Chapter 2

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Natural colours are generally defined as materials extracted, isolated or otherwise derived from plants, animals, or microorganisms that are capable of imparting a distinguishing colour when added to foodstuffs.

2.1 Pigments – Classification

2.1.1 Based on origin

Pigments can be classified based on their origin as natural, synthetic, or inorganic. Natural pigments are produced by living organisms such as plants, animals, and microorganisms. Natural and synthetic pigments are organic compounds. Inorganic pigments are found in nature or reproduced by synthesis (Bauernfeind, 1981).

2.1.2 Based on chemical structure of the chromophore

Pigments can be classified according to the chemical structure of the chromophore as: (i) Chromophores with conjugated systems (carotenoid, anthocyanin, betalain, caramel and synthetic pigment) and (ii) Metal-coordinated porphyrins (myoglobin, chlorophyll, and their derivatives) (Wong, 1989).
2.1.3. Based on the structural characteristics of the natural pigments

Natural pigments are also classified based on their structural characteristics as: Tetrapyrrole derivatives (chlorophylls and heme colours), Isoprenoid derivatives (carotenoids and iridoids), N-heterocyclic compounds different from tetrapyrroles (purines, pterins, flavins, phenazines, phenoxazines, and betalains), Benzopyran derivatives (anthocyanins and other flavonoid pigments), and Quinones (benzoquinone, naphthoquinone, anthraquinone and melanins). (Bauernfeind, 1981; Hari et al., 1994).

2.1.4. Based on application as food additives

Based on the application of the pigments as food additives, the Food and Drug Administration (FDA) has classified pigments into two types. (i) Certifiable (synthetic pigments and lakes) and (ii) Exempt from certification (pigments derived from natural sources such as vegetables, minerals, or animals, and synthetic counterparts of natural derivatives) (FNB, 1971; Frick and Meggos, 1988; Wong, 1989).

2.2. Characteristics of major pigments
2.2.1. Tetrapyrrole Derivatives

Tetrapyrrole derivatives have pyrrole rings in linear or cyclic arrays. Linear array is very common in algae Rhodophyta and Cryptophyta. In the cyclic compounds, the heme group (the porphyrin ring bonded to an iron atom) is present in hemoglobin and myoglobin of animals, in cytochromes, peroxidases, catalases, and vitamin B_{12} as a prosthetic group. However, chlorophylls (Fig. 2.1) constitute the most important subgroup of pigments within the tetrapyrrole derivatives. They are mainly present in the chloroplasts of higher plants and in other groups such as
algae and bacteria (Britton, 1991; Counsell et al., 1979; Hari et al., 1994; Lichtenhaler, 1987; Rudiger and Schoch, 1988).

2.2.2. Isoprenoid Derivatives

Among the isoprenoid derivatives the most common and most important natural pigments are the carotenoids. They have drawn the attention of chemists, biochemists, biologists, food science and technologists, and pharmacists, for more than a century.

Carotenoids are a class of fat soluble pigments responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colours of some birds, insects, fish, and crustaceans. In plants, algae and photosynthetic bacteria these pigments play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts, and molds,
where they may carry out a protective function against damage by light and oxygen. Although animals appear to be incapable of synthesizing carotenoids, many animals incorporate carotenoids from their diet. Within animals, carotenoids provide bright colouration, serve as antioxidants, and can be a source for vitamin A activity (Britton, 1991; Ong and Tee, 1992). Some familiar examples of carotenoid colouration are the oranges of carrots and citrus fruits, the reds of peppers and tomatoes, and the pinks of flamingoes and salmon (Pfander, 1992). Some 600 different carotenoids are known to occur naturally (Ong and Tee, 1992), and new carotenoids continue to be identified (Mercadante, 1999). It is estimated that nature produces about 100 million tons of carotenoids annually (http://www.industrialorganica.com/carotenoids.html).

Carotenoids are a class of hydrocarbons (carotenes) and their oxygenated derivatives are known as xanthophylls. They consist of eight isoprenoid units joined in such a manner that the arrangement of isoprenoid units is reversed at the center of the molecule so that the two central methyl groups are in a 1, 6-position relationship and the remaining non terminal methyl groups are in a 1, 5-position relationship. All carotenoids may be formally derived from the acyclic $C_{40}H_{56}$ structure, having a long central chain of conjugated double bonds by hydrogenation, dehydrogenation, cyclization or oxidation, or any combination of these processes (Fig. 2.2).

![Fig. 2.2. Acyclic $C_{40}H_{56}$ structure](image-url)
In Greek, “xantho” means yellow, and “phylls” stands for leaves, which is comparable to “chlorophylls” (green leaves). Structures of some important carotenoids are given in Fig. 2.3.

