A pigment is a material that changes the color of reflected or transmitted light. The pigment appears color due to a molecule-specific structure called chromophore. This structure captures the energy and the excitation of an electron from an external orbital to a higher orbital is produced; the non absorbed energy is reflected and/or refracted, which is captured by the eye, and generates neural impulses that are transmitted to the brain where they could be interpreted as a color (Hari et al., 1994).

The use of pigment as colorants is practiced since prehistoric times (Figure 1). Archaeologists have uncovered the evidence that, early humans used paint for aesthetic purposes, such as body decoration. The use of pigment in prehistoric time was further proved, when pigments and grinding equipments reported in a cave at Twin Rivers, near Lusaka, Zambia which were between 350,000 and 400,000 years old (Kassinger, 2003). In Europe, the use of pigment was practiced during the Bronze Age. The earliest written record of the use of natural dyes was found in China dated 2600 BC (Gokhale et al., 2004). In Indian, it was even known from Indus Valley period (2500 BC) (Aberoumand, 2011). Henna was used even before 2500 BC, while saffron is mentioned in the Bible (Gulrajani, 2001). In Egypt, mummies have been found wrapped in colored cloth, the chemical analysis of this cloth showed the presence of alizarin, a pigment extracted from madder. The cochineal dye was used by the people of Aztec and Maya culture period of Central and North America. By the 4th century AD, dyes such as woad, madder, weld, indigo and a dark reddish-purple came into existence (Rymbai, 2011) which further encouraged using pigments for various purposes.
The addition of color to food started in Egypt, when candy makers added natural extracts in 1500 BC. Similarly, the use of natural colorants in food was seen in Japan in the shosoin text of the Nara period (8th century), which contains references regarding coloring soybean and adzuki-bean cakes (Aberoumand, 2011). The coloring continued till 19th century with new colorants such as the saffron, from the local area (Meggos, 1995).

In 1856, the first synthetic color (mauvine) was developed by Sir William Henry Perkin which brought the revolution in the history of colorant (Walford, 1980). The synthetic color captured the market due to ease to produce, less expensive, superior in coloring properties and only a tiny amount was needed to color. The synthetic color can be blended easily and didn't impart unwanted flavours to foods. Since then, the industrial revolution started, synthetic color were used rapidly (Downham and Collins, 2000). Sellers at the time offered more than 80 artificial coloring agents. Many color additives at that time, had never been tested for its toxicity or other adverse effects, which ultimately lead to pose adverse effect on the health and environment with rise of diseases leading to death due to their carcinogenic nature. This drawback of synthetic color increased the demand of natural pigment all around the globe (Manikprabhu and Lingappa, 2013).

Natural pigments can be obtained either from plants or microorganisms. It is a well known practice to extract the natural colors from the plant sources, but the yield is very low and they have low eco-efficiency. Extraction of colors from the microbial source is an upcoming field. Various types of microorganisms like bacteria and fungi produce pigments. Natural colors from these sources can be extracted using simple and effective protocols (Raisainen et al., 2002).
Figure 1: Spotted horse panel of Pech Merle Cave; showing the use of color in ancient life. (Barbara, 2013)
2.1. Pigment producing microorganisms

Microorganisms provide a readily available alternative source of naturally derived pigments. The production of natural pigments utilizing microbial biosynthesis has received greater interest in recent years (Indra et al., 2014). A large number of microorganisms can produce pigment (Table 1). However, the use of pigments from microbial source must satisfy several criteria in order to use them commercially such as, the capability to tolerate high pH, temperature, mineral concentration, yield, its non-toxicity, easily separable from the cell mass and to use a wide range of carbon and nitrogen sources (Kamla et al., 2012).

Wide variety of fungi produce characteristic pigments such as species of Drechslera produce maroon, brown (hydroxyanthraquinones), red (catenarin), bronze (cynodontin) and red brown (tritisporin) pigments; similar compound, erytroglaucin a red pigment is produced by Aspergillus glaucus with addition of orange (auroglaucin) and yellow (flavoglaucin) pigment. Pigments like aurofurasarin and rubrofusarin from Fusarium culmorum, melanin from Phellinus robustus; boletol from Boletus luridus; citromycetin, chrysogenin, citrinin, fulvic acid from Penicillium were also reported (Nelson et al., 2002). Fungus such as Monascus, Aspergillus, Penicillus, Paecilomyces variotii, Aspergillus carbonarius, Trichoderma virens, Curvularia lunata and Alternaria alternata also produced pigments (Babitskaya et al., 2000). Many pigments producing fungi even isolated from marine ecosystem like Hortaea werneckii, Phaeotheca triangularis and Trimmatostroma salinum which produce pigment with improved pigment stability (Lathadevi et al., 2014). Other than fungi, bacteria like Exiguobacterium aurantiacum, Exiguobacterium profundum, Flavobacterium sp., Bradyrhizobium sp. and Agrobacterium aurantiacum were also reported for the pigment production (Sasidharan et al., 2013; Laurent Dufosse, 2006).
Further, pigment production from halo tolerant bacteria *Halobacterium* (Asker and Ohta, 1999), psychrotrophic bacterium *Janthinobacterium lividum* (Yoshitoshi *et al.*, 2003) and endophytic bacteria *Paracoccus sphaerophysae* (Zhen Shan *et al.*, 2011) were also reported, which suggest pigment producing microorganisms are ubiquitous however, compared with other bacterial groups the pigment production is more widely present in actinomycetes (Marroquin and Zapata, 1954). The genus like *Streptomyces, Nocardia, Micromonospora, Thermomonospora, Actinoplanes, Microbispora, Streptosporangium, Actinomadura, Rhodococcus and Kitasatospora* (Sandeep and Menaka Devi, 2014) produce wide variety of pigments, among them *Streptomyces* is considered as highest pigment producing genus (Conn and Jean, 1942). Many species of this genus like *S. griseus, S. griseoviridis, S. coelicolor* (Darshan and Manonmani, 2015) *S. cyaneus* (Simone *et al.*, 1999), *S. vietnamensis* (Hong-hui Zhu *et al.*, 2007), *S. peucetius* (Arcamone, 1998) and *S. echinoruber* (Gupta *et al.*, 2011) were reported to produce pigments with good biological activity.
Table 1: List of some pigment producing microorganisms

