A broad diversity of microorganisms inhabiting the earth’s surface is capable to survive in extreme climatic conditions. These microorganisms are referred as extremophiles. Extreme environments possess various factors which are incompatible with most of the life forms. Both eukaryotic and prokaryotic microorganisms are found in extreme environments like environments having extremely low (psychrophiles-cold loving) and high temperatures (thermophiles-heat loving), acidic conditions (acidophiles- low pH), alkaline conditions (alkaiphiles- high pH), high pressure (barophiles/piezophiles), high salinity (halophiles-salt loving), etc. Temperature is one of the most important factors that governs growth and survival of microorganisms. According to temperature requirement, microorganisms can be classified into- psychrophiles (-2 ºC to below 20 ºC), mesophiles (20-40 ºC) and thermophiles and hyperthermophiles (45-113 ºC) (Seckbach and Oren, 2000). Morita (1975) described pyschrophiles as organisms with minimal growth temperature to be ≤ 0ºC, while optimal and maximal growth temperatures are 15 ºC and 20 ºC, respectively. On the other hand, psychrotolerants have the ability to survive at low temperature but have optimal and maximal growth temperature above 15 ºC and 20 ºC.

Several reports on thermophillic fungi have been placed on records (Johri et al. 1999; Maheshwari et al. 2000; Ghazifard et al. 2001) while there are relatively very few reports on psychrophilic fungi. Diversity of filamentous and non-filamentous fungi originated from colder regions is yet to be explored with focused attention. Fungi (ascomycota and basidiomycota) are remarkable organisms having the potential to thrive themselves under stress (extreme) conditions by producing secondary metabolites such as enzymes, pigments, antimicrobials, antioxidants, etc., and these secondary metabolites possess various applications in biotechnology.

### 2.1 Cold temperature adapting microorganisms

As described earlier, temperature is the most significant factor for affecting the growth of microorganisms differentiating them between cold and hot temperature loving or tolerating organisms. The 85 % of total earth’s biosphere is exposed to low temperature
i.e. below 5 ºC (Margesin et al. 2007). In such harsh conditions, various structural and functional adaptations are required in microorganisms for successful survival and colonization (Bej and Mojib 2010; Gostincar et al. 2010). Significantly, these colder regions exhibit colonies of cold loving microorganisms representing the ascomycota group. The ascomycota found to be dominant in comparison of any other filamentous fungi (Devi et al. 2012; Timling et al. 2014). Other colder areas such as Antarctic regions have been reported to inhabit filamentous as well as non-filamentous fungi in abundance (Ray et al. 1992; Connell et al. 2006). Kostadinova et al. (2017) also investigated a group of psychrotolerant filamentous fungi belonging to division ascomycota, deuteromycota, zygomycota and basidiomycota, from the soil of Antarctic region. Isolation of several fungi from permafrost, which is a thick surface layer of soil permanently exposed to freezing temperature, has also been reported (Gilichinsky et al. 2005). A good diversity of basidiomycetes and ascomycetes fungi consisting genera Penicillium, Aspergillus, Geomyces and Cladosporium was recorded in Arctic permafrost (Ozerskaya et al. 2009). In contrast to this, very few fungi were isolated from permafrost of Antarctic region. Margesin (2008; 2009) also reported numerous filamentous fungi from permafrost soil of Arctic and Antarctic regions. Along with soil and permafrost, low temperature ecological niche also consist of habitats that may include snow, glaciers, cold lakes, oceans and caves exhibiting wide range of fungal diversity. These cold habitats have also been reported to exhibit several bacterial genera (López et al. 2009; Xu et al. 2003; Arnau et al. 2015).

Apart from this, Indian Himalayan region (IHR) is also considered as major source of filamentous fungi with the potential to thrive in cold environmental conditions. The study carried out from low temperature environments in IHR reported the dominance of genera Aspergillus, Penicillium and Cladosporium (Pandey and Palni 1998; Pandey et al. 2008). The genera Aspergillus, Penicillium, Cladosporium and Trametes also possess the unique characters such as tolerance to wide range of pH and high salt concentration and production of secondary metabolites such as enzymes and pigments (Pandey et al. 2007; Rinu and Pandey 2010; Dhakar et al. 2014a; Pandey et al. 2016). Along with filamentous fungi, IHR also possesses several cold loving genera of Pseudomonas and Bacillus (Jain and Pandey 2016b). Therefore, IHR has become the main focus of the microbial diversity in last two decades and, thus, receiving full attention for the exploration of both psychrotolerant prokaryotes and eukaryotes.
(Pandey and Palni 1998). Glaciers of IHR including Roopkund, Kafni, Gangotri and Pindari have been studied for microbial diversity (Pradhan et al. 2010; Shivaji et al. 2011; Srinivas et al. 2011). Indeed, the microorganisms (eukaryotes or prokaryotes) from these regions have been explored for their biotechnological applications in terms of production of cold active enzymes (Kudus et al. 2011, 2012; Pandey et al. 2016), biodegradation (Dhakar et al. 2014a; Kaira et al. 2015), and plant growth promotion (Pandey et al. 2004; Rinu et al. 2012; Jain and Pandey 2016a). Various studies have been carried out for the production of pigments from different genera of filamentous fungi (Penicillium, Aspergillus, Paecilomyces, Talaromyces) and bacteria (Serratia, Pseudomonas, Bacillus) (Cho et al. 2002; Chadni et al. 2017; Oren 2009). However, no reports on pigment production by microorganisms from IHR have been described yet.

2.2 Classification and identification of Penicillium

Penicillium is the best known genus among filamentous fungi that can be represented as brush like structure i.e. Penicillus (little brush) microscopically (Link 1809) having both natural as well as industrial importance. The structure of Penicillium contains conidiophores which may be simple or branched and are terminated by clusters of flask shaped phialides, also called sterigmata. The tip of the phialides consists of spores called conidia. According to British mycologist Ainsworth (1973) fungi is divided on the basis of presence or absence of plasmodium which includes two divisions namely Myxomycota and Eumycota Fig.3.

The taxonomic position of Penicillium according to Alexopoulos et al. (1996) is as follows-

- Kingdom: Fungi
- Phylum: Ascomycota
- Class: Eurotiomycetes
- Order: Eurotiales
- Family: Trichomaceae
- Genus: Penicillium

Initially the morphological methods including the study of colony characters and diameters on the specific medium and the microscopic characters consisting of
production of conidiophore branching patterns were traditionally used for the classification and identification of the genus *Penicillium* (Raper and Thom 1949; Pitt 1979). Conidiophores were categorized according to branching pattern which includes monoverticillate, biverticillate and terverticillate. Further, the biochemical studies came into interest to increase the efficient identification of the genus (Cruickshank and Pitt 1987). Later, it was concluded that only morphological, microscopical and biochemical studies were not enough for the accurate identification of fungi. Therefore, molecular identification (DNA sequencing) has become the main focus in the year 1990. By using standardized short DNA sequences (DNA barcoding), identification of any eukaryotic organism is possible. The barcode CO1 (Cytochrome oxidase c) was reported to be the most suitable for genus *Penicillium*, while doubtful results were found in other genera. The identification of most of the fungi was not possible from the barcode because it was impossible to generate universal primers due to the inconsistent results. Therefore, the most suitable and authenticated barcode for fungi was accepted to be Internal Transcribed Spacer (ITS region).