Fig. 2.3. Structures of some important carotenoids

Xanthophylls were found in algae, and lutein, a component of xanthophylls were found in egg yolks. Lutein is the carotenoid pigment found in amaranth, red paprika, mustard green, and marigold flower petals. Important carotenoids are the orange, carotenes of carrot, apricot, peach, citrus fruits; the red lycopene of watermelon and apricot; the yellow orange xanthophylls of peach (Pfander, 1992).
Some carotenoids serve as precursors for vitamin A synthesis. A molecule of orange beta-carotene is converted into two molecules of colourless vitamin A within the body. Alpha-carotene, gamma-carotene and cryptoxanthin are also known as precursors for vitamin A synthesis. However, because of minor difference in chemical structure one molecule of each of these pigments yields only one molecule of vitamin A (Britton, 1991; Ong and Tee, 1992).

Recently, iridoids, a group of plant isoprenoid compounds, have acquired some relevance. Saffron (Crocus sativus L.) and Cape jasmine fruit (Gardenia jasminoides Ellis) are the best-known iridoid-containing plants, but their colours are influenced by carotenoids (Sacchettini and Poulter, 1997).

2.2.3. N-Heterocyclic compounds different from tetrapyrroles
a. Pterins

Pterins were first isolated as the colouring agents from the butterfly wings (hence the origin of their name, from the Greek “pteron”, wing) and they are known to perform many roles in colouration in the biological world (Smith and Ramfrez, 1960). The pterin ring system (Fig. 2.5) is probably present in every form of life. They are responsible for colour in some insects, in vertebrate eyes, human urine, and bacteria (Lactobacillus casei and Streptomyces faecalis R) (Forrest, 1962; Hari et al., 1994).

![Fig. 2.5. Structure of Pterin](image)
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b. Flavins

Flavins are widely spread compounds, synthesized by all living cells of microorganisms and plants. Flavin is a tricyclic heteronuclear organic ring (Fig. 2.6) whose biochemical source is the vitamin riboflavin. The flavin moiety is often attached with an adenosine diphosphate to form flavin adenine dinucleotide (FAD), and in other circumstances, is found as flavin mononucleotide (FMN), a phosphorylated form of riboflavin. It is in one or the other of these forms that flavin is present as a prosthetic group in flavoproteins. Other sources are a wide range of leafy vegetables, meat and fish (Counsell et al., 1979; Forrest, 1962; Hari et al., 1994).

![Fig. 2.6. Structure of flavin](image)

c. Phenazines

Many phenazine compounds (Fig. 2.7) are found in nature and are produced by bacteria such as *Pseudomonas* sp., *Streptomyces* sp., and *Pantoea agglomerans*. These phenazine natural products have been implicated in the virulence and competitive fitness of producing organisms. For example, the
phenazine pyocyanin produced by Pseudomonas aeruginosa contributes to its ability to colonise the lungs of cystic fibrosis patients. Similarly, phenazine-1-carboxylic acid, produced by a number of Pseudomonas, increase survival in soil environments and has been shown to be essential for the biological control activity of certain strains (McDonald and Mavrodi, 2001; Turner and Messenger, 1986).

![Fig. 2.7. Structure of phenazine](image)

d. Phenoxazines

Phenoxazines are found in fungi and insects (Fig. 2.8) and are structurally related with phenazines (Hari et al., 1994). They impart yellow, golden yellow, and dark brown colours. In invertebrate animals, phenoxazines are represented by the group called ommochromes. Interestingly some microorganisms produce this pigment that show antibiotic activities. Streptomycetes sp. produces the pink-red phenoxazine “actinomycin”, a chromopeptide antibiotic of commercial importance (Delgado-Vargas et al., 2000).

![Fig. 2.8. Structure of phenoxazine](image)
e. Betalaines

Betalain pigments are water soluble vacuolar yellow (betaxanthins) and violet (betacyanins) pigments (Fig. 2.9) that replace anthocyanins in most plant families of the order Caryophyllales. They are also found in some fungal species of Amanita and Hygrocybe. Betalains are conjugates of the chromophore betalamic acid which are derived from dihydroxyphenylalanine by an oxidative 4, 5-extradiol ring opening mechanism (Wohlpart and Mabry, 1968).

![Fig. 2.9. Structure of betalaines](image)

2.2.4. Benzopyran derivatives

The most studied benzopyran derivatives are the flavonoids (Fig. 2.10). These are phenolic compounds with two aromatic rings bonded by a C3 unit (central pyran ring) and divided in to 13 classes based on the oxidation state of the pyran ring and on the characteristic colour: anthocyanins, aurons, chalcones,
yellow flavonols, flavones, uncoloured flavonols, flavanones, dihydroflavonols, dihydrochalcones, leucoanthocyanidins, catechins, flavans, and isoflavonoids. Each type of flavonoid can be modified by hydroxylation, methylation, acylation, and glycosylation to obtain a great natural diversity of compounds. Flavonoids are water soluble and are widely distributed in vascular plants. More than 5000 flavonoids have been chemically characterized (Harborne, 1993; Koes et al., 1994).