<table>
<thead>
<tr>
<th>SL. No</th>
<th>Pigment</th>
<th>Microorganisms</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Prodigiosin</td>
<td>Serratia marcescens</td>
<td>Red</td>
</tr>
<tr>
<td>2.</td>
<td>Indigoidine</td>
<td>Corynebacterium insidiosum</td>
<td>Blue</td>
</tr>
<tr>
<td>3.</td>
<td>Zeaxanthin</td>
<td>Staphylococcus aureus</td>
<td>Yellow</td>
</tr>
<tr>
<td>4.</td>
<td>Canthaxanthin, Ankaflavin, Monascorubramin, Rubropunctatin</td>
<td>Monascus spp.</td>
<td>Orange, pink, Yellow, red, Orange</td>
</tr>
<tr>
<td>5.</td>
<td>Prodigiosin like pigment</td>
<td>Rugamonas rubra</td>
<td>Red</td>
</tr>
<tr>
<td>6.</td>
<td>Prodigiosin like pigment</td>
<td>Streptoverticillium rubrirectili</td>
<td>Red</td>
</tr>
<tr>
<td>7.</td>
<td>Pyocyanin blue</td>
<td>Pseudomonas aeruginosa</td>
<td>Green</td>
</tr>
<tr>
<td>8.</td>
<td>Astaxanthin</td>
<td>Haematococcus pluvialis</td>
<td>Red</td>
</tr>
<tr>
<td>9.</td>
<td>β carotene</td>
<td>Dunaliella salina</td>
<td>Orange</td>
</tr>
<tr>
<td>10.</td>
<td>Canthaxanthin</td>
<td>Bradyrhizobium sp.</td>
<td>Orange/Dark red</td>
</tr>
<tr>
<td>11.</td>
<td>Xanthomonadin</td>
<td>Xanthomonas oryzae</td>
<td>Yellow</td>
</tr>
<tr>
<td>12.</td>
<td>Astaxanthin</td>
<td>Phaffia rhodozyma</td>
<td>Red</td>
</tr>
<tr>
<td>13.</td>
<td>Prodigiosin like pigment</td>
<td>Serratia rubidaea</td>
<td>Red</td>
</tr>
<tr>
<td>14.</td>
<td>Prodigiosin like pigment</td>
<td>Vibrio gaogenes</td>
<td>Red</td>
</tr>
<tr>
<td>15.</td>
<td>Prodigiosin like pigment</td>
<td>Alteromonas rubra</td>
<td>Red</td>
</tr>
<tr>
<td>16.</td>
<td>Violacein</td>
<td>Janthinobacterium lividum</td>
<td>Purple</td>
</tr>
<tr>
<td>17.</td>
<td>Anthraquinone</td>
<td>Penicillium oxalicum</td>
<td>Red</td>
</tr>
<tr>
<td>18.</td>
<td>Astaxanthin</td>
<td>Xanthophyllomyces dendrorhous</td>
<td>Pink-red</td>
</tr>
<tr>
<td>19.</td>
<td>Lycopene, β-carotene</td>
<td>Blakeslea trispora</td>
<td>Red, Yellow-orange</td>
</tr>
<tr>
<td>20.</td>
<td>Melanin</td>
<td>Saccharomyces neoformans</td>
<td>Black</td>
</tr>
<tr>
<td>21.</td>
<td>Naphtoquinone</td>
<td>Cordyceps unilateralis</td>
<td>Deep blood red</td>
</tr>
<tr>
<td>22.</td>
<td>Riboflavin</td>
<td>Ashbya gossypi</td>
<td>Yellow</td>
</tr>
<tr>
<td>23.</td>
<td>Rubrolone</td>
<td>Streptomyces echinoruber</td>
<td>Red</td>
</tr>
<tr>
<td>24.</td>
<td>Torularhodin</td>
<td>Rhodotorula spp.</td>
<td>Orange-red</td>
</tr>
<tr>
<td>25.</td>
<td>Zeaxanthin</td>
<td>Flavobacterium spp.</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

Gupta et al., 2011
2.2. List of some natural pigments

2.2.1. Carotenoids

Carotenoids are the pigment responsible for the yellow, orange, red, and purple color in a wide variety of plants, animals, and microorganisms (Botella and Rodriguez, 2006).

Microorganisms producing carotenoids are *Dunaliella* sp., *Blakeslea trispora*, *Phycomyces blakesleeanus*, *Mucor circinelloides*, *Fusarium sporotrichioides*, *Agrobacterium aurantiacum*, *Paracoccus carotinifaciens*, *Gordonia jacobea* (Laurent Dufosse, 2006), *Sporidobolus salmoncolor*, *Rhodosporium paludigenum* and *Rhodotorula glutinis* (Reeba et al., 2015).

There are over 600 known carotenoids which split into two classes; xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). Among various carotenoids, the most important are alpha carotene, beta-carotene, beta-cryptoxanthin, lutein, lycopene, xanthophylls, violaxanthin, neoxanthin, zeaxanthin and canthxanthin (Rymbai et al., 2011). The market value of carotenoids has been forecast to reach a value of $1.2 billion by 2018 (Indra et al., 2014).

All carotenoids are tetraterpenoids, meaning that they are produced from 8 isoprene molecules and contain 40 carbon atoms. Carotenoids absorb wavelengths ranging from 400-550 nanometers (violet to green light). The most prominent function of carotenoids is their contribution to harvest light energy by absorbing light and passing the excitation energy on to chlorophyll, thereby extending the wavelength range of the light that can be harvested (Saskia and Volker, 2011). They protect chlorophyll from photo damage, some used as anticancer and some against obesity (Armstrong and Hearst,
1996; Irwandi Jaswir et al., 2011). Carotenoids are used as vitamin supplements and play an important role in protecting from oxidative stress. In addition, carotenoids are used commercially as food colorants, animal feed supplements and more recently in nutraceuticals, cosmetic and pharmaceutical purposes (Garrido-Fernandez et al., 2010). Epidemiological studies have shown that people with high β-carotene intake have a significantly reduced the risk of lung cancer (Alija et al., 2004).