The ITS region is the most widely used sequence marker for identification and characterization of fungi and its universal primers are also available (Schoch et al. 2012). In order to study the environmental fungal diversity, ITS region is being used in last few years. Basically, ITS region is a fragment of ribosomal DNA (rDNA) between 18S and 28S ribosome, including ITS1-5.8S-ITS2 component. The 5.8S in ITS component was considered to be conserved while the other two ITS exhibited some variability (Boysen et al. 1996). Skouboe et al. (1999) also reported the identification and characterization of *Penicillium* spp. by using ITS region. Diversity of *Penicillium* spp. isolated from the agriculture soil of Iran was investigated by using ITS region. The identified species were as follows: *P. aurantiogriseum, P. citrinum, P. commune*, etc. The study supported the rapid identification of various species from the respective genus (Javadi et al. 2012). Apart from ITS, β-tubulin, RNA polymerase II largest subunit (RPB2) gene and Calmodulin (CaM) have also been considered as secondary markers for identification (Samson et al. 2004; Houbraken et al. 2012; Visagie et al. 2014).

Later, the polyphasic approach (morphological and molecular) was considered as an efficient tool to identify at the species level. Earlier, morphological characteristics
were considered as potent tool for identification but due to the limitations in genomic and proteomic level, molecular tools were also included for identification purpose. Apart from morphological and molecular methods, physiological parameters such as effect of temperature and pH, carbon and nitrogen sources, organic solvents and mineral salts as supplements were also taken into consideration to increase the efficiency of identification. The profile of secondary metabolites produced by the specific species also supported the identification of the species. Kozlovsky et al. (2009) also reported that the potential production of secondary metabolites is a component of the physiological and biochemical identification.

Fig. 3 Classification of fungi (Ainsworth 1973)

2.3 Distribution of Penicillium

According to the Dictionary of the Fungi (10th ed.), there are over 300 species of genus Penicillium (Kirk et al. 2008). Penicillium are familiar as blue and green moulds that mainly feed on citrus fruits (P. digitatum and P. italicum), cheese and meat (P. roqueforti and P. camembertii) (Geisen et al. 2001) and are considered as one of the most dominant genus in various ecosystems including forests, beaches, deserts, and
cultivated lands (Pitt et al. 2000). Along with the genus *Penicillium*, *Aspergillus* and *Paecilomyces* are also being considered as important genera. *Penicillium* spp. are ubiquitous in soil environment preferring cool and moderate climate for their growth and have the ability to produce powerful organic acids that can degrade surface of many rocks such as carbonate, marble and granite (Sterflinger 2000). Houbraken and Samson (2011) reported that *Penicillium* grows in less water availability and low or high temperature. Cantrell et al. (2011) reported the dominance of *Aspergillus* and *Penicillium* species in low temperature and saline habitats, along with this, diversity and applications of various species of genus *Penicillium* were also described in low temperature environments such as arctic glaciers (Sonjak et al. 2006), Indian Himalaya (Pandey et al. 2008), cold environments (Margesin and Miteva 2011) and Antarctica (Kostadinova et al. 2009). Some *Penicillium* spp. was found to be restricted to particular habitats (Filtenborg et al. 1996). The distribution of fungi in forest soils have also been reported (Mohanty and Panda 1998; Manoharchary et al. 2005; 2008). In addition to this, the physiological parameters including temperature, pH and salt effect the genus *Penicillium* and tend to produce variety of secondary metabolites such as pigments, enzymes, exudates, antibiotics, etc., that were found to be attributed towards survival of these fungi under extreme conditions and act as therapeutic agents in various biotechnological applications (Dhakar et al. 2014a). Several species of *Penicillium* have been identified till date and were explored for their ecological as well as industrial importance.

### 2.3.1 Ecological and industrial importance of *Penicillium*

*Penicillium* spp. play major role in plant growth promotion activities and biodegradation or bioremediation processes (Pandey et al. 2007; Pandey et al. 2008; Hossain et al. 2014; Dhakar et al. 2014b). Sartaj et al. (2011) reported that the species of *Penicillium* support the plant growth in tomato crop by colonizing their root to resist them against plant pathogens. An endophyte isolated from *Ixeric repens* identified as *Penicillium citrinum* was found to produce plant growth hormones which promote the growth in plants (Khan et al. 2008). The presence of elemental phosphorus in soil is very low and is unavailable for plants. Therefore, microorganisms in soil help them to obtain the phosphorus from soil by solubilizing it, and are known as Phosphate Solubilizing Microorganisms (PSMs). The species of cold tolerant *Penicillium*, *Aspergillus* and
Paecilomyces, isolated from IHR, have been investigated for phosphate solubilization activity (Rinu et al. 2012). Secretion of various organic acids is involved in the phenomenon of phosphate solubilization. Pandey et al. (2008) reported the diversity of psychrotolerant Penicillium spp. with phosphate solubilizing activity. Along with this, Penicillium is also involved in biodegradation and bioremediation process. The fungi are good producers of many hydrolytic enzymes (laccases, lipases, amylases and cellulases, etc.) and are involved in many biogeochemical cycles. Laccase is the most efficient lignin degrading enzyme secreted by basidiomycetes, ascomycetes and deuteromycetes. Dhakar et al. (2014a; 2015) reported the lignin degrading enzymes secreted by psychrotolerant fungi Penicillium pinophilum and Aspergillus niger. A lipid degrading lipase enzymes are also secreted by wide range of fungi including Penicillium, Candida, Aspergillus, etc., (Singh and Mukhopadhyay 2012; Thota et al. 2012; Pandey et al. 2016). These hydrolytic enzymes are secondary metabolites and have been therapeutic agents in various industries such as paper and pulp, pharmaceuticals and cosmetics, biodegrading, nanotechnology, etc.

Apart from hydrolytic enzymes, microorganisms also secrete other secondary metabolites, such as, pigments that have various biotechnological applications. Natural pigments are produced by plants, animals, microorganisms and insects. Microorganisms are being more explored for pigments because of their availability throughout the year. Other important factors are: their stability at different temperatures and pH and their rapid and easy production in low cost medium as well as their growth that is independent of weather conditions (Manikprabhu and Lingappa 2013). It has been reported that fungal secondary metabolites are extremely important to our health and nutrition and have tremendous economic impact (Adrio et al. 2003). Their synthesis is regulated by various biotic factors like oxygen, light, temperature and humidity. With the establishment of growth of fungal colony under natural conditions, parts of the mycelium may switch biochemical activity to pathways of secondary metabolites. Instead of producing new fungal building materials they also produce compounds called secondary metabolites. Keller et al. (2005) described secondary metabolites as bioactive substances and suggested that their production is often correlated with a specific stage of morphological differentiation. Natural pigments are categorized according to their structures and are synthesized by the metabolic pathways which includes, amino-acid derived pathways, the shikimic acid pathway for the biosynthesis of aromatic amino
acids, mevalonic acid pathway for synthesising sterols, terpenoids, carotenoids, and polyketide pathways for quinones and azaphilones (Wasser 2011).

2.4 Categorization of natural pigments and their derivatives

2.4.1 Tetrapyrrole derivatives

These compounds consist of pyrrole ring in linear or cyclic array. The most important subgroup of tetrapyrrole derivatives is chlorophyll, present mainly in higher plants, ferns, mosses, most of the green algae and prokaryotic organisms. These compounds tend to absorb light in the visible region, mainly absorb red and blue light and transmit green light. Along with chlorophyll, a blue–green pigment named phytochrome is also present in green plants that have the ability to control metabolic and developmental processes. Phytochrome consist of a protein covalently linked to bilin chromophore involve in the regulation of many enzymatic activity at biochemical and transcriptional level. Fig. 4 represents the structure of phytochrome. It was reported that phytochrome is involved in the germination, flowering, ripening, anthocyanin and protein synthesis and is also found in algae (Rhodophyta, Cryptophyta) (Delgado-Vargas et al. 2000).