Fig. 2.10. Structure of a typical flavanoid

2.2.5. Quinones

Quinones include large number of structural variants and have a great number of colouring compounds. Their basic structure (Fig. 2.11) consists of a desaturated cyclic ketone that is derived from an aromatic monocyclic or polycyclic compound. Coenzyme Q, represented by the ubiquinone has the quinone structure. This group is widely present in animals, plants and microorganisms and plays an important role in electron transport system in their cells.
Quinones can be classified based on their structure as benzoquinones, naphthoquinones, anthraquinones, dibenzoquinones, dianthraquinones, and dinaphthoquinones. Large number of quinines is found due to their variability in the kind and structure of substituents. Quinones are found in plants: plastoquinones are found in chloroplasts of higher plants and algae; ubiquinones are ubiquitous in living organisms; menaquinones are found in bacteria; naphthoquinones in animals; and anthraquinones in fungi, lichens, flowering plants, and insects. Many quinones are byproducts of the metabolic pathways and a few organisms (fungi) produce large quantities. In general, quinones produce yellow, red, or brown colourations, while quinine salts show purple, blue, or green colours (Hari et al., 1994; Thomson, 1962b).

![Basic structure of quinone](image)

**Fig. 2.11. Basic structure of quinone**

### 2.2.6. Melanins

Melanins are nitrogenous polymeric compounds and exist as a mixture of macromolecules with indole ring. They are responsible for the black, grey and brown colouration in animals, plants and microorganisms. There are three different types of melanin (Brown and Salvo, 1994; Hari et al., 1994; Thomson, 1962a; Thomson, 1962b).
(i) Eumelanins - widely distributed in vertebrate and invertebrate animals.
(ii) Phaemelanins - macromolecules in mammals and birds.
(iii) Allomelanins - present in seeds, spores and fungi.

Neuromelanin is the dark pigment present in pigment bearing neurons. The loss of pigmented neurons from a specific nucleus is seen in a variety of degenerative diseases. Dark skin protects against ultraviolet light which causes mutations in skin cells, which in turn cause skin cancers. Light-skinned persons have about a tenfold greater risk of dying from skin burn under equal sun conditions. Furthermore, dark skin prevents UV-A radiation from destroying the essential B vitamin folate, which is needed for the synthesis of DNA in dividing cells. The melanins (Fig. 2.12) act as a protective screen in humans and other vertebrates and in some fungi melanins are essential for their vital cycle.

Fig. 2.12. Structure of melanin
2.3. **Microbial pigments**

Among the natural sources of colourants, microorganisms offer great scope and hope. The ease of cultivation, extraction, the genetic diversity in microbes and sophistication of technology has made their choice more feasible (Juailova *et al.*, 1997). Among the different organisms bacteria, yeast, algae, fungi, and actinomycetes appear more efficient and attractive sources of biocolourants. List of some of the microorganisms and their pigment is given in Table 2.1.

There are a number of microorganisms, which have the ability to produce pigments in high yields, including species of *Monascus* (Hajjaj *et al.*, 2000) and *Serratia* (Williams *et al.*, 1971a). The red pigments, produced in solid-state cultures by several species of the genus *Monascus*, have been traditionally used in many Asian countries for colouring and securing a number of fermented foods (Francis, 1987).
### Table 2.1. List of some of the pigment producing microbes