### 2.2.2. Melanin

Melanin is a pigment commonly found in all living kingdoms. The presence of melanin in representatives of almost every large taxon suggests an evolutionary importance (Przemyslaw and Maja, 2006). Microorganisms like *Colletotrichum lagenarium*, *Magnaporthe grisea*, *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Aspergillus fumigates* (Kim et al., 2003) and many species of the genus *Streptomyces* produce melanin (Panchanathan et al., 2013).

Structurally melanin is a diverse high molecular pigment produced by the oxidation of the amino acid tyrosine, followed by polymerization in a specialized group of cells known as melanocytes.

Melanin confers resistance to Ultra violet light by absorbing a broad range of the electromagnetic spectrum and preventing photo-induced damage. Melanin has many applications, it is used for mimicry, protects damage against high temperatures and chemical stresses. It is widely used in cosmetics, photo protective creams and eyeglasses. Melanin producing microorganisms are used for immobilization of radioactive waste such as uranium. In addition, melanin synthesis genes from bacteria have been used as
reporter genes to screen recombinant bacterial strains. Other reports have shown the anti-HIV properties of melanin as well as their usefulness for photo voltage generation and fluorescence studies. Melanin is also used to generate monoclonal antibodies for the treatment of human metastatic melanoma (Shripad et al., 2013; Przemyslaw and Maja, 2006).

### 2.2.3. Prodigiosin

Prodigiosin is a red pigment first isolated from *Serratia marcescens* which was the initial member of a class of naturally-occurring polypyrroles, possessing a common characteristic pyrrolylpyrromethene skeleton (Dale and Mona, 1987).

Apart from *Serratia marcescens*, prodigiosin has also found to produce by *Pseudomonas magneslorubra*, *Vibrio psychroerythrous*, *Serratia rubidaea*, *Vibrio gazogenes*, *Alteromonas rubra*, *Rugamonas rubra* and *Streptoverticillium rubrirectici* (Darshan and Manonmani, 2015).

The maximum production of prodigiosin occurs after cellular multiplication has ceased (Robert, 1972). Prodigiosins are strong therapeutic molecules, especially for their immunosuppressive properties and anticancer properties. Four possible mechanisms are suggested to prodigiosins such as pH modulators, cell cycle inhibitors, DNA cleavage agents and mitogen activated protein kinase regulators. These molecules when combined with some other anticancer agents can greatly help in fighting cancer. Prodigiosin show apoptosis of haematopoietic cancer cells. Prodigiosin also show insecticidal, antifungal, antibacterial and anti-malarial activities (Abigail et al., 2004; Kamble and Hiwarale, 2012).
2.2.4. Violacein

Violacein is a violet colored pigment, first described to be obtained from a gram-negative bacteria *Chromobacterium violaceum* which was isolated from the Amazon River in Brazil. Apart from *Chromobacterium violaceum*, violacein has also been reported to produce by several other Gram-negative bacteria like *Collimonas* sp., *Duganella* sp., *Janthinobacterium lividum*, *Microbulbifer* sp. *Pseudoalteromonas luteoviolacea*, *Pseudoalteromonas tunicata*, and *Pseudoalteromonas ulvae* inhabiting different environments like soil, glacial and seas (Sergio *et al*., 2011).

Violacein consists of three structural units, i.e., 5-hydroxyindole, an oxindole, and 2-pyrrolidone (Tsutomu, 2011).

Violacein has a variety of biological activities, including antiviral, antibacterial, antiulcerogenic, anti-leishmanial, anticancer and enzyme modulation properties (Azamjon *et al*., 2011). Violacein reported to show antiviral activity against herpes simplex virus and poliovirus. Purified violacein exhibited high toxicity towards cultures of ciliated protozoa such as *Spumella* sp. and *Ochromonas* sp. (Nelson *et al*., 2007)

2.2.5. Riboflavin

Riboflavin also called as vitamin B₂ is water soluble pigment, exhibits a strong yellowish-green fluorescence which was first isolated by A.W. Blyth in 1879. Riboflavin structure was confirmed by R. Kuhn and F. Weygand in 1934 (Kutsal and Ozbas, 1989) which suggests that, it has two distinct parts, a ribose sugar unit and a three-ring flavin structure, known as the lumichrome.

Riboflavin is an essential vitamin that needs to be supplemented in the diet of humans at a concentration of 1.1- 1.3 mg per day. Riboflavin acts as a
structural component of the coenzymes flavin mononucleotide and flavin adenine dinucleotide. Both coenzymes catalyze non-enzymatic oxidation-reduction reactions by functioning as dehydrogenating hydrogen carriers in the transport system involved in ATP production. For over 30 years, riboflavin supplements have been used as part of the phototherapy treatment of neonatal jaundice. Riboflavin co-treatment with β blockers shows improvement against migraine headaches (Feroz, 2010). Riboflavin in combination with UV light has been shown to be effective in reducing the ability of harmful pathogens found in blood products. When UV light is applied to blood products containing riboflavin, the nucleic acids in the pathogens are damaged, rendering them unable to replicate and cause disease (Goodrich et al., 2006).

2.2.6. Anthocynins

Anthocyanins are naturally occurring compounds that impart color to fruits, vegetables, and plants. They are water-soluble and the most important pigment after chlorophyll. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salt and belong to a parent class of molecules called flavonoids. To date, there are 17 known naturally occurring anthocyanins (Konga et al., 2003; Cooper-Driver and Bhattacharya, 1998).

Anthocyanins imparts color to the flowers and fruits which attracts insect and animals, which helps plant for pollination and seed dispersal and hence they are of considerable value in the co-evolution of plant-animal interactions (Konga et al., 2003). The biological activity of anthocyanins includes antioxidant, anticancer, and anti-allergic (Jin Hwan et al., 2011).
Anthocynins helps in tissue inflammation or capillary fragility and also as anti-tumour agent (Konga et al., 2003; Kamei et al., 1998).