![Fig. 4 Structure of phytochrome](image)
2.4.2 Isoprenoid derivatives

Also termed as terpenoids, possess a big family of natural compounds and are present in all kingdoms facilitating multiple functions including production of hormones and pigments. According to their structures quinones, carotenoids and iridoids exist under this group. However, iridoids are plant isoprenoid compounds and quinones are considered in another group because some of the compounds were produced by different biosynthetic pathway (Delgado-Vargas et al. 2000).

\[
\text{Fig. 5 Structure of carotenoid derivatives (A) Astaxanthin, (B) } \beta\text{-carotene, and (C) zeaxanthin}
\]

Keller et al. (2005) reported major terpenes synthesized by fungi which include carotenoids, gibberellins, indole-diterpenes and trichothecenes. Terpenes are linear or cyclic in structure and are basically composed of several isoprene units. It has been depicted that terpenes are also involved in the development and growth processes of plants and microorganisms. Terpenes are classified according to the number of carbon atoms and are divided into volatile mono and sesquiterpenes, less volatile diterpenes, non-volatile triterpenoids and sterols and carotenoid pigments. A filamentous fungus Neurospora intermedium produces pigments through carotenoid biosynthetic pathway (Gusdinar et al. 2014). A number of enzymes including geranylgeranyl pyrophosphate synthase, phytoene synthase, carotene desaturase and lycopene cyclase catalyse the
carotenoid biosynthetic pathways (Kirti et al. 2014). Carotenoids are widest group of pigments that consist of eight isoprenoid units. They have been distributed in higher plants, animals, algae, fungi and bacteria, and are responsible for the red, orange, yellow colours of different fruits, flowers, insects, fungi, birds, etc. Carotenoids are yellow to orange-red pigments produced by microbes belonging to genera Myxococcus, Streptomyces, Mycobacterium, Agrobacterium and Sulfolobus (Malik et al. 2012). They make a group of coloured terpenoids with antioxidant properties which are widespread in the plant and animal kingdoms, as well as in fungi and in photosynthetic and non-photosynthetic microorganisms (Phadwal 2005). Several derivatives of carotenoids including astaxanthin and zeaxanthin have been reported to be produced by various yeast and fungal species. Fig. 5 shows structure of derivatives of carotenoids, all consisting of isoprene units. Latha and Jeevaratnam (2010) reported a red pigment from yeast Rhodotorula glutinis, and found that the pigment contains β-carotene, toluene and torularhodin compounds. Various derivatives of carotenoids such as plectaniaxanthin in ascomycetes and canth-axanthin in Cantharellus cinnabarinus have been found (Goodwin 1992).

2.4.3 Benzopyran derivatives

Benzopyran derivatives are phenolic compounds with two aromatic rings bonded by (carbon 3) C3 unit. They consist of compounds which are categorized on the basis of oxidation state and characteristic colour and divided into 13 classes including anthocyanins, aurons, chalcones, yellow flavonols, flavones, uncolored flavonols, flavanones, dihydroflavonols, dihydrochalcones, leucoanthocyanidins, catechins, flavans, and isoflavonoids. Fig. 6 shows the representing structure of flavonoid derivatives. Most of the studied groups of these compounds are flavonoids. They are water soluble and produce colours in the range from orange to blue in petals, fruits, leaves and yellow colour in flowers. Production of flavonoids from endophytic fungi (Aspergillus spp.) isolated from Ginkgo biloba L. has also been reported (Qiu et al. 2010). Flavonoids act as antioxidants by scavenging free radicals.
2.4.4 Polyketides

Polyketides are a class of secondary metabolites produced by bacteria, fungi, and plants, and are formed by combination of acetate units, basically derived from acetyl co-enzyme A. Polyketides are synthesized by a group of enzymes classified as type I, II and III polyketide synthases (PKSs), commonly found in microorganisms. The classes of polyketides consist of derivatives of quinones, including anthraquinones, napthaquinones, hydroxyanthraquinones and azaphilone compounds. The basic structure of quinone consists of a desaturated cyclic ketone that is derived from an aromatic monocyclic or polycyclic compound (Fig. 7). Variety of quinones are found in different life forms, such as plastoquinones found in chloroplasts of higher plants and algae, menaquinones in bacteria, naphthoquinones in animals and anthraquinones in fungi, lichens, flowering plants and insects. The colouration by quinones has been reported to produce yellow, red, or brown whereas salts of quinone show purple, blue, or green colours (Thomson 1962; Hari et al. 1994). A number of derivatives of quinones have been found among various living organisms possessing various functions. Anslow and Raistrick (1938) reported the production of pigments like fumigatin and spinulosin from Aspergillus fumigatus and Penicillium spinulosum, respectively, and found these pigments as toluquinone.

In past years, polyketide pigments have been used for identification and species differentiation of ascomycetous fungi (Frisvad and Samson 2004). Earlier reports described Monascus spp. as well-known organisms for the production of several
polyketide pigments possessing certain range of colouration such as ankaflavin and monascin (yellow), monascorubarin and rupropunctatin (orange), monascorubramine and rubropunctamine (purple-red) as well as some mycotoxins. These pigments were reported to have various biotechnological applications. However, recent studies have highlighted the potential of using polyketide pigment produced by fungi as food colorants by *Monascus* spp.

**Fig. 7** Basic structure of Anthraquinone

**Fig. 8** Types of natural pigments
The genera *Penicillium aceulatum* and *P. pinophillum* were also reported to produce polyketide pigment (monascus-like azaphilone), with not any production of mycotoxins and are found to be suitable as food grade pigments (Mapari et al. 2008). Other *Penicillium* strains including *P. purpurogenum* and *P. funiculosum* have been reported as novel producers of monascus-like azaphilone pigments (Mapari et al. 2009). With their importance in drug and pharmaceutical industries they are also being exploited in dyeing and textile industries due to the production of huge range of pigments. The flow chart of various kinds of natural pigments shown in Fig. 8.

### 2.4.5 Melanin

This is nitrogenous polymeric compound having indole ring. Generally the black, grey and brown pigmentation in animals, plants and microorganisms is due to the presence of melanin pigments. Varieties of melanin have been reported in living organisms, such as eumelanins (Fig. 9) have been widely distributed in vertebrate and invertebrate animals, phaemelanins present in mammals and birds, while plant seeds, fungi and insects consist of allomelanins (Brown and Salvo 1994; Takano et al. 1997). Gonclaves et al. (2012) reported the characterization of melanin pigment produced by *Aspergillus nidulans*.

![Fig.9 Structure of melanin derivative (Eumelanine)](image-url)
2.4.6 Alkaloids

Alkaloids are organic compounds consisting of one nitrogen atom in a heterocyclic ring. Although plants are the main source of alkaloids, but there are some reports that suggest their presence in fungi also, such as ergot (Claviceps spp) and Psilocybe spp. (Hoffmann 2003). The genus Claviceps produce numerous secondary metabolites having pharmacological importance and are being used in medicines; the genus Penicillium is also reported to produce ergot alkaloids having therapeutic applications. A secondary metabolite quinoline, also known as alkaloid mycotoxin (Pohl et al. 2011) produced by Penicillium oxalicum, has been reported to have an aniprotozoal activity (Ruisi et al. 2007). The structure of alkaloid (quinoline) is shown in Fig. 10. A wide variety of other medicinally important alkaloids have been produced through chemical modification of naturally occurring alkaloids (Carlile et al. 2000).