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pigment</th>
<th>Colour</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Janthinobacterium lividum</em></td>
<td>Violacein</td>
<td>Purple</td>
<td>Matz <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><em>Xanthomonas oryzae pv. oryzae</em></td>
<td>Xanthomonadin</td>
<td>Yellow</td>
<td>Rajagopal <em>et al.</em>, 1997</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Zeaxanthin</td>
<td>Yellow</td>
<td>Hammond and White, 1970</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Pyocyanin</td>
<td>Blue-green</td>
<td>Baron and Rowe, 1981</td>
</tr>
<tr>
<td><em>Phaffia rhodozyma</em></td>
<td>Astaxanthin</td>
<td>Red</td>
<td>Florencio <em>et al.</em>, 1998</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin</td>
<td>Red</td>
<td>Kobayashi <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><em>Serratia rubidaea</em></td>
<td>Prodigsin like pigment</td>
<td>Red</td>
<td>Moss, 2002</td>
</tr>
<tr>
<td><em>Vibrio gazogenes</em></td>
<td>Prodigsin like pigment</td>
<td>Red</td>
<td>Moss, 2002</td>
</tr>
<tr>
<td><em>Alteromonas rubra</em></td>
<td>Prodigsin like pigment</td>
<td>Red</td>
<td>Moss, 2002</td>
</tr>
<tr>
<td><em>Rugamonas rubra</em></td>
<td>Prodigsin like pigment</td>
<td>Red</td>
<td>Gerber, 1975</td>
</tr>
<tr>
<td><em>Streptoverticillium rubrisaticuli</em></td>
<td>Prodigsin like pigment</td>
<td>Red</td>
<td>Gerber, 1975</td>
</tr>
<tr>
<td><em>Bradyrhizobium</em></td>
<td>Canthaxanthin</td>
<td>Orange</td>
<td>Lorquin <em>et al.</em>, 1997</td>
</tr>
<tr>
<td><em>Corynebacterium insidiosum</em></td>
<td>Indigoidine</td>
<td>Blue</td>
<td>Starr <em>et al.</em>, 1966</td>
</tr>
<tr>
<td><em>Micrococcus roseus</em></td>
<td>Canthaxanthin</td>
<td>Orange-pink</td>
<td>Cooney <em>et al.</em>, 1966</td>
</tr>
<tr>
<td><em>Dunaliella salina</em></td>
<td>β-carotene</td>
<td>Orange</td>
<td>Jacobson and Wasileski, 1994</td>
</tr>
</tbody>
</table>
The microorganisms such as *Monascus*, *Rhodotorula*, *Bacillus*, *Achromobacter*, *Yarrowia* and *Phaffia* produce a large number of pigments. Commonly found microbial pigments are carotenoids and astaxanthin. Carotenoids are yellow, orange and red pigments, which are widely distributed in nature. They are utilized as food or feed supplements and as antioxidants in pharmaceutical formulations (Miura *et al.*, 1998).

Several microorganisms have been shown to produce astaxanthin. They include *Agrobacterium aurantiacum* (Misawa *et al.*, 1995), *Phaffia rhodozyma* and *Haematococcus pluvalis* (Johnson and An, 1991). Among them *Phaffia rhodozyma* is a potential candidate for commercial production due to its high astaxanthin content (Andrews *et al.*, 1972). Astaxanthin added to poultry feed can improve the colour of both egg yolks and flesh (Johnson *et al.*, 1980). Astaxanthin possesses an unusual antioxidant activity, which has caused a surge in the nutraceutical market for the encapsulated product. Astaxanthin has potent antioxidant activity and may have a role in decaying or preventing degenerative diseases in human and animals (Schroeder and Johnson, 1993). Research on the health benefits of astaxanthin is very recent and has mostly been performed *in vitro* or at the pre-clinical level with humans (Higuera-ciapara *et al.*, 2006). The medical literature suggests that β-carotene may exhibit anticancer activities and aid in reducing the incidence of cardiovascular diseases (Sies and Krinski, 1995). Further, health benefits such as cardiovascular disease prevention, immune system boosting, bioactivity against *Helicobacter pylori*, and cataract prevention, have been associated with astaxanthin consumption (Ciapara *et al.*, 2006).

The species of *Monascus* produces several natural pigments, including primary colour, red and the secondary colour, orange during the solid state fermentation. These colours are widely used in Asia as food colourants. *Monascus* are traditionally used in oriental countries, originally in China and Thailand, to
prepare fermented rice with strong red colour, which finds several applications ranging from conferring colour to products such as wine, cheese and meat, to medicinal uses and as a meat preservative (Wong and Koehler, 1981).

A maroon dye was extracted from the rhizome of *Arnebia nobilis* (Indrayan *et al.*, 2004). Phycoerythrin pigment was isolated from the cyanobacterium, *Nostoc muscorum* (Ranjitha and Kaushik, 2005). The nutritional requirements for pyoverdine production by *Pseudomonas aeruginosa* was reported by Barbhaiya and Rao, (1985b). The pigment Xanthomonadin produced by the genus *Xanthomonas* has a role against photo damage (Rajagopal *et al.*, 1997).

