INTRODUCTION

From a global ecosystem perspective, life as we know it on Earth depends on resources such as water, energy, and favourable environmental conditions. Energy is an essential and vital component for development, and the global economy literally runs on energy. Demand for energy and its resources are increasing every day due to rapid outgrowth of population and urbanization. Major energy demand is fulfilled from conventional energy resources like coal, petroleum and natural gas. Fossil fuels, including oil, coal and natural gas, are providing about 85% of energy globally. These sources are in the verge of getting exhausted. It was estimated that the oil sources might be depleted by 2050 (Goyal et al., 2008). Moreover, the process of obtaining energy from these sources causes atmospheric pollution, resulting in problems like global warming and acid rain. This has triggered recent interest in alternative sources to petroleum-based fuels. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable and readily available (Meher et al., 2004). Negative environmental impacts, limited sources and rising prices of fossil fuels pose significant environmental and socio-economic challenges. Globally, major national and international initiatives are underway to identify, revive, research and recommend renewable sources of energy.

Worldwide consumption of fuels is undoubtedly unstable causing world economic crisis; the worst compared to other economic recession that took place at different era. This factor has urged all nations, especially the government and academics to find another alternative to replace the usage of petroleum. Therefore, there is a rising demand to globally provide renewable energy by means of a sustainable and ethical approach. Sustainable development is a concept that has become significant and increases the awareness of its necessity.

There are many alternatives nowadays. There are three generations of biofuel. Biofuels made from sugar, starch, vegetable oil, or animal fats using conventional technology are first-generation biofuels. These feed stocks could instead enter the animal or human food chain, and as the global population has risen their use in producing biofuels has been criticised for diverting food away from the human food chain, leading to food shortages and price rises. Second generation biofuel production
processes are in development. These allow biofuel to be derived from any source of biomass, not just from food crops such as corn and soybeans but also from waste cooking oil. Algae fuel, biofuel from algae is a third generation biofuel, it also called oilgae.

Biofuels are derived from a biomass-product of carbon fixation by modern living photosynthetic organisms; plants, microalgae, and cyanobacteria. Traditional natural organic fossil fuels (coal, peat, oil, and gas) are products of long-term geological deposition of plant-type biomass consisting of carbon and hydrogen. Fossil fuels were formed from a biomass by its anaerobic degradation under heat and pressure in the earth’s crust over millions of years. Obviously, in terms of human life (or even of tens or hundreds of generations) scale, such fuels are considered as non-renewable natural resources (Sarsekeyeva et al., 2015).

According to data, provided by International Energy Agency (http://www.iea.org), recently consumed primary energy sources are mainly represented by oil (36.0%), coal (27.4%), and natural gas (23.0%) that add up a total of 86.4% of the energy (fossil and nonfossil) consumed in the world. The nonfossil energy sources include hydropower plants (6.3%), nuclear (8.5%), and other sources of energy (geothermal, solar, tidal, wind, wood, and waste burning) with a sum of 0.9%. However, over the last 5 years, a trend has been observed toward reduction of the consumption of traditional energy sources, with a simultaneous increase in the use of non-traditional sources (up to 25% in 2015), including solar energy, wind, biomass use, etc. (Sarsekeyeva et al., 2015).