![Basic structure of Quinoline](image)

Fig. 10 Basic structure of Quinoline

2.4.7 Betalains

Betalains were called carophyllinenroth and named as chromoalkaloids (Piatelli 1976), but they do not belong to alkaloid group due to the presence of several carboxyl groups. They are classified according to their structures into betaxanthin (yellow in colour) and betacyanins (red-purple in colour). The presence of betalains was found in higher plants such as Caryophyllales and also among higher fungi such as Amantia, Hygrocube and Hygrosporus (Mabry et al.1963; Strack et al. 1993). Betalains are structurally related to alkaloids but shows no toxic effect in the human body, therefore betalains represent safe alternative to some synthetic food colorants. Fig. 11 shows structure of betalains. There are very few applications of betalains in pharmaceuticals, but in recent years their antiviral and antimicrobial activities have been reported. Along with pharmaceutical effects, betalains have several applications in food such as in gelatin desserts, confectionaries, poultry, dairy and meat products (Delgado-Vargas et al. 2000).
As it is now understood that these pigments are responsible for different colouration in all types of living organisms. Moreover, these secondary metabolites are also responsible for the protection of particular organisms from environmental stress, due to occurrence of antioxidant or antimicrobial properties. In view of exploration of different pigments for industrial purposes, microorganisms are attaining great interest due to the production of combination of pigments, their fast growing nature under laboratory conditions, and requirement of not so expensive medium for growth. These are the advantages of microorganisms over other living organisms like, plants, insects, and animals to be exploited for biotechnological applications.

2.5 Pigments produced by microorganisms

At present, there is a need of many natural compounds as there are many health problems generated due to synthetic compounds (Downham and Collins 2000; Knecht and Humpf 2006; Yang 2006). According to Haxo (1955), these pigments also play role as antioxidants in respiration, light filters for protection of photosensitive enzymes and sexual reproduction influenced by carotenoids.

2.5.1 Bacterial pigments

Pigment producing bacteria are found in various ecological niches, such as soil (Zhu et al. 2007), desert soil (Liu et al. 2009), rhizospheric soil (Peix et al. 2005), fresh water

Fig. 11 Basic structure of Betalains
Asker et al. 2008), and marine water (Franks et al. 2005). Indeed, their presence is also reported in extreme climatic conditions such as low and high temperature regions as well as in salt tolerating regions. Among all the groups of bacteria, various genera of actinobacter such as *Streptomyces*, *Nocardia*, *Micromonospora*, *Thermomonospora*, *Actinoplanes*, *Microbispora*, *Streptosporangium*, *Actinomadura*, *Rhodococcus*, and *Kitasatospora* have been reported to produce wide variety of pigments (Marroquin and Zapata 1954; Rana and Salam 2014).

Bacteria have also been explored to produce pigments, such as, carotenoids, melanins, violacein, prodigiosin, pyocyanin (Ahmed et al. 2012; Venil et al. 2014). As carotenoids consist a major group of pigments they are described by a huge class of bacteria, e.g., lycopene from *Streptomyces chrestomyceticus*, and lutein and zeaxanthin from *Flavobacterium* sp. A carotenoid pigment named canthaxanthin was reported from photosynthetic bacterium *Bradyrhizobium* sp and *Halobacterium*. While, *Agrobacterium aurantiacum* has been reported to produce astaxanthin pigment (Dufosse 2006). In the recent years, two bacteria namely *Paracoccus carotinifaciens* and *Halobacterium salinarium* have been targeted for characterizing new biological sources of astaxanthin (Calo et al. 1995; Tsubokura et al. 1999). A violet coloured pigment named violacein was first isolated from gram negative bacterium *Chromobacterium violaceum*. Further, its production has also been reported from various microorganisms, such as, *Collimonas* sp, *Duganella* sp, *Janthinobacterium lividum*, *Microbulbifer* sp, *Pseudoalteromonas luteoviolacea*, *Pseudoalteromonas tunicata*, and *Pseudoalteromonas ulvae* inhabiting different environment like soil, marine (Yada et al. 2008; Aranda et al. 2011), glacier (Lu et al. 2009), sea surface (Hakvag et al. 2009) and rhizosphere (Aranda et al. 2011). A wide range of microbes including *Colletotrichum lagenarium*, *Cryptococcus neoformans* (Langfelder et al. 2003), *Vibrio cholerae*, *Alteromonas nigrifaciens* (Soliev et al. 2011), and *Streptomyces* spp. (Manivasagan et al. 2013) have been reported to produce melanin pigments. Melanin plays a great role in bacterial survival as it protects the organisms against UV light, high temperature and chemical stress. *Serratia marcescens* was found to produce red coloured prodigiosin pigment for the first time (Boger and Patel, 1987). Bacteria including *Pseudomonas magneslorubra*, *Vibrio psychroerythrous*, *Vibrio gazogenes*, *Alteromonas rubra*, *Rugamonas rubra* and *Streptoverticillium rubrireticuli* (Darshan and Manonmani 2015) were also found to produce prodigiosin pigment. A blue coloured pigment named
Pyocyanin was firstly isolated from *Pseudomonas aeruginosa* by Hassan and Fridovich (1980). Pyocyanin has been used as bio-control agent and possess antimicrobial activity. The list of pigment producing bacteria available through literature is given in Table 1.

**Table 1 Pigment producing bacterial species**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Pigment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micromonospora lupine</em></td>
<td>Anthraquinone</td>
<td>Igarashi et al. 2007</td>
</tr>
<tr>
<td><em>Streptomyces sp</em></td>
<td>Carotenoid</td>
<td>Dharmaraj et al. 2009</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Prodigiosin red</td>
<td>Venil and Lakshmanaperumalsamy 2009</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Pyocyanin blue</td>
<td>El-Fouty et al. 2015</td>
</tr>
<tr>
<td><em>Chromobacterium sp NIIST (MTCC 5522)</em></td>
<td>Violacein</td>
<td>Sasidharan et al. 2015</td>
</tr>
<tr>
<td><em>Hahella Chejuensis</em></td>
<td>Prodiginines</td>
<td>Kim et al. 2007</td>
</tr>
<tr>
<td><em>Pedobacter sp</em></td>
<td>Carotenoid</td>
<td>Correa Llanten et al. 2012</td>
</tr>
<tr>
<td><em>Vogesella indigofera</em></td>
<td>Blue pigment</td>
<td>Gu and Cheung et al. 2001</td>
</tr>
<tr>
<td><em>Bacillus nakamurai</em></td>
<td>Black pigment</td>
<td>Dunlap et al. 2016</td>
</tr>
<tr>
<td><em>Agrobacterium aurantiacum</em></td>
<td>Astaxanthin</td>
<td>Tuli et al. 2015</td>
</tr>
<tr>
<td><em>Flavobacterium spp.</em></td>
<td>Zeaxanthin</td>
<td>Tuli et al. 2015</td>
</tr>
<tr>
<td><em>Flavobacterium sp</em></td>
<td>4-Ketonostoxanthin 3’-sulphate</td>
<td>Yokoyama et al. 1996</td>
</tr>
<tr>
<td><em>Xanthomonas oryzae</em></td>
<td>xanthomonadin</td>
<td>Malik et al. 2012</td>
</tr>
</tbody>
</table>