2.2.7. Pyocyanin

Pyocyanin is a blue, secondary metabolite produced and secreted by the Gram negative bacterium *Pseudomonas aeruginosa* (Hassan and Fridovich, 1980) which is composed of 2 subunits of Nmethyl-1-hydroxyphenazine (Norman et al., 2004). To synthesize pyocyanin, specific genes must be functional. *MvfR* is a gene which produces a transcription factor which activates *phnAB* genes. These genes produce the molecule quinolone which then regulates operons 1 and 2 of *phzRABCDEFG* which are the key to the synthesis pyocyanin (Dmitri et al., 2001). Pyocyanin is reported to have many biological activities like anti-bacterial, anti-fungal and as bio-control agent (Sheeba et al., 2014).

Due to the above wide availability, applications and eco-friendly nature of pigments, they found great demand in the global market.
2.3. Market trends of pigments

There are no reliable published statistics on the size of the color market; however, 47% of the market is occupied by synthetic colors followed by 27% natural colors, 20% nature identical colors and 11% caramel. As from the above statistics, it is clear that synthetic color is the major capturer of the market, but due to recent studies on synthetic color it was found that most of the synthetic colors are carcinogenic which greatly increased the demand of natural color with a predicted annual growth rate of 5-10% (Downham and Collins, 2000).

The world market of the natural sources was estimated in 1987, as US$ 35 million. Currently the market for the natural pigments is probably up to 600 million, but the cost of the natural colors in most cases is higher than the synthetic colors of similar shades, but this hurdle can be overcome by the mass production of the natural colors which would bring the cost down (Babitha et al., 2004).

2.4. Applications of pigments

2.4.1. Pigments in textile industry

The textile industry uses approximately 1.3 million tons of dyes and dye precursors, which are synthesized synthetically. Dyeing to textile is a simple process; involve dipping the textile in the pigment extract. Variation in color texture is achieved by changing the dipping time and the temperature of the dye bath. Further, the dyeing performances, differs depending on the types of fiber and also on the ability to maintain its color under several external conditions such as perspiration, washing and rubbing/crocking.

Dye like berberine has a high affinity for protein fibers such as wool and silk, whereas it shows low affinity for cellulosic fibers because of the lack of ionic charges on cotton surface compared to protein fibers. Polysaccharide
like chitosan used in pre-treatment of cotton fibers prior to dyeing which increases the cationic sites on cotton and subsequently the affinity of dye toward fiber, dye uptake. Enzyme like alkaline protease enhances the quality of natural dye by partial or complete damage of cuticle facilitating easier penetration of natural dye molecules into fibers. Treatment with ultraviolet radiation has also showed a crucial role in dying process. The effect of ultraviolet radiation on henna showed higher color strength and shade of fabrics than compared to that of non-irradiated henna (Masoud and Siyamak, 2014).

Usually, synthetic dyes are used in textile industries however; they have some limitations such as:

(i) Their production process requires hazardous chemicals, creating worker safety concerns.

(ii) They may generate hazardous wastes.

(iii) These dyes are not environment friendly.

In this regard, pigments from natural source are used to overcome this problem (Chidambaram et al., 2013).

Microorganisms produce a large variety of stable pigments such as carotenoids, flavonoids, quinones, and rubramines with higher yield and lower residues when compared to the plant and animal pigments (Hobson and Wales, 1998). Besides, some microbial colorants, especially anthraquinone type compounds, have shown remarkable antibacterial activity in addition to providing bright colors (Frandsen et al., 2006), which could serve as functional dyes in producing colored antimicrobial textiles.
Pigment like prodigiosin from *Vibrio* spp. suggests that, it could be used to dye many fibers including wool, nylon, acrylics and silk. Pigment from *Serratia marcescens* can color five types of fabric, namely acrylic, polyester microfiber, polyester, silk and cotton using tamarind as a mordant (Yusof, 2008). Similar textile-dyeing ability was also reported for *Janthinobacterium lividum* (Shirata *et al.*, 2000) which gave a good color tone when applied to silk, cotton, wool (bluish-purple, all natural fibers), nylon and vinylon (dark blue, both synthetic fibers). In the view of the vast availability of the microbial pigment, their affinity towards different textile, cost effectiveness and non toxic nature of microbial pigments may increase their market trend and could replace the synthetic colors which are toxic to mankind and nature.

### 2.4.2. Pigments as food grade colorant

The development of foods with an attractive appearance is an important goal in the food industry. To attract the food, many colors either synthetic or natural are added. In recent days, food producers are turning from synthetic color to natural colors, since synthetic color has demonstrated negative health issues (Aberoumand, 2011). Among various natural colorants, microbial colorants play a significant role as a food coloring agent, because of its cheap production, easier extraction, higher yields through strain improvement, no lack of raw materials and no seasonal variations (Malik *et al.*, 2012). Many pigments from microbial sources such as red pigment from *Monascus* sp., astaxanthin from *Xanthophyllomyces dendrorhous*, arpink red from *Penicillium oxalicum*, riboflavin from *Ashbya gossypii*, β-carotene from *Blakeslea trispora* and lycopene from *Erwinia uredovora* and *Fusarium*...
*sporotrichioides* (Selvakumar *et al*., 2009) are added to the food to increase its appeal. Similarly, canthaxanthin an orange yellow pigment produced by *Haematococcus lacustris* is used in poultry for the appearance of color shade of the yolk. Further, canthaxanthin is also used in cosmetics and foods, particularly in dairy products such as cheese, confectionery in soft and hard candy, fish products, meat products, fruit products, beverages, snacks, beer and wine. Pigments like riboflavin (vitamin B2) used in beverages, instant desserts, ice creams and tablets.

Pigments like anthocyanin used as preservative, as anthocyanin has antagonistic activity against bacteria, viruses and fungi thus protect food from microbial spoilage. Similarly, carotenoids can act as sunscreen to maintain the quality of food by protecting from intense light (Chattopadhyay *et al*., 2008).