Continued use of fossil fuels is now widely recognized as unsustainable because of diminishing supplies and contribution of these fuels to increased carbon dioxide (CO\textsubscript{2}) concentrations in environment. Fossil fuel combustion accounts for approximately 99% of total global CO\textsubscript{2} emissions, excluding those from forest fires and use of wood fuel (IEA, 2007). Rise in actual global CO\textsubscript{2} emissions increased by 4.2% during 2011, and reached 34.5 billion tons in 2012. Transport accounts for 19% global energy use representing 23% of energy-related carbon dioxide (CO\textsubscript{2}) emissions and growth projections estimate a nearly 50% increase in transport energy use and CO\textsubscript{2} emissions by 2030 and more than 80% by 2050 (IEA, 2009). Main sources of CO\textsubscript{2} emissions are fossil fuel combustion for transport, electricity, thermal energy generation, and other small industrial sources. Moreover, the increase in CO\textsubscript{2} is mainly due to energy-related human activities over the past decades determined by
strong economic growth in developing countries like India and China (European Expert Group on Future Transport Fuel, 2011). Due to emission of large amount of green house gases (GHGs) and associated global warming, the search for renewable energy sources such as nuclear, photovoltaic, hydroelectric and wind energy are becoming increasingly popular as alternative sources, but they cannot necessarily fulfill the demand of fuel in transport sector (Palmer et al., 2011). According to IEA (2008) transportation sector alone accounts for 62.3% of fossil oil consumption compared to other energy sources. As a result, transportation covers a significant share approximately 22% of global fossil fuel combustion-related CO$_2$ emissions (Oliver et al., 2013). Thus, biofuel stands out as the ideal solution, and it could be rapidly implemented as it will reduce not only the dependency on imported oil but also reduce CO$_2$ emissions of transportation sector.

With rapidly rising world energy demand and high fossil fuel prices, an intense interest is being focused on the photosynthetic plants/organisms which can store lipids in the form of triacylglycerides (TAGs) and further transesterified to biodiesel as an alternative to fossil fuels (Mohan et al., 2011). Therefore, raw materials containing higher content of fatty acid (FA) should be chosen for biofuel production (Lei et al., 2012), in this regard biological sources viz., rapeseed, canola, sunflower, soybean oils, beef tallow and many other oils have been used for the production of bio-diesel esters, which are thought to be an alternative of fossil fuels (Sakthivel et al., 2011). Still biodiesel derived from these edible crops using today’s technology does not represent an effective alternative to substitute conventional fuel due to high costs of production and land use competition with edible crops. This leads to transition from the first (edible crops) and second generation (lignocellulosic biomass from dedicated non-edible crops like switch grass and agricultural waste) to a third generation of biofuel, such as photosynthetic microorganisms, as a promising option of sustainable biofuel production (Quintana et al., 2011). Microorganisms such as bacteria, yeasts, molds and algae can accumulate lipids at more than 20% of their biomass and are defined as oleaginous species (Karatay and Donmez, 2011). It has been reported that biofuel production from photosynthetic microorganism is more advantageous as well as higher than best oil producing crop plants due to their rapid growth rates, relatively simple genetic system and limited effect on the food supply (Kim et al., 2012).
Biofuel research is not just a matter of finding the right type of biomass and converting it to fuel, but it must also find environmentally and economically sound uses for the by-products of biofuels production. Biofuels target a much larger fuel market and so in the future will play an increasingly important role in maintaining energy security. Major biofuel products include biomethanol, bioethanol, biobutanol, biomethane, biohydrogen, and biodiesel. Among them, only bioethanol and biodiesel are presently produced at industrial scale, and they comprise about 90% of the biofuel market (Mata et al., 2010). Currently, fuels make up approximately 70% of the global final energy market. In contrast, global electricity demand accounts for only 30% (Hankamer et al., 2007). Yet, despite the importance of fuels, almost all CO$_2$ free energy production systems under development are designed to drive electricity generation (e.g., nuclear, photovoltaic, wind, geothermal, wave and hydroelectric). Given the above situation, there is presently a debate as to which fuels from biomass with their yield potentials appear most attractive. Several biofuel candidates were proposed to displace fossil fuels in order to eliminate the vulnerability of energy sector (Korres et al., 2010; Singh et al., 2011b). Much of the discussion over biofuels production has focused on higher plants such as corn, sugarcane, soyabean, algae, oil-palm and others (Pandey, 2011; Gnansounou et al., 2008) and the problems associated with their use, such as the loss of ecosystems or increase in the food prices. While most bioenergy options fail on both counts, several microorganism-based options have the potential to produce huge amounts of renewable energy without disruptions. Cyanobacteria and their superior photosynthesis capabilities can convert up to 10% of the sun’s energy into biomass, compared to about 1% recorded by conventional energy crops such as corn or sugarcane, or about 5% achieved by algae. Photosynthetic microorganisms like cyanobacteria and microalgae can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner and at rates high enough to replace a substantial fraction of our society’s use of fossil fuels (Li et al., 2008).