2.4. Prodigiosin

2.4.1. Occurrence and structure

Prodigiosin is a tripyrrole first characterized from *Serratia marcescens*, which forms beautiful pillar box red colonies. Its name is derived from "prodigious" - something marvellous. The prodigiosin tripyrrole was shown to be localized in extracellular and cell-associated vesicles and in intracellular granules (Kobayashi and Ichikawa, 1991). Secondary metabolites related to prodigiosins are produced by a wide variety of bacteria including *Serratia marcescens*, *Serratia rubidaea*, *Vibrio gazogenes*, *Vibrio psychroerythrous*, *Pseudomonas magneslorubra*, *Alteromonas rubra*, *Rugamonas rubra*, *Streptomyces longisporus*, *Streptomyces spectabilis* and *Streptoverticillium rubrireticuli* (Variyar *et al.*, 2002). Prodigiosin, C$_{20}$H$_{25}$N$_{3}$O, has an unusual structure with three pyrrole rings and is a pyrrylidypyrrylmethene; two of the rings are directly linked to each other, and the third is attached by way of a methene bridge (Gerber, 1975; Qadri and Williams, 1972). The highly conjugated system of seven double bonds (Fig. 2.13) presumably accounts for the intense pigmentation.
A novel endospore forming *S. marcescens* subsp. *sakuensis*, isolated from activated sludge (Ajithkumar *et al.*, 2003) and two novel halophilic bacterial strains named *Zooshikella ganghwensis*, isolated from Korean tidal flats (Yi *et al.*, 2003), have been reported to produce prodigiosin. Further, a novel red pigment, 2,2V-[3-methoxy-1’ amyl-5’-methyl-4-(1”-pyrryl)] dipyrryl-methene (MAMPDM), was isolated from an alkalophillic *Micrococcus* sp. (Variyar *et al.*, 2002).

A family of natural red pigments called prodigiosins is synthesized from *Serratia marcescens* (Han *et al.*, 1998). The members of this family include prodigiosin, cycloprodigiosin hydrochloride (cPrG-HCl), undecylprodigiosin, metacycloprodigiosin and desmethoxyprodigiosin. Immunosuppressive properties have been attributed to members of the prodigiosin family, which includes undecylprodigiosin, metacycloprodigiosin and cycloprodigiosin hydrochloride (cPrG.HCl), with a mechanism of action different from that of other well known immunosuppressants such as cyclosporin A, FK506 and rapamycin (Kataoka *et al.*, 1995; Songia *et al.*, 1997; Tsuji *et al.*, 1990).
Several bacteria produce metabolites similar to prodigiosin and there has been considerable confusion with respect to naming them. To some extent, “prodigiosin” is used in the literature in a generic sense to include a family of similar materials. In devising trivial names for a group of related compounds it is useful to define a basic nucleus. Two such possibilities have been used for the prodigiosin-like materials (Gerber, 1975). The completely stripped down nucleus, devoid of all substituents, is termed “prodigiosene,” while the portion common to most of the natural products, and containing a 6-methoxy substituent, is termed “prodiginine”. Hence, prodigiosin could also be referred to as either 2-methyl-3-pentylprodiginine or 2-methyl-3-pentyl-6-methoxyprodigiosene. Prodigiosenes are synthesized by members of two families of Actinomycetales, Actinomycetaceae and Streptomycetaceae. Nocardia madurae, N. pelletieri, and Streptomyces longisporus ruber each synthesize two pigments, one of which has a cyclic side chain attached to the prodigiosene nucleus. Ability to produce these cyclic compounds may be characteristic of species of Actinomycetales. Pigments of lower molecular weight are produced by S. marcescens. Whereas, N. madurae, N. pelletieri, and S. longisporus ruber produce pigments of higher molecular weights (Table 2.2) (Williams, 1973).

Four structural types based on the prodiginine nucleus can be recognized:

1. **Presence of straight chain alkyl substituents:**

   1A. Alkyl substituents at both positions 2 and 3.

   The prototype is prodigiosin itself with a methyl group at position 2 and a pentyl group at position 3. Higher homologues with methyl at position 2 and either hexyl or heptyl at position 3 (along with prodigiosin) have been isolated from Pseudomonas magnesiorubra, the marine psychrophilic bacterium Vibrio psychroerythreus, a sewage bacterium (Gerber, 1975). A river bacterium, Rugamonas rubra produced prodigiosin (Austin and Moss, 1986). Norprodigiosin
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(2-methyl-3-pentyl-norprodiginine) is formed by the *Serratia marcescens* mutant OF (Qadri and Williams, 1973).

**1B. Alkyl substituents at position 2 only.**

Prodigiosin-like materials with an undecyl chain at position 2 were first fully characterized from *Streptomyces longisporus ruber* (Harashima *et al.*, 1967; Wasserman *et al.*, 1966) and with a nonyl sidechain from *Actinomadura madurae* (Gerber, 1975).

**2. Ring formation between positions 2 and 4**

A structure with a cyclononyl ring linked to positions 2 and 4, and carrying an additional ethyl substituent was isolated from *Streptomyces longisporus ruber* generally known as metacycloprodigiosin, is probably identical with streptorubin A from *Streptomyces rubrireticuli var. pimprina* (Gerber, 1975). Some organisms like *Streptomyces hiroshimensis*, produce both metacyclo-prodigiosin and undecylprodiginine. This is also true for an actinomycete isolated from leek roots and belonging to the *Streptoverticillium baldaccii* cluster (Brambilla *et al.*, 1995).