The use of natural pigment as a food grade colorant not only increased the appeal of the food, but also used as a preservative for maintaining the good quality of the food which makes it superior than synthetic color.

### 2.4.3. Pigments as antimicrobial agent

Pigments like carotenoids, melanins, flavins, quinones, monascins, violacein, and indigo are good antimicrobial agents. Pyocyanin and pyorubin produced from *Pseudomonas aeruginosa* showed a distinct antibacterial effect against *Citrobacter* sp., which usually associated with urinary tract and wound infections. Pigment obtained from *Streptomyces hygroscopicus*, even showed good antimicrobial activity against drug resistant pathogens such as methicillin and vancomycin resistant strains of *Staphylococcus aureus* and β-lactamase producing culture of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella* sp. (Selvameenal *et al*., 2009; Berlanga *et al*.,
2000). Pigment from *Monascus ruber* showed antimicrobial activity against food borne bacteria (Francielo *et al.*, 2014).

Further, inhibition of human pathogenic bacteria like *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Vibrio cholera* was observed by endophytic fungal pigment of *Monodictys castaneae* (Kushwaha *et al.*, 2014). Hence, apart from coloring agent microbial pigments could be a new source for novel drugs.

### 2.4.4. Pigments as antioxidant

An antioxidant is a molecule that delay or inhibit cellular damage by donating electrons to a rampaging free radical and neutralize through their free radical scavenging property (Lobo *et al.*, 2010). The increase in free radicals in the body increases the chance of occurrence of chronic diseases such as cancer, diabetes, cardiovascular and autoimmune disorders (Branislav *et al.*, 2011). Microbial pigments like carotenoid, and naphthaquinone have been shown to have a potent antioxidant activity due to their biological functions. Bacterial pigment like xanthomonadin showed antioxidant activity by inhibiting photodynamic lipid peroxidation in liposome and offered protection against photo damage. Violacein has shown protection against oxidative damage in gastric ulceration by stimulating mucosal defence mechanism (Hardeep *et al.*, 2014). Hence it could be said that, the use of microbial pigment as antioxidants thought to possibly prevent the increasing incidence of many diseases such as cancers and heart disease.
2.4.5. Pigment as anticancer agent

Pigments like carotenoids have good anticancer activity on human T-cell leukemia, which causes fatal malignancy due its free radical scavenging property (Ratih and Se-Kwon, 2011; Lobo et al., 2010). Pigments obtained from Monascus spp. showed remarkable activity against different cancer cells like monascin has inhibitory activity on mouse skin carcinogenesis which is induced by peroxynitrite and ultraviolet light, while ankaflavin showed inhibitory activity on Hep G2 and A549 human cancer cell lines. Similarly, monaphilone A and monaphilone B, exhibits antiproliferative effect against HEp-2 human laryngeal carcinoma cell line. Moreover, rubropunctatin could induce the apoptosis, mediated by tumor necrosis factor and inhibit proliferation of human gastric adenocarcinoma (Yanli et al., 2012). Further, pigments from Streptomyces sp. have also reported for anticancer activity (Arcamone, 1998).

Pigment like prodigiosin has been tested against more than 60 cancer cell lines and showed a good anticancer activity with an average inhibitory concentration of 2.1 μM. The effectiveness of prodigiosin is due presence of multiple cellular targets which make it a good anticancer agent (Darshan and Manonmanis, 2015). In the view of the above, microbial pigment could be potential useful therapeutic agents against cancer cells in the near future.

2.4.6. Pigments as stain and fluorescence-based indicators

Many fluorescent pigments from bacteria are used to check the progress of specific reactions. A key example of this is phycoerythrin. Phycoerythrin is a fluorescence-based indicator used to detect the rate of damage caused by free peroxy radicals. The pigment initially shows
fluorescence, as peroxy radicals are added to the pigment, dark spots appear where radicals have reacted with the pigment, causing the overall fluorescence emission to decrease over time. This assay can then be used to predict the rate of peroxy radical scavenging in human plasma (DeLange and Glazer, 1989).

Pigment from *Monascus* is being excellent in having staining property under a visible light, having fluorescence whose wavelength being different from its excitation wavelength, being useful not only as a staining in usual endoscopy but also as a fluorescent dye for interstitial staining in confocal endoscopy, to give a vivid stained image useful in detection of a small affected region. Further, it stains only the cytoplasm without staining cell nuclei, thus indicating that these colors have reduced cellular mutagenicity (Yamamoto *et al.*, 2006).

### 2.4.7. Pigments as anti-obesity agent

Obesity may define as an excessive body weight in the form of fat. Study indicates that obesity causes many diseases like diabetes mellitus, cardiovascular disease, certain forms of cancer and sleep-breathing disorder. Therefore, the necessity of discovering anti-obesity agent is needed for the safety of the people.

Pigments like fucoxanthin and neoxanthin showed a significant suppressive effect on obesity. Studies suggest that oral treatment with fucoxanthin significantly reduced the abdominal white adipose tissue (Ratih and Se-Kwon, 2011). Similarly, xanthigen not only promoted weight loss, but also reduced liver fat content, and improved liver function (Abidov *et al.*, 2010). Hence, the pigments can be used as anti-obesity agent.
2.5. Fermentation

A lot of attention is now paid to the biotechnological synthesis of the colors through the bacteria. The production of the natural colors has two fundamental approaches; first is to find new sources of colors and then enhance their color production capacity. The other approach is to obtain enhanced and consistent yields from the already recognized good sources of the colorants either through the strain improvement or through optimizing the process parameters to maximize the pigment yield.

The appropriate use of the fermentation physiology together with the metabolic engineering could allow the efficient mass production of the colorants. With the advances in the gene technology, attempts have been made to create cell factories for the production of pigments through the heterologous expression of biosynthetic pathways from either already known or novel pigment producers.