Oxygenic photosynthetic microorganisms, including cyanobacteria and microalgae, have recently drawn attention as a new feedstock for next generation biofuels. Algal biomass has several properties over terrestrial energy crops for biofuels production; they are considered to be among the most efficient organisms
regarding the solar energy conversion and they do not compete with food crops, as they can be cultivated with relatively simple requirements in non-arable land. Cyanobacteria are photoautotrophic microorganisms, which utilize light energy and inorganic nutrients (carbon dioxide, nitrogen, phosphorus etc.) and synthesize valuable biomass compounds, such as lipids, proteins, carbohydrates, pigments etc. The productivity and photosynthetic efficiency of cyanobacteria (3 to 9%) is higher as compared to plants and algae, they are considered as a promising alternative for producing biofuels with cyanobacteria having certain advantages over algae and plant. Cyanobacteria offer a diverse spectrum of valuable products for the production of biofuels and biochemicals, however, the feasibility of cyanobacterial production depends on lowering costs and increasing efficiency in various aspects such as strain improvement, photosynthetic capacity, and harvesting and isolation of products from cyanobacteria.

Today we stand on the threshold of creating and using a fourth-generation biofuels, which is based on fast growing, rapidly renewable, and genetically engineered resource- procaryotic photosynthesizing cells of cyanobacteria. Cyanobacteria have a high potential for production of biofuel because of their significant advantageous properties:

1. Cyanobacteria do not claim arable lands and do not compete with farmers for crop areas and other resources.
2. Cyanobacteria grow and accumulate biomass rapidly.
3. Cyanobacteria directly fix atmospheric or water-dissolved CO$_2$, and they require only sunlight, water, and a minimum set of inorganic trace elements for growth. Cyanobacteria are capable of handling the excess CO$_2$ (the emissions we are trying to avoid) directly into hydrocarbons for biofuels. They can be also used to recover the waste water from organic and inorganic contaminants.
4. Plasticity of cyanobacterial metabolism allows for directed biosynthesis of lipids in controlled photobioreactors.
5. Many strains of cyanobacteria may be easily and stably transformed. Therefore, they can be used as convenient platforms for the genetic modification of metabolic pathways. Some researchers also support
cyanobacteria because fuel from engineered cyanobacteria is excreted outside the cell, in contrast to eukaryotic algae, in which fuel production takes place inside the cell (Sarsekeyeva et al., 2015).

6. Lipid accumulation in oleaginous algae is mostly achieved by imposing stress (i.e., adverse environmental conditions or abiotic factors). In contrast, enrichment of lipids in cyanobacteria can be accomplished by over-expressing enzymes of thylakoid membranes, which are positively correlated with photosynthetic and biomass-production rate (Mullineaux, 1999). Thus, the highest density of lipids in cyanobacteria biomass occurs together with the highest biomass-production rate.

7. As entire genome sequence of *Synechocystis* PCC 6803 is published, so cyanobacteria are much more amenable to metabolic engineering to improve lipid content beyond that of the wild type (Sheng et al., 2011). Due to these reasons genetic modification of cyanobacteria has gained appreciable attention in recent years in increasing and modifying the accumulation of lipids, alcohols, hydrocarbons, polysaccharides, and other energy storage compounds.

Traditionally, identification of cyanobacteria has relied on morphological, physiological, and ecological characteristics (Castiglioni et al., 2004), but these characteristics vary under different environmental or growth conditions, thus, are confusing and lead to uncertain identifications. For example, the presence of nitrogen sources like NH$_4^+$, NO$_3^-$ inhibits the formation of heterocyst and presence of phosphate inhibits the formation of akinetes in cyanobacteria. Due to above reasons, assessment of biodiversity using morphological and physiological variables provides misleading information (Kumari et al., 2009). Therefore, molecular approaches based on PCR techniques and DNA fingerprinting has been adopted for taxonomical studies and proved to be more reliable than conventional ones in various aspects of identification of cyanobacterial population and to uncover cryptic variations of strains or closely related species (Oinam et al., 2011).