### 2.5.2 Fungal pigments

Fungi are reported as potent pigment producing microorganisms (Babitha et al. 2007). Pigments produced by fungi are acceptable currently as they are non-toxic (Duran et al. 2002). Filamentous fungi have the potential to produce an extraordinary range of
pigments that include several chemical classes such as carotenoids, flavins, melanins, quinones, phenazines and monascins (Dufosse et al. 2014). It has been described that the microorganisms belonging to several classes of fungi are known to produce a wide range of excreted water-soluble pigments with the low productivity (Mapari et al. 2005). However, food colourants from ascomycetous fungi have been explored with successful attempts. In earlier studies, special attention was given only on the strains of genus Monascus for its potential to produce huge range of natural pigments. However, several other were also found with the ability to produce pigments in high quantities, such as the species belonging to the genera Paecilomyces, Penicillium, Aspergillus, Trichoderma. These fungi were found to be the potential producers of natural pigments mainly of red, yellow, orange and brown colours (Engstrom et al. 1982; Suhr et al. 2002; Cho et al. 2002; Carvalho et al. 2003; Dufossé, 2006; Méndez-Zavala et al. 2007; Hernández-Rivera et al. 2008; Mendez et al. 2011). Earlier studies also confirmed that Monascus purpureus and species of Emericella and Penicillium pose no toxic effects (Martinkova et al. 1995; Youssef et al. 2008) as well as the pigment produced by these fungi are biodegradable (Daniel 2007; Devi 2014) and contain negligible amount of phenolic components (Alvarez et al. 2002; Cheng et al. 2004). Mapari et al. (2008) described the production of monascus like-pigments from strains of Penicillium having applications in food industries as they are not associated with mycotoxin citrinin.

Basidiomycetes including Rhodosporidium, Sclerotium, Sclerotinia, Sporidiobolus and Ustilago have been reported to be the potent producers of carotene. However, genera of ascomycetes (Aspergillus, Cercospora, Penicillium and Aschersonia) and zygomycetes (Phycomyces, Blakeslea and Mucor) were also found to be responsible for the production of carotenes (Avalos and Carmen Limon 2015). Another major group of pigments such as anthraquinones, naphthaquinones, melanins, alkaloids and flavins were also reported by genera Penicillium (Dhale and Vijay-Raj 2009), Aspergillus (He et al. 2012), Trichoderma (Chitale at al. 2012). Several reports suggested that the pigment anthraquinones and their derivatives identified from various fungi exhibit antibacterial, antifungal, cytotoxic and antiprotozoal activities (Hancock and Farmer 1993; Manojlovic et al. 2000; Nagia and EL-Mohamedy 2007). Production of yellow pigment (anthracene-glycoside asperflavin ribo furanoside) from a marine originated fungi Microsporin sp has been reported by Li et al. (2006). Various studies suggest that pigments produced by marine endophytic fungi help them to mimic and to
increase the beauty of associated life forms (Dufosse et al. 2014). In view of natural pigments, the pigments obtained from filamentous fungi satisfy several criteria regarding their toxicity, stability, and their application for industrial purposes. Although many fungi were reported for non-toxic and stable pigments production, the development of fermentation derived pigments need high capital investment in terms of media components. To counter balance the production cost, researchers have shown a great interest in the use of waste or industrial side streams for the fermentation processes in the development of microbial pigments (Panesar et al. 2015). Several pigments from filamentous fungi are already used for industrial production while some are in the development stage. Table 2 shows various fungi involved in the production of varied pigments.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Pigment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monascus purpureus</td>
<td>Ankaflavin, monascin, Rubropunctatin, monascorubrin</td>
<td>Chen and Johns 1993; Tuli et al. 2015</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Rubroglaucin, asperxanthin</td>
<td>Gould and Raistrick 1934</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>Hydroquinones, lactoflavin</td>
<td>Zajic and Kuehn 1962</td>
</tr>
<tr>
<td>Cordyceps unilateralis</td>
<td>Naphthoquinone</td>
<td>Tuli et al. 2015</td>
</tr>
<tr>
<td>Fusarium sp JN158</td>
<td>Benzoquinone</td>
<td>Zheng et al. 2017</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>Anthraquinone</td>
<td>Nagia and El-Mohamedy 2007</td>
</tr>
<tr>
<td>Talaromyces verruculosus</td>
<td>Red pigment</td>
<td>Chadni et al. 2017</td>
</tr>
<tr>
<td>Stephylium lycopersici</td>
<td>Anthraquinone</td>
<td>Li et al. 2017</td>
</tr>
<tr>
<td>Penicillium oxalicum</td>
<td>Anthraquinone</td>
<td>Sardaryan et al. 2004</td>
</tr>
<tr>
<td>Penicillium aculeatum</td>
<td>Ankafalvin</td>
<td>Afshari et al. 2015</td>
</tr>
<tr>
<td>Penicillium purpurugenum</td>
<td>Red pigment</td>
<td>Mendez et al. 2011</td>
</tr>
<tr>
<td>Penicillium citrinum</td>
<td>Citromycetin</td>
<td>Iwanoff 1932</td>
</tr>
<tr>
<td>Penicillium sclerotiorum</td>
<td>Carotene</td>
<td>Mase et al. 1957</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>Furfural</td>
<td>Chitale et al. 2012</td>
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<tr>
<td>Trichoderma versicolor</td>
<td>Red and blue pigment</td>
<td>Tudor et al. 2013</td>
</tr>
<tr>
<td>Xylaria polymorpha</td>
<td>Red and blue</td>
<td>Tudor et al. 2013</td>
</tr>
<tr>
<td>Xylaria euglossa</td>
<td>Phlegmacin</td>
<td>Wang et al. 2005</td>
</tr>
<tr>
<td>Neurospora intermedia</td>
<td>Mixture of carotenoids</td>
<td>Khiabani et al. 2011</td>
</tr>
</tbody>
</table>

### 2.5.3 Yeast pigments

Natural pigments such as carotenoids, anthraquinones etc., have also been produced by yeast along with bacteria and fungi. Latha and Jeevanratnam (2010) investigated pigment produced by *Rhodotorula glutinis* DFR-PDY and depicted the presence of carotenoids. Also, the co-culture of *R. glutinis* with *Lactobacillus helveticus* produced the pigment carotenoid (Frengova et al. 1994). The red yeast *Xanthophyllomyces dendrorhous* (previously known as *Phaffia rhodozyma*) has been regarded as one of the promising microbiological production systems for natural astaxanthins (Dominguez Bocanegra et al. 2007). Variety of carotenoids mainly astaxanthin, lycopene and β-carotenoids have been reported from *P. rhodozyma* (Andrews et al. 1976) and was considered as the major source of natural astaxanthin.

### 2.6 Parameters for the pigment production

In order to optimize the process of pigment production various factors or parameters have been used. These parameters play major role in development as well as production of secondary metabolites. Factors affecting the growth and pigment production by filamentous fungi are described below.

#### 2.6.1 Temperature and pH

Cellular growth and metabolite production is mainly affected by temperature. Thus, temperature is suggested to be a factor that may be utilized to regulate enzymatic processes connected to pigment production by the fungal cell (Mendez et al. 2011). Along with temperature, filamentous fungi also get affected by change in hydrogen ion concentration inside the fungal cell. The pH of the environment affects most of the
processes taking place inside the cell including, cellular metabolism, nutrient absorption, etc. The cellular growth and pigment production of various species may get affected by pH.