**3. Ring formation between positions 3 and 4**

The only example of this structural type is a cyclized form of prodigiosin itself, usually known as cycloprodigiosin; originally isolated from a marine bacterium, *Alteromonas ruber* (Gerber and Gauthier, 1979). Cycloprodigiosin was also found, together with prodigiosin itself, in the anaerobic marine bacterium *Vibrio gazogenes* (Gerber, 1983; Harwood, 1978; Laatsch and Thomson, 1983) and as its hydrochloride in *Pseudoalteromonas denitrificans*, isolated from the sea near Japan (Kawauchi *et al.*, 1997).
4. Ring formation between position 2 of the monopyrryl unit and position 10 of the dipyrryl unit.

Compounds described generically as “macrocyclic prodiginines” have been isolated from Actinomadura pelletieri (formerly Nocardia pelletieri) and Actinomadura madurae (formerly N. madurae) (Gerber, 1975). These structures contain a bridge with several –CH₂– groups between the first and third pyrrole rings. These “macrocyclic prodiginines” are apparently unique to the two organisms named. Finally, there has been unfortunate nomenclature confusion between the red pigment prodigiosin and a material referred to in Russian literature as “prodigiosan”. The latter is a polysaccharide or lipopolysaccharide also isolated from Serratia marcescens. Moreover, in some cases, the Russian word for prodigiosan was translated as prodigiosin to take only one example, “activation of mononuclear phagocytes by a lipopolysaccharide (prodigiosin)” (Panin et al., 1996).
<table>
<thead>
<tr>
<th>Order</th>
<th>Genus and species</th>
<th>Trivial name of pigment</th>
<th>Prodigiosene nomenclature</th>
<th>Molecular weight (Da) and Formula</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriales</td>
<td>Serratia marcescens</td>
<td>Predigiosein</td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{30}H_{28}N_{12}O_{12}</td>
<td>Rapoport and Ildyenko, 1962</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{29}H_{26}N_{12}O_{12}</td>
<td>Harri et al., 1970</td>
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<td></td>
<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{28}H_{24}N_{12}O_{12}</td>
<td>Wassermann et al., 1968</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{27}H_{22}N_{12}O_{12}</td>
<td>Gerber, 1969</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{26}H_{20}N_{12}O_{12}</td>
<td>Gerber, 1970</td>
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<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{25}H_{18}N_{12}O_{12}</td>
<td>Harasima et al., 1967</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{24}H_{16}N_{12}O_{12}</td>
<td>Wasserman et al., 1966</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{23}H_{14}N_{12}O_{12}</td>
<td>Wasserman et al., 1969</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2-Methyl-3-ethyl-4,6-dimethoxyprodigiosein</td>
<td>C_{22}H_{12}N_{12}O_{12}</td>
<td>Wasserman et al., 1971</td>
</tr>
</tbody>
</table>
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2.5. Microbial production of Prodigiosin

2.5.1. Submerged Fermentation (SmF)

Prodigiosin (5-[(3-methoxy-5-pyrrol-2-ylidene-pyrrol-2-ylidene) methyl]-2-ethyl-3-pentyl-1H-pyrrole) was isolated from Serratia, Pseudomonas and Streptomyces (Giri et al., 2004), actinomycetes and a few other bacteria (Grimont and Grimont, 1978) and was observed as a typical alkaloid compound produced as a secondary metabolite. It has a unique structure consisting of three pyrrole rings and a pyrrolylpyrromethene skeleton with a C-4 methoxy group (Bennett and Bentley, 2000). Prodigiosin appeared only in the later stages of bacterial growth (Williams et al., 1971b). The actinomycete Streptomyces coelicolor A3(2) produced a closely related linear tripyrrole, undecylprodigiosin, and a cyclic derivative, butyl-meta-cycloheptylprodiginine in a 2 : 1 ratio (Tsao et al., 1985).

A bifurcated pathway has been proposed for the synthesis of prodigiosin culminating in the enzymic condensation of the terminal products of the two pathways, 4-methoxy-2, 2-bipyrrole-5-carboxyaldehyde and the monopyrrole, 2-methyl-3-n-amyl-pyrrole (MAP). The precursors for prodigiosin were shown to be acetate, serine, alanine, methionine and proline (Williams, 1973). Recently, the mechanism of proline incorporation into a pyrrole moiety has been shown biochemically and a pathway for synthesis of undecylprodigiosin proposed (Cerdeno et al., 2001; Thomas et al., 2002). Several investigations indicated that the pyrrole groups of prodigiosin arise from amino acids (Shrimpton et al., 1963; Stavri and Marx, 1961; Tanaka et al., 1972; Williams et al., 1971a) and acetate (Cushley et al., 1971).