Most cyanobacteria are photo lithoautotrophs, which means that sun light serves as the primary energy source, electrons are obtained from an inorganic source (i.e., water) and CO$_2$ is the sole carbon source. Like other organisms, cyanobacteria additionally require sources of nitrogen, sulphur, and phosphorous for the production
of new biomass (Ludwig and Bryant, 2012). Cyanobacteria are equipped with numerous mechanisms that allow them to survive under conditions of nutrient starvation, some of which are unique to these organisms (Schwarz and Forchhammer, 2005). Additionally, recent studies have shown that transcriptome reprogramming plays a central role in the acquisition of a phenotypic state under specific perturbations (Bonneau et al., 2007; Komili and Silver, 2008; Brauer et al., 2008; Lopez-Maury et al., 2008; Jiao et al., 2011). Thus, identification of all differentially regulated genes in cyanobacteria, regardless of the amplitude of changes in transcript levels, is very important to understand cellular strategies when cells are perturbed (Singh et al., 2010). Understanding the response to nitrogen deprivation, nitrogen fixation, and diazotrophic growth in cyanobacteria will shed light on basic mechanisms of bacterial genetic regulation and physiology. In addition, it may help to develop better strains of cyanobacteria for production of renewable chemicals and biofuels (Flaherty et al., 2011).

For the growth of cyanobacteria, the intensity of sunlight and CO₂ are the main factors. There are also other parameters (biotic and abiotic) that greatly influence the growth of cyanobacteria and biosynthesis of lipids with high fatty acid profile. Other parameters include salinity, pH, temperature, presence of organic and inorganic nutrients. They flourish in water that is salty, brackish or fresh, in cold and hot springs, and in environments where no other microalgae can exist. Cyanobacteria comprise a large component of marine plankton with global distribution. A number of freshwater species are also able to withstand relatively high concentrations of sodium chloride. It appears that many cyanobacteria isolated from coastal environments tolerate saline environments (i.e. are halotolerant) rather than require salinity (i.e., are halophilic). As frequent colonisers of euryhaline (very saline) environments, cyanobacteria are found in salt works and salt marshes, and are able to grow at combined salt concentrations as high as 3-4 molar mass (Reed et al., 1984).

Accumulation of lipid in cyanobacteria can be greatly influenced by different abiotic factors. Production of biodiesel depends on high value of lipids present in cyanobacteria. High value fatty acid accumulation varies from strain to strain with change in different abiotic parameters. Strains which can thrive well in varied environmental conditions and those produce high value lipid can be given priority and should be promoted for production of alternate renewable energy. They can be
cultured and cultivated in large bioreactors providing best conditions for production in huge amount. Those strains which are not so efficient for biodiesel production can also be manipulated by using advanced techniques and by providing suitable abiotic conditions.

Present non-renewable resources are not sufficient for increasing population. Petroleum resources will almost deplete in the near future. Use and combustion of such resources cause great harm like, global warming and pollution to the environment. Such problem should be overcome by a better harmless way. Use of biodiesel will become the alternate source of energy. Many research articles have been published to improve the efficiency of biodiesel derived from microalgae which are as efficient as petroleum. They will be less harmful and will serve the environment in an eco-friendly way. Many researchers and scientists have been exploring technology for advancement of this new technology to achieve this goal. Thus, there is a need to indentify fast growing cyanobacterial strains producing high amount of lipids with good fatty acid profile from freshwater lakes of Kumaun region, Uttarakhand, India.

Keeping the above facts in mind, present study will be conducted with the following objectives:

1. Isolation, identification and molecular characterization of cyanobacterial strains from Kumaun region of the Uttarakhand.

2. Lipid/fatty acid profiling of promising cyanobacterial strains through chromatographic techniques.

3. Transient expression of candidate gene(s) with respect to lipid content under abiotic stress.