Chintapenta et al. (2014) reported the production of red pigment by a mangrove Penicillium sp at temperature 25 °C and pH 3. Hailei et al. (2011) used co-culture of Penicillium sp and Candida tropicalis for the enhanced production of a red pigment at temperature 30 °C that was found to be non-toxic. This experiment suggests that co-culturing is a promising way to produce a pigment potentially useful for colour applications. Similarly, 30 °C temperature with optimum pH 7 were found to be optimum for the production of red pigment by Talaromyces verruculosus (Chadni et al. 2017). Esinoza-Hernandez et al. (2013) reported three novel pigment-producing Penicillium spp. strains showing optimum temperature for pigment production at 24 °C. Filamentous fungi Monascus spp. were also found to produce maximum amount of pigment at 30 °C and pH 6 in several reports (Velmurugan et al. 2011; Padmavathi and Prabhudessai 2013). A Fungus named Isaria farinosa was studied for its production of red pigment where 27 °C temperature with pH 5 were obtained as optimum conditions (Velmurugan et al. 2010a). On the other hand, optimum temperature and pH were achieved to be 15 °C and 5 pH, respectively, in case of psychrotolerant Penicillium pinophilum that produced maximum red coloured pigment (Maharana 2016); this is the only report that showed low temperature requirement for pigment production. The reports on the effect of temperature on cell growth along with pigment production by various species of fungi indicated the optimum temperature ranges from 24 to 30 °C.

Reports from literature suggested the production of pigment by filamentous fungi mainly in the range between 3-9 pH. The effect of pH depends on the species of fungi as well as on the type of pigment produced. Baneshi et al. (2014) reported the optimum pH for the pigment production by Monascus purpureus in acidic range that is 3. Chen and Johns (1993) reported the cell growth and ankaflavin production by M. purpureus at pH 4. These reports suggested the pigment production toward acidic condition. However, many filamentous fungi also showed better pigmentation at alkaline pH, such as, Hernandez et al. (2014) noted the optimum pH value for pigment production for Pycnoporus was 6.5. Similarly, Afshari et al. (2015) also reported the highest pigment production by Penicillium aculeatum ATCC 10409 at pH 6.5. It has
been reported that at lower pH, there is predominance of yellow pigments while at higher pH, red pigment was found to be dominated (Yongsmith et al. 1993). Similarly, Chintapenta et al. (2014) also reported pH 3 as optimum temperature for red pigment production, whereas at pH 2, there was a yield of yellow pigment instead of red by *Penicillium* strain (DLR-7). Baker and Tatum (1998) reported naphthasarians production at pH below 4 while at pH 8 only dimeric naphthaquinone was produced.

### 6.2 Carbon source

Carbon source exists as main source of energy and metabolite production by heterotrophic microorganisms. It has been reported that maltose, soluble starch, ethanol and glucose have been claimed to be superior over other carbon sources in liquid media (Yoshimura et al. 1975; Lin and Demain 1991; Chen and Johns 1993). Various types of carbon sources are metabolized by different species of fungi. Gunasekaran and Poorniammal (2008) reported the growth as well as pigment production in medium containing various carbon sources including glucose, fructose, dextrose, lactose, sucrose, maltose, mannose, galactose, soluble starch, xylose or glycerol and maximum pigment production was obtained in glucose. Utilization of carbon sources and production of metabolites may vary with the microorganism itself. Pisareva and kujumdzieva (2010) reported glucose as best carbon source for growth and pigment production by *Monascus pilosus* while other carbon sources such as lactose and galactose inhibit the growth and production of pigment. On the other hand, Chitale et al. (2012) reported the maximum pigmentation by *Trichoderma viride* in the presence of lactose and xylose while sucrose, fructose, mannose and mannitol suppressed the pigment production. In case of *Monascus pupleus*, maltose was found to be better than glucose in presence of peptone as nitrogen source (Chen and Johns 1993). Other than this, soluble starch and glucose or sucrose were found to be the best for pigment production by *Paecilomyces sinclarii* (Cho et al. 2002) and *Isaria farinosa* (Velmurugan et al. 2010a), respectively. On the whole, the best carbon source depends upon the strain, other media components and the target pigment (Obgonna et al. 2016).

Nimnoi and Lumyong (2011) studied *Monascus pupleus* CMU001 and reported that maximum pigment production took place in the PD medium and growth development was obtained in the presence of glucose. This study indicates that the optima carbon source for growth may not be the optima for pigment production. Also, Pradeep et al.
(2013) investigated *Fusarium moniliforme* and reported maximum biomass production in the presence of glucose while maximum pigmentation was achieved in the presence of potato dextrose medium.

### 2.6.3 Nitrogen source

Various nitrogen sources also support the growth and pigmentation produced by filamentous fungi. The effect of both organic and inorganic nitrogen sources on cell growth as well as pigment production has been investigated. Earlier studies confirm that organic nitrogen is more favourable for biomass and pigment production by fungi in comparison to inorganic nitrogen. Lin and Demain (1995) reported that peptone and yeast extract facilitate the pigment production in *Monascus* sp, while ammonium sulphate and ammonium nitrate showed inhibitory effect on cell development and pigmentation of the respective fungus. Similarly, nitrogen source including yeast extract, meat extract, peptone, and monosodium glutamate were found to be optimum for the production of pigment in *Isaria farinosa* (Velmurugan et al. 2010a). On the other hand some studies described that, in *Monascus* sp organic nitrogen sources facilitate growth and do not favour pigment production (Carles and Shepherd 1997). Cho et al. (2002) reported that highest pigment production was supported by various peptones in filamentous fungi; highest biomass was achieved in the presence of peptone in *Fusarium moniliforme* (Pradeep et al. 2013). Patil et al. (2015) also suggested that *Penicillium purpurogenum* obtain its maximum pigment in the presence of peptone. Gunasekaran and Poorniammal (2008) also reported that organic nitrogen such as peptone, peptone-yeast extract mixture, tryptone and monosodium glumate showed positive effect on red pigment production by *Penicillium* sp and among all peptone gave highest yield for red pigment production. Apart from this, nitrogen sources also affect the pH of the medium due to which pigment produced is also affected. Shi et al. (2015) reported that *Monascus* sp produced predominantly yellow pigment in a medium containing peptone but when ammonium sulphate was added as nitrogen source, red pigment predominated.

### 2.6.4 Mineral salts

Along with various carbon and nitrogen sources, various mineral salts also act as important factor affecting pigment production in several microorganisms (Fogarty and
Tobin 1996; An et al. 2001). Mineral ions such as K\(^+\), Mg\(^+\), Zn\(^+\) played significant role in the increase of biomass and pigment production (Pradeep et al. 2013). Patil et al. (2015) reported that among various mineral salts (KCl, MgSO\(_4\), CoCl, CuSO\(_4\), ZnSO\(_4\)). MgSO\(_4\) enhanced the pigment production by *Penicillium purpurogenum*. In case of *Paecilomyces sinclari* a detrimental effect was shown by calcium chloride whereas other bio-elements appeared to have no notable or detrimental effect (Cho et al. 2002). Zinc ions are also reported to be suitable for enhanced pigment production in *Monascus* sp (Bau and Wong 1979). It has been reported that some metal ions are supposed to affect the production of particular group of pigment such as in case of *Penicillium rhodozyma* iron ion decreased astaxanthin production (An et al. 2001).