Optimization of fermentation processes is an important ‘strategy’ needed to achieve high-level production of valuable fermentative products. Medium optimization is one of the important processes for getting maximum pigment yield and it involves several factors such as medium components, operating conditions such as pH, temperature, aeration and agitation, etc. In this strategy one factor is optimized while keeping other factors kept constant (Chidambaram et al., 2013).

pH is the important factor which has a strong effect on the biosynthesis of metabolites such as pigments. Metabolically, pH is associated with changes in the activities of proteins, which control the activities such as cellular growth, production of primary and secondary metabolites, fermentation, and the oxidation processes of the cell (Alejandro Mendez et al., 2011). The pigment production by microbes is seen in a wide range of pH (Latha et al., 2005). However, the optimum pH for pigment
production varies with microorganisms for example, Cho et al., (2002) inoculated *Paecilomyces sinclairii* in the medium maintained at pH ranging from 3-10, and found the optimum pigment production at pH 6.0.

Haisheng Wang et al., (2009) inoculated *Duganella* sp. in nutrient broth for the period of 45 h and he found the optimum pigment production at pH 6.7 with initiation of pigment production after 10 h.

Similarly, among different pH values (from 3.0 to 6.0) Joshi et al., (2011) found maximum pigment production of *Sarcina* sp. at pH 5.5.

The Gram positive bacterium *Exiguobacterium aurantiacum* when inoculated in the medium of varying pH (4-10), the pH optimum for pigment production was found at pH 7 (Fatima et al., 2013).

Afshari et al., (2015) found the maximum pigment production by *Penicillium aculeatum* at pH 6.5.

It’s also observed that at different pH level different pigments were produced by the same microorganisms. Generally, the suitable pH for growth and pigment production of *Monascus* spp. is 5.5-6.5. Chen and Johns, (1993) found optimum production of yellow pigment by *Monascus purpureus* at pH 4.0, but when the pH is increased to pH 6.5 red pigment is produced (Yanli et al., 2012). The result suggests that the type of pigment formation is directly influenced by pH.

Like pH, temperature also play very important role in pigment production. The pigment production is seen in a wide range of temperature, from 5-45°C (Fong et al., 2001; Swati et al., 2012) with optimum varying with microorganism.

Cho et al., (2002) when incubated *Paecilomyces sinclairii* in a temperature ranging from 10 to 40°C and found the optimum pigment production at 25 °C.
Similarly Linawati et al., (2002) found the optimum pigment production by *Serratia marcescens* at 30°C.

Joshi et al., (2011) and Afshari et al., (2015) incubated *Sarcina* sp. and *Penicillium aculeatum* in a temperature range 25-35 °C and found the optimum pigment production at 30 and 35°C respectively.

It is also noticed that the increase in temperature beyond certain level has changed the absorption maxima of the pigment, for example in *Monascus* sp. it was found that at 30 °C the absorption maximum of the pigment found at 500 nm but beyond 30 °C, the spectral lines shifted towards 400 nm, which corresponded to yellow pigment.

Moreover, the temperature has a direct effect on the yield of the pigment. In *Monascus* sp. the pigment yield was optimum at 40 °C, above and below this temperature the yield decreased drastically (Carvalho et al., 2005; Sumathy et al., 2007).

The medium component such as carbon, nitrogen and trace elements plays an important role in the economical production of metabolites by microorganisms via fermentation. Therefore, application of a cheap and readily available substrate as a fermentation medium can be a good strategy for attaining significant production of pigment (Thiyam et al., 2014).

Each microorganism has a particular demand for carbon, nitrogen and trace elements. Yuan et al., 2009 found sucrose and casein was the most suitable carbon and nitrogen source for cell growth and pigment formation in *Janthinobacterium lividum*. 
In *Serratia marcescens*, Linawati *et al.*, (2002) observed the pigment production in citric acid, lactic acid, yeast extract and beef extract but not in the presence of glucose.

Production of pigments like prodigiosin was significantly increased upon cultivating in a medium containing sucrose and glycine. Furthermore, the inorganic supplement, KH$_2$PO$_4$, greatly accelerates the cell growth and subsequently accelerated the prodigiosin production. However, other inorganic components did not provide any outstanding results, even a negative effect. Concentrations of nitrogen source like potassium nitrate, tryptophan and beef extract had significant effects on violacein production.

The yield of violacein by *Duganella* sp. B2 reached 1.62 g/l under the optimized conditions and was increased approximately 4.8-folds (Chidambaram *et al.*, 2013).

The concentration of carbon, nitrogen and trace elements also effect the pigment production. Microorganism like *Paecilomyces sinclairii* produce pigment in a carbon source (soluble starch) at concentration 1.5% and nitrogen source (meat peptone) at 1.5% (Cho *et al.*, 2002) while *Duganella* sp., produce pigment in presence of soluble starch 1.3%, beef extract 0.153 %, potassium nitrate 0.11%, ammonium ferrous sulfate 0.008 %, dipotassium hydrogen phosphate 0.025 %, magnesium sulfate 0.075 % and L-tryptophan 0.074 % (Haisheng Wang *et al.*, 2009) suggesting the media component optimization is necessary for the maximum yield of the pigment.

In recent years, raw materials and by-products of agro-industrial origin have been proposed as low-cost alternative carbohydrate sources for microbial metabolite production, with the view of minimizing environmental and their disposal problems.
The microbes produce pigments by a fermentation process, which may be solid state (SSF) or submerged fermentation (SmF). The rates of utilization of various nutrients differ in each substrate, and so does productivity. In SSF technique, the substrates are utilized very slowly and steadily, so the same substrate can be used for longer fermentation periods. SSF offers several advantages including simpler techniques, less capital investment, lower levels of end-product inhibition and catabolic repression, lower amounts of waste output, better product, and higher yield. SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content (Thiyam et al., 2014 a). The substrates used in SSF supply the basic nutrients to the microorganisms and serve as an anchor for the cells. Interestingly, recent studies report that SSF provides a more adequate habitat for fungi, resulting in high pigment production in a relatively low-cost process when agro-industrial wastes are used as substrate such as rice bran, wheat bran, coconut oil cake, sesame oil cake, palm kernel cake, groundnut oil cake, cassava powder, spent brewing grain, corn cob and jackfruit seed powder (Palanivel Velmurugan et al., 2011).