Many studies were carried out to improve prodigiosin production. A classical syntrophic cross-feeding method showed that prodigiosin was formed via a bifurcated pathway, which depended upon a number of genes coding for the
enzymes involved (Block, 1961). Many factors, such as temperature, pH, dissolved oxygen level, light and medium composition influenced the production of prodigiosin (Heinemann et al., 1970; Rjazantseva et al., 1995; Sole et al., 1994). Prodigiosin secretion was enhanced on addition of SDS into the cultures (Feng et al., 1982). An integrated fermentation-separation system was employed to eliminate the toxic effects of metabolites on cell growth and alleviate the feedback repression by end product (Bae et al., 2001).

Species of *Serratia marcescens* are the major producers of prodigiosin (Furstner, 2003). Secondary metabolites may be located in the cell envelope of microorganisms, as is true of prodigiosin (Purkayastha and Williams, 1960), although prodigiosin is not released into the medium, as is characteristic of many secondary metabolites. The production of prodigiosin in *S. marcescens* is susceptible to temperature and is substantially inhibited at temperatures higher than 37°C (Giri et al., 2004). Conventional media used for the biosynthesis of prodigiosin by *S. marcescens* strains are complex media that are rich in a variety of nutrients (Furstner, 2003; Giri et al., 2004; Yamashita et al., 2001). Certain nutrients, such as thiamine (Goldschmidt and Williams, 1968) and ferric acid (Silverman and Munoz, 1973), are particularly crucial for prodigiosin production, whereas phosphate (Witney et al., 1977), adenosine triphosphate, and ribose (Lawanson and Sholeye, 1975) have inhibitory effects on prodigiosin yield. It was observed that novel peanut seed broth gave rise to a significant enhancement of prodigiosin production (Giri et al., 2004). Moreover, it was reported that the addition of silica-gel carriers to a liquid culture of *S. marcescens* led to marked increases in cell growth and the production of prodigiosin (Yamashita et al., 2001). In addition, since prodigiosin is often located on the cell envelope, the addition of surfactants, such as sodium dodecyl sulphate (SDS), could also enhance the recovery efficiency for prodigiosins (Feng et al., 1982). Identification of optimized medium composition to achieve more efficient production of a prodigiosin-like
pigment (PLP) from \textit{S. marcescens} SMΔR, which is a SpnR-defective isogenic mutant of \textit{S. marcescens} SS-1 was reported (Wei and Chen, 2005). LB broth was shown to be an effective growth medium for \textit{S. marcescens} SMΔR, leading to the production of a biosurfactant and also a prodigiosin-like-pigment (Homg \textit{et al.}, 2002; Wei \textit{et al.}, 2004). The components of LB broth (tryptone, NaCl, and yeast extract) were examined individually for their effects on prodigiosin production. The effect of vegetable oil supplementation on prodigiosin production was also reported (Wei and Chen, 2005).

A \textit{Serratia marcescens} mutant for prodigiosin production was obtained by UV mutation with rational screening methods and a two-step feeding strategy was used to increase its productivity. In flasks, the mutant strain B6 gave a 2.8-fold higher prodigiosin production than that of the parent strain with glycerol as a carbon source. In a 5 liter bioreactor, with a two-step feeding strategy in which glucose was selected as the initial carbon source in the fermentation media, glycerol was fed as a ‘prodigiosin inducer’ (Tao \textit{et al.}, 2005).

2.6. Purification and characterization of Prodigiosin

Water insoluble, lipophilic pigments are usually extracted with water-miscible organic solvent, such as acetone, methanol, ethanol, or mixtures thereof, to allow better solvent penetration. Dried materials can be extracted with water-immiscible solvents. The extract usually contains a substantial amount of water, which can be removed by partition to hexane, petroleum ether, diethyl ether, or dichloromethane or mixtures of these solvents. The chromatographic behavior and the ultraviolet and visible absorption spectrum provide the first clues for the identification of pigments. Both the position of the absorption maxima ($\lambda_{\text{max}}$) and the shape (fine structure) of the spectrum reflect the chromophore. Spectra is taken, stored, and subsequently compared with those of standards (Amaya, 2001).
It is now recommended that the following minimum criteria be fulfilled for identification of an unknown compound (Liaaen-Jensen, 1971; Pfander et al., 1994).

- The visible absorption spectrum ($\lambda_{\text{max}}$)
- Thin layer chromatogram (Rf)
- High performance liquid chromatogram (Retention time)
- Mass Spectrum (Molecular mass)
- NMR (Chemical shift data)

Prodigiosin (2-methyl-3-pentyl-6-methoxyprodigiosene) was extracted from a 72 h culture of *Serratia marcescens* in Nutrient broth with absolute ethanol and petroleum ether; followed by drying in a boiling water bath; and dissolution of the residue in 50% ethanol (Rosenzweig and Stotzky, 1980).