Apart from the above mentioned parameters, some other factors such as light, incubation period, moisture content have also been found to affect the biomass as well as pigment production by various fungal species. Velmurugan et al. (2010b) investigated various genera namely *Monascus purpureus*, *Isaria farinosa*, *Emericella nidulans*, *Fusarium verticilliode* and *Penicillium purpurogenum* and found that they exhibit good biomass and pigment under dark conditions as compared to being exposed to light of different wavelength. Tudor et al. (2012) studied about the moisture content and reported that *Trametes versicolor* and *Xylaria polymorpha* were stimulated to form pigments at moisture content below 28 % and 38 % in *Acer saccharum* and *Fagus grandfolia*, respectively.

### 2.7 Stability of pigments at different parameters

Microbial pigments are sensitive to light, heat, acidity, air and water activity; therefore, it is necessary to check the stability of pigment produced by microorganisms in order to meet industrial standards. Several fungal pigments have been reported in the literature that must satisfy criteria regarding toxicity and stability (Malik et al. 2012). Velmurugan et al. (2011) investigated the stability of pigment produced by *Monascus purpureus* KACC 42430 at different temperatures, pH and salt concentrations and resulted in thermostability up to temperature 100 ºC and change in colour at different pH range. Mapari et al. (2009) studied pigment produced by *Penicillium aculeatum* at various pH range and found pigment stability at neutral pH. *Monascus* pigment, when added to sausages, showed 92 % to 98 % stability at 4 ºC for three months (Fabre et al. 1993).
Mahesh et al. (2014) reported that pigment produced by *Fusarium solani* and *Penicillium pseudostromaticum* showed stability at different range of temperature with variation in colour intensity at different pH. In an earlier study by Hailei et al. (2011), the pigment was found to be stable at wide range of pH and temperature and showed its solubility in wide range of polar solvents.

### 2.8 Purification and characterization processes of fungal pigments

Many filamentous fungi accumulate a complex mixture of pigments, thus, it is essential to purify and quantify the pigments that are present in the complex mixture. Initially, there is a need for extracting the crude pigment; this step removes lot of impurities and makes the pigment partially purified. Purification and quantification processes of extracted pigment consist of chromatographic and spectrophotometric techniques as well as some chemical tests. Chromatographic techniques involve Thin Layer Chromatography (TLC), Column Chromatography (CC), and High Performance Liquid Chromatography (HPLC) for purification process while characterization and identification consist of some higher techniques such as UV/Vis spectroscopy, Fourier Transform Infrared spectroscopy (FT-IR), Liquid Chromatography Mass Spectrometry (LC/MS), Gas Chromatography Mass Spectrometry (GC/MS) and Nuclear Magnetic Resonance (NMR). UV/Vis spectroscopy gives information about the presence of rings, carbonyl groups and isomeric effects. FT-IR gives information about different functional groups present in the sample. HPLC coupled with a MS detector form LC/MS is useful for identifying all non-volatile compounds and is considered very convenient technique to identify carotenoids whereas GC couple with an MS detector (GC/MS) detects all volatile compounds or compounds having high boiling point. Lu et al. (2010) analysed and identified astaxanthin and its precursors from *Xanthophyllomyces dendrorhous* by using LC-APCI-MS technique. While, Latha and Jeevanratnam (2010) purified and characterize the pigment produced by *Rhodotorula glutinis* by using TLC, HPLC and NMR and successfully obtain three compounds β-carotene, torulene and torularhodin. Kumar et al. (2011) determined melanin pigment by characterizing the pigment produced by *Aspergillus bridgeri* using FT-IR and Electron Paramagnetic Resonance (EPR).

Combinations of different organic and inorganic solvents are applied in chromatographic techniques. Mahesh et al. (2014) detected the presence of amide
groups, aromatic rings and keto groups in the pigment produced by *Penicillium* and *Fusarium*. The FTIR analysis of pigment produced by filamentous fungi (*Fusarium solani* and *Penicillium pseudostramaticum*) showed presence of amide groups, aromatic rings and keto groups. Orange colour pigment produced by *Thraustochytrium* CHN-1 strain was found to contain astaxanthin and other carotenoid derivatives analysed by HPLC-MS (Carmona et al. 2002). Souza et al. (2016) identified the pigments oosporein, orrevactaene and dihydrotichodimerol from *Lecanicillium aphanocladii*, *Epicoccum nigrum* and *Penicillium flavigenum* by using MS/MS and NMR techniques. Another ascomycetous fungi *Xylaria euglossa* was also exploited for pigment production and a new pigment, phlegmacin A 8,8’-di-O-methyl ether, along with known fungal pigment (S)-torosachrysone-8-O-methyl ether and emodin-6,8-di-O-methyl ether were identified by using NMR technique (Wanga et al. 2005). Mohammed (2015) analysed huge range of compounds present in red pigment produced by *Monascus* sp by GC/MS technique.

Apart from this, phytochemical analysis was carried out to test the presence of secondary metabolites such as alkaloids, phenols, flavonoids, carotenoids, and anthraquinones. These phytochemical tests depict the presence of compounds that gives confirmation of the compound. Phytochemical screening of *Monascus* pigment was done by Meera and Anchana (2015) and depicted the presence of flavonoids, proteins and amino acids.

### 2.9 Applications of pigments

Natural pigments, particularly microbial pigments, are highly in demand as natural food colouring agents. Because of their wide range of properties, they are being exploited in several biotechnological industries including dyeing and textiles, pharmaceuticals, food and beverages etc., shown in Fig. 12. Apart from this, pigments from microbes are also act as surviving agent to thrive under stress conditions and also perform defence mechanism against pathogens.

#### 2.9.1 Pigments in dyeing and textile industry

Synthetic dyes are used in dyeing and textile industries due to their easy and cheap synthesis, stability towards light, temperature and moisture. However, these synthetic
pigments are highly toxic to human health and possess toxic, carcinogenic as well as mutagenic properties leading to various health problems to all living being (Srikanlayanukul et al. 2006). During dyeing and finishing operations, about 200,000 tons of dyes are lost as effluents every year, due to their stability against light, temperature and water. These waste materials remain persist in environment making it polluted. To overcome these problems, natural pigments are being explored. Microbial pigments are eco-friendly colorants applicable to dye various textile fabrics (Chadni et al. 2017). Yusof (2008) reported pigment from Serratia marcescens with tamarind as mordant which is able to dye five types of fabric including acrylic, polyester, microfiber, silk and cotton. Pigments from filamentous fungi including species of Trichoderma and Aspergillus were reported to dye silk fabric (Devi 2014). Anthraquinone pigment isolated from Fusarium oxysporum was also found to dye wool fabric (Nagia and El-Mohamedy 2007). In another study, Velmurugan et al. (2010b) also aimed to evaluate the dyeing potential of pigment produced by five filamentous fungi (Monascus purpureus, Isaria sp, Emericella sp, Fusarium sp and Penicillium sp) for leather fabric. Recently, Chadni et al. (2017) reported dyeing of cotton fabric with a red coloured pigment produced by Talaromyces verruculosus.