In contrast to SSF, in SmF the substrates are utilized quite rapidly; hence need to be constantly replaced/supplemented with nutrients. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture content (Reeba et al., 2015).

Various agro-waste extract are used for the production of pigment using SmF. Agro-waste such as cotton seed meal extract are used for the production of pyocyanin by Pseudomonas aeruginosa (El-Foulya et al., 2015). Similarly, Chandi et al., (2010) used tomato waste for the production of carotenoids by Rhodotorula glutinis. From
Apart from use of agro-waste for the production of pigment at low cost, there are some other methods which can enhance the pigment production. There are many recent indications that production of pigments greatly depends on interactions of microorganism. Some dual microbial systems have been characterized at the molecular level, and several small signalling molecules are known (Chidambara et al., 2013).

2.6. Co–culture method

Bacterial populations in natural habitats are complex communities containing many species that exhibit competition in order to survive with limiting nutritional resources. Competition for limited resources and antagonism are characteristics of these micro-habitats, which favour various defence mechanisms that rely mainly on the production of bioactive secondary metabolites. The defence mechanism depends on the following factors:

(1) The ability of metabolites to prevent invasion by a competitive strain, (2) the ability of metabolites to facilitate the invasion of an established competitor's habitat, and (3) the ability of metabolites to mediate direct "head-to-head" interactions by competitive strains (Slattery et al., 2001). However, in pure cultures, these expressions remain silent (Yamanaka et al., 2005). To overcome this, co-culture was developed which recently become an emerging tool to increase the chemical diversity of secondary products during in vitro fermentation (Nutzmann et al., 2011). Though the co-culturing technique is in demand in recent years, however the techniques aroused thousands of years ago where the mixed species used for the production of many foods and beverages like yogurt, cheese, soy sauce and wine (Terry, 2013). Co-
cultivation is expected in the future to routinely complement other experimental approaches that likewise aim at diversifying secondary compound production by microorganisms such as mutagenesis, the one strain many compounds approach (Wei et al., 2010) or treatment of microbes with epigenetic modifiers (Cichewicz, 2010). Co-cultivation is an ecologically driven approach that tries to mimic the natural situation where a given microbe is always embedded in a more or less complex microbial community and exposed to a multitude of chemical signals that are exchanged between the different taxa that compose this community. Compounds produced by these hosts are likely to have an influence on this microbial community and may even act as stimulating clues that induce the production of certain microbial compounds that may not be accumulated in the absence of these clues (Andreas et al., 2014).

2.7. Streptomyces coelicolor and actinorhodin

*Streptomyces* are Gram-positive soil bacteria that produce numerous secondary metabolites. A total of 75-80% bioactive molecules have been isolated from *Streptomyces* hence the genus is considered as industrial important microorganism (Berdy, 2012). The genus *Streptomyces* was first proposed by Waksman and Henrici (1943). At the time of writing the genus encompasses of 696 valid species (http://www.bacterio.net/-allnamessz.html). Among the genus, *Streptomyces coelicolor* is the best-characterized genetically, and its entire genome has been sequenced, which revealed that it has 8,667,507 base pair linear chromosome, with high GC content (72.12%). In total it has 7,825 genes predicted which include more than 18 clusters that code for enzymes characteristic of secondary metabolites. The enzymes include type I and type II polyketide synthases (PKSs), chalcone synthases, non-ribosomal peptide synthetases, terpene cyclases, and others
which produce, actinorhodin, prodigines, desferrioxamines G1 and E, eicosapentaenoic acid, hopanoid biosynthesis, tetrahydroxynaphthalene biosynthesis, geosmin biosynthesis, carotenoid isoreneriatine, butyrolactones, calcium dependent antibiotic and siderophores named coelichelin and coelibactin (Rekha and Mervyn, 1997; Bentley et al., 2002).

*S. coelicolor* sporulates in specialized cells called aerial hyphae. These cells are produced during the course of a life cycle of several days duration (Adams et al., 1998). The first cells produced after the germination is the substrate hyphae. These hyphae are subdivided by cross walls at relatively infrequent intervals and grow by elongating and branching, giving rise to long substrate filaments that can contain dozens of chromosomes. Within 48 hours of germination, aerial hyphae begin to appear in the developing colony. These filaments stand up in the air, forming a white layer of fuzz on the colony surface called the aerial mycelium. Unlike the substrate hyphae, the aerial hyphae undergo extensive cell division that divides them into chains of uni-nucleoidal compartments. Each of these compartments then matures into a spore. While septation and spore formation are taking place in the aerial mycelium, the substrate mycelium produces pigment which is blue in color (Justin et al., 1999).

Actinorhodin (Figure 2) an antibiotic belongs to polyketide, its production can be easily visualized, as it is a colored pigment. It was first described by Brockmann and Pini in 1947 when they recognized its inhibitory effect against *Staphylococcus aureus* (Wright and Hopwood, 1976).

This pigment is a pH indicator; it appears blue in alkali and red in acid pH and usually produced in stationary phase. Various actinorhodin congeners like α, β, γ and ε-actinorhodin were also reported (Zhang et al., 2006). The genes involved in actinorhodin production reside in a single cluster (SCO5076 to SCO5092). The
Biotechnological production of biocolor from microorganisms

production begins with the synthesis of a 16 carbon polyketide backbone by a type II polyketide synthase complex, encoded by *actI*-ORF1, *actI*-ORF2 and *actI*-ORF3. The resulting octaketide is modified by a ketoreductase, an aromatase and a cyclase, leading to a predicted bicyclic intermediate. Next, the *actVI* gene product catalyses the formation of a pyran ring, establishing the (3S, 15R) stereochemistry found in the ACT-type benzoisochromanequinones, and leading to the formation of the three-ringed molecule (S)-DNPA (4-dihydro-9-hydroxy-1-methyl-10-oxo-3-H-naphtho[2,3-c]-pyran-3-(S)-acetic acid). (S)-DNPA is converted to dihydrokalafungin, and the current opinion is that either dihydrokalafungin or one of its derivatives is the last three ringed intermediate in the pathway, covalent coupling of two of these molecules leads to the formation of the six-ringed actinorhodin molecule (Kapile et al., 2007).

