by changing growth conditions or by forming nanocomposites may be useful in various biotechnological applications.

Taxonomic status and evolutionary history of diatoms is still not transparent. The ability to form silica cell walls in algae may have arisen in the chrysophytes or synurophytes, which make silicified scales (Pickett-Heaps et al. 1990). Although they are all heterokont algae, however, phylogenetic analysis indicate that diatoms constitute
separate lineages and are not derived from them (Saunders et al. 1995; Medlin et al. 1996; Goertzen and Theriot, 2003; Andersen, 2004). Phylogenetic analysis based on SSU rDNA sequences and presence of fucoxanthin as a major carotenoid strongly suggest that Bolidomonas species could be similar to the heterokonts which eventually gave rise to the diatom lineage (Medlin et al. 2000). This hypothesis is also consistent with the most recent eukaryotic phylogenetic trees (Baldauf et al. 2000).

Silica is one of the major constituents of natural soil which exists in different forms of quartz. Geological Survey of India recorded the occurrence of highest amount of friable quartzite belonging to the Shillong groups of rocks sporadically along easternmost part of Nagaon district of Assam, viz., Jiajuri, Borhola, Thanajuri and Chapanala. By employing different methods of beneficiation, about 75% of the glass sand may be recovered from this friable quartzite (Goswami, 2006). No any extensive investigation has been carried out to characterize the diatom, having the genetic ability to deposit silica over their cell surface in characteristics nanoporous forms from these silica rich regions of Assam.

The presence of abundant silica in natural habitats creates a specific type of ecological niche. Moreover, silica is an absolute requirement for growth and development of diatoms. However, the diatoms of North-East region of India is still largely unexplored and unexploited, therefore, the proposed study has been conceptualized with the following objectives:

1. To survey the aquatic and semi-aquatic habitats for diatom species from few silica rich sites of Nagaon district of Assam.
2. To optimize conditions for *in-vitro* culture of diatom species.
3. To characterize the nanostructures of diatom species by using SEM and AFM.
4. To develop a SSR *i.e.*, 18S ribosomal RNA based phylogenetic analysis of the diatom species.