Gupta and Aggarwal (2016) investigated the pigment produced from Penicillium minioluteum and used it for dyeing of wet blue goat nappa skin. Microbial pigments also have potential to produce different colour tones in different textile fabrics. Pigment from Janthinobacterium lividum shows a bluish purple colour tone on silk, cotton, and wool, while dark blue is seen with nylon and vinylon (Shirata et al. 2000). Poornimal et al. (2013) in a study, observed the high affinity of silk fabric towards Thermomyces pigments. Similarly, pigments from strains of Streptomyces also showed dyeing efficiency with respect to the material (Kramar et al. 2014). Due to easy availability, affinity towards different textiles, cost effectiveness and non-toxic nature, microbial pigments are replacing synthetic pigments and becoming advantageous to mankind.

2.9.2 Pigments in pharmaceutical industry

Apart from being used as natural colorants, microbial pigments also act as antimicrobial, antioxidant and anticancer agents and used in pharmaceutical industry. An important carotenoid derivative astaxanthin is red coloured pigment with great
commercial value and pharmaceutical applications. Further, *Monascus purpureus* produces pigments which help in the inhibition of hepatitis virus replication by interfering with viral RNA polymerase activity (Sun et al. 2012).

Pigments including carotenoids, flavins, quinones, monascins, prodigiosin and melanins have been reported as antimicrobial agents (Malik et al. 2012). An endophytic fungal species *Monodictys castaneae* was reported to inhibit the growth of human pathogenic bacteria *Staphylococcus aureus, Klebsiella pneumoniae* and *Vibrio cholera* (Visalakchi and Muthumary 2010). Similarly, Berlanga et al. (2000) and Selvameenal et al. (2009) reported that the pigment from *Streptomyces hygroscopicus* have potential to inhibit pathogens including strains of *Staphylococcus aureus, Escherchia coli, Pseudomonas aeruginosa* and *Klebsiella* sp. Meera and Anchana (2015) observed the antibacterial activity of pigment isolated from *Monascus* sp against *Bacillus* spp., *Escherchia coli*
and *Pseudomonas* sp. In addition to this, pigment produced by filamentous fungi *Monascus purpureus* has been reported to have antifungal activity against *Aspergillus*, *Trichoderma*, *Mucor*, *Penicillium* and *Fusarium* species and antibacterial activity against *Bacillus*, *Pseudomonas*, *Escherichia* and *Streptomyces* spp. (Ungureanu and Ferdes 2010). Along with this, there are several reports on antimicrobial activities of fungi such as *Sporobolomyces* sp (Manimala and Murugesan 2014), *Fusarium* sp (Geweely 2011; Mani et al. 2015), *Aspergillus* sp and *Penicillium* sp (Geweely 2011; Teixeira et al. 2012) against various species of fungi and bacteria.

Increment of free radicals in the human body enhances the chance of chronic disease such as cancer, diabetes, autoimmune disorders (Rankovic et al. 2011). In order to avoid the formation of these free radicals in the body antioxidants are used. Antioxidants are free radical scavenging molecules that neutralizes it by donating electrons. Pigments as secondary metabolites are known for their antioxidant activity also. Microbial pigments such as carotenoids and naphtoquinones possess good antioxidant property (Tuli et al. 2015). Similarly, anthraquinones from *Stemphylium lycopersici* (Li et al. 2017) and melanin from *Streptomyces glaucescens* NEAE-H (El-Naggar and El-Ewasy 2017) also tend to exhibit antioxidants properties. Carotenoid pigment derived from marine bacterium *Pedobacter has been* demonstrated as antioxidant and has the capacity to protect itself against oxidative-damage (Correa Llanten et al. 2012).

Astaxanthin pigment isolated from various microbes possesses powerful antioxidant activity (Nakano et al. 1999) and also reported to exert protective effect against chronic disease such as cancer (Hussein et al. 2006). Efforts to use microbial pigments as anticancer agents have laid the foundation for successful treatments. Wang et al. (2012) reported a pigment prodigiosin from *Pseudomonas* sp. 1020R that showed cytotoxic activity against U937 leukemia cells, indeed, this pigment also showed anticancer activity against 60 different cancer cell lines. Melanin pigment isolated from *Streptomyces glaucescens* NEAE-H has been reported for anti-cancer activity against skin cancer cell line (El-Naggar and El-Ewasy 2017). In a study, Huang et al. (2011) investigated fungus *Alternaria* sp ZJ9-6B and obtain a derivative of anthraquinone which showed anti-cancer activity against human breast cancer cell lines. Monascin pigment obtained from *Monascus* sp showed inhibitory activity against mouse skin
carcinogenesis. Similarly, anti-proliferative effect against HEP-2 human laryngeal carcinoma cell lines was shown by pigments such as monaphilone A and monaphilone B (Feng et al. 2012). The pigment produced by *Hahella chejuensis* is reported to have immune suppressant and antitumor properties (Kim et al. 2008). In view of these reports, it is considered that microbial pigments can be potential therapeutic agents against various chronic diseases and can be used as novel drug.

**2.9.3 Pigments in food and cosmetic industry**

Initially, the natural sources of food colorants were plants and animals. Later, due to increase in demand of food colorants they have become inadequate which led to the use of synthetic colourants (Obgonna et al. 2016). Due to the negative impact of these synthetic pigments towards human health, microbes are being explored for natural colourants. Food colourants are added to improve the quality of food and to make it more attractive in appearance for consumers. The red and yellow polyketide pigments produced from filamentous fungi, *Monascus* sp has been used as food colourants for dried fermented rice in South East Asia for more than thousand years. Some species of *Monascus* are also reported to produce mycotoxins named citrinin, therefore, its use is banned in United States (US). Some filamentous fungi such as *Penicillium* and *Talaromyces* are found to produce monascus like polyketide azophilone pigments without producing citrinin or other mycotoxins (Mapari et al. 2008).

Filamentous fungi, belonging to species of *Penicillium* and *Aspergillus*, have been reported to produce hydroxyanthraquinoid (HAQN) pigments (Dufosse et al. 2014). Red pigments from *Monascus* sp, astaxanthin from *Xanthophyllomyces dendrorhous*, Arpink red™ from *Penicillium oxalicum*, lycopene from *Erwinia uredovora* and *Fusarium sporotrichioides* were reported to be added in food to increase its appeal (Dharmaraj et al. 2009). Another derivative of carotenoid pigment known as canthaxanthin is used in foods, such as, cheese, candy, beverages, snacks, beer and wine. Riboflavin pigments are also reported to be used in beverages and ice creams (Chattopadhyay et al. 2008). Apart from addition in food, these microbial pigments are also used in cosmetics, such as red pigment from *Penicillium oxalicum* is reported to be used in cosmetic as well as in food industry (Sardaryan et al. 2004). Pigment known as zeaxanthin produced from *Flavobacterium* sp is reported to be used in poultry feeds to
accentuate the colour of yolk of eggs (Alcantara and Sanchez 1999), and also it has been used in cosmetic and food industry as well.

2.9.4 Pigments as bio-indicators

Apart from being used in dyeing, pharmaceutical, food colouring and cosmetics industries, microbial pigments are also used as bio-indicators. A pigment known as phycoerythrin is reported to be used to predict the rate of peroxy radical scavenging in human plasma (Delange and Glazer 1989). Pigments are also used to detect heavy metals. *Vogesella indigofera* has been reported to produce blue coloured pigment under normal environmental condition, whereas, when exposed to heavy metal like hexavalent chromium, the pigment production was absent (Gu and Cheng 2001). *Pantoea agglomerans* has been reported to monitor temperature variations as it produced deep blue colour at temperature below 10 ºC (Fujikawa and Akimoto 2011).