![Figure 2: Structure of actinorhodin](image)
2.8. Nanoparticles

“Small is beautiful and small is powerful” (Khanna, 2008), the important statement holds well when we talk about nanoparticles. Nanoparticles, generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are composed of (Song et al., 2009).

The concept of nanoparticles was first presented by Richard Feynman through his famous lecture, entitled “There’s a plenty of room at the bottom” at the American Institute of Technology (Nasreen et al., 2014). Though the concept of nanoparticles was first presented by Richard Feynman, but nanoparticles were used since antiquity.

Since ancient times, gold and silver in different forms has been widely used as a medicine for treatment of various diseases. Gold in the form of Swarna bhasma (gold ash) (Pradeep and Anshup, 2009) and silver in the form of Rajath Bhasma (Rajath is silver and Bhasma means fine powder) was practiced in medicine (Kalishwaralal et al., 2010). Hippocrates the father of medicine, promoted the use of silver for healing the wounds (Wesley Alexander, 2009). With the present day understanding of nanoscience, one can clearly get enlightened that these formulations contained gold and silver nanoparticles (Kalishwaralal et al., 2010).

Among various nanoparticles, gold and silver nanoparticles are of great interest having distinctive properties, such as good electrical conductivity, chemical stability, catalytic, antibacterial activity (Prathna et al., 2011; Castro et al., 2011), subwavelength imaging, data storage, sensor devices and as hyperthermia agents to heat-kill cancer cells (Zhou et al., 2012; Hauck et al., 2008).

Generally, nanoparticles are synthesized by several methods such as physical, chemical and biological (Dubey et al., 2010; Filippo et al., 2010).
nanoparticles using chemical and physical methods are rapid but these methods often involve the use of hazardous chemicals which may pose environmental risks (Prathna et al., 2011). Compared to physical and chemical methods, biological synthesis using microbes and plants was regarded as a safe and ecofriendly process (He et al., 2008). Several biological synthesis methods, using microbes like Cladosporium cladosporioides (Balaji et al., 2009), Neurospora crassa (Castro et al., 2011), Fusarium oxysporum (Duran et al., 2005) and Streptomyces griseus (Derakhshan et al., 2012) have been suggested as safe, cost effective, possible eco-friendly and alternatives ways to chemical and physical methods (Saifuddin et al., 2009). Parallel to microbes mediated synthesis, several rapid plant mediated synthesis method using crude plant parts extracts like Sorbus aucuparia (Dubey et al., 2010), Cocculus hirsutus (Bar et al., 2012) and Chenopodium album (Dwivedi and Gopal, 2010) were also reported (Raisanen et al., 2002).

For the formation of nanoparticles first the microorganisms should develop a mechanism, which can withstand the toxic environments caused due to heavy metal. To counter this effect, microorganisms harbor numerous metal resistance gene clusters enabling cell detoxification via a number of mechanisms such as complexation, efflux, or reductive precipitation. Microorganisms use different mechanisms for the formation of nanoparticles; however nanoparticles are usually formed by the following ways:

Metal ions are first trapped on the surface or inside of the microbial cells. The trapped metal ions are then reduced to nanoparticles in the presence of enzymes. In general, microorganisms impact the mineral formation in two distinct ways. They can modify the composition of the solution so that it becomes supersaturated or more supersaturated than it previously was with respect to a specific phase. A second means
by which microorganisms can impact mineral formation is through the production of organic polymers, which can impact nucleation by favoring (or inhibiting) the stabilization of the very first mineral seeds. This section reviewed the possible formation mechanisms for some typical nanoparticles such as gold and silver nanoparticles (Xiangqian Li et al., 2011).

2.9. Acid detection

The accurate and definitive identification of microorganisms is one of the cornerstones forming the joint foundation of the fields of microbiology. The first credible approaches to the systematic classification of bacteria began in the latter part of the 19th century, which separated groups of bacteria on the basis of morphology, size, and motility. A pioneer investigator during this period was Ferdinand Cohn who introduced the concept of a diversity of microorganisms and argued that, within species; varieties emerged and transmitted their characteristics to the next generation. The subsequent development of agar-based media led to the in vitro isolation and propagation of pure cultures. This singular event fueled the first substantive biochemical investigations of bacterial species (Michael and Sharon, 2001). Biochemical tests used for identification and classification of bacteria largely based on their reactions in a series (Baron, 1996), some can be classified, or distinguished from one another by the ability to grow on different substrates and/or production of different end products, produce specific enzymes. One of the common substrate used in biochemical tests is carbohydrates, which can be fermented or oxidized via aerobic or anaerobic respiration. When a carbohydrate is fermented it produces acid (Prakash et al., 2012). This acid production can be detected by the addition of a pH indicator like congo red, phenol red, methyl red etc., that mainly belong to azo dye which change according to the pH (Aneja, 2003).
Azo dyes are aromatic compounds with one or more –N=N– groups, constitute the largest class of synthetic dyes (Anjali et al., 2007). Azo dyes are commonly used in general for biochemical test (Manikprabhu and Lingappa, 2013), but it has found that their effluent caused serious damage, since they would significantly affect the photosynthetic activity of hydrophytes by reducing light penetration (Liang et al., 2013; Brown and De Vito, 1993). Though many bacteria are capable to degrade such type of dyes, aerobically and an aerobically, but often the metabolic products, usually aromatic amines are noxious or even more dangerous than the starting dye (Dawkar et al., 2009). It is very difficult to treat such water waste practicing traditional biological processes (Poon et al., 1999). However, various physicochemical decolorization technique guidelines were reported. Few, however been accepted by the industries to treat dye water waste, but lack to implement due to the high cost and low efficiency (Asgher et al., 2007). So natural pigments are required, which substitute synthetic dye.