The pigment from the cell pellet of *Serratia marcescens* isolated from soil was extracted with acetone, mixed with ethyl acetate fraction, and dried with sodium sulphate. The extracts were evaporated and a wavelength scan was done from 200 to 700 nm. A solvent mixture in 2.5:2.5:0.5 ratio of dichloromethane, chloroform and acetone was used for effective separation of the impurities extracted along with the pigment by thin layer chromatography. Silica column of mesh size 80–100 was used for separation of the non coloured impurity from the pigment. The purified sample showed a single peak absorbance at 535 nm in the UV spectrophotometer. It was further analysed for determination of molecular weight using mass spectrophotometer. The pure prodigiosin pigment analysed by mass spectrophotometry showed a molecular weight of 324 Da (Giri et al., 2004).

Prodigiosin was extracted by shaking the environmental isolate *Serratia marcescens* 2170 cells with acidic methanol (1 ml of 1N HCl: 24 ml of methanol) and the supernatant was evaporated under vacuum. Atmospheric pressure liquid
chromatography of the extract was performed on silica gel with chloroform and methanol as solvents. The isolated pigment was redissolved in methanol and analyzed by electrospray ionization mass spectrometry (ESI-MS) using a VG-Quattro triple quadrupol mass spectrometer. The isolated pigment was repurified by subsequent semipreparative HPLC. A Nucleosil C_{18} reversed-phase column (250x4 mm, 10 µm) was used with a 0 % to 100 % linear gradient in 30 minutes (A: 10 mM ammonium acetate, pH 7.0, B: 100 % acetonitrile). The elution was monitored both using diode-array UV detector and by ESI-MS (Montaner and Perez-Tomas, 2001; Tomas and Montaner, 2003).

After fermentation of *Serratia marcescens* from the soil, the broth was drained from the internal adsorbent bioreactor (IAB), and then 1 l of a 95 % (v/v) acidified ethanol (pH 3.0) solution was added to the IAB. Attached pigments were extracted by circular desorption using an impeller in the IAB at 200 rpm for 5 h, and then concentrated and centrifuged. It was then isolated using phase separation with water and chloroform. Red prodigiosins were placed in a down-filled chloroform phase and then concentrated by evaporation. Prodigiosin was separated by silica gel column chromatography (2.5×30 cm; Kieselgel 60; Merck, Darmstadt, Germany). It was eluted with a mixture of hexane: ethyl acetate (2:1; v/v). The concentrated pigment was separated by development in a 95:5 (v/v) mixture of chloroform: methanol using TLC. Single red prodigiosin (Rf value: 0.43) was then collected and dissolved in acetone. The pigment was repurified by further TLC (chloroform: methanol: diethyl ether = 6:2:2), and finally purified by preparative HPLC (with a C_{18} column (2.5×10 cm). It was isocratically eluted with a mixture of methanol: water (7:3, v/v) that was adjusted to pH 3.0 with 0.1 N HCl at a flow rate of 20 ml/min. A large open stainless steel grid with a pore size of 0.5 cm was used as the support of the internal adsorbent. The concentration of the red prodigiosin produced was estimated by measuring the absorbance at 535 nm using a double beam UV-visible spectrophotometer in acidified methanol (0.01 N HCl...
4 ml + methanol 96 ml) (Goldschmidt and Williams, 1968). The molecular mass of the pigment purified was determined using an electrospray-ionization mass spectrometer. The $^1$H- and $^{13}$C-NMR spectra of the pigment sample were recorded after dissolved in CDCl$_3$. The chemical shifts were referenced to an internal TMS (trimethylsilyl) signal. A FT-IR spectrum of the pigment was recorded (Song et al., 2006).

Prodigiosin from the environmental isolate 2170 was extracted from pigmented suspensions with acidic methanol (1 ml of 1N HCl: 24 ml of methanol) and then centrifuged (6,800xg for 15 minutes). The solvent of the supernatant was then evaporated under vacuum. Atmospheric pressure liquid chromatography of the extract was performed on silica gel with chloroform and methanol as solvents. The eluted fractions were pooled and the chloroform/methanol extract was vacuum evaporated, redissolved in H$_2$O and lyophilized. (Montaner et al., 2000). The isolated pigment was redissolved in methanol and analyzed by electrospray ionization mass spectrometry (ESI-MS) using a VG-Quattro triple quadrupole mass spectrometer. The isolated pigment was repurified by subsequent semipreparative HPLC. A Nucleosil C$_{18}$ reversed-phase column was used with a 0 % to 100 % linear gradient in 30 minutes (A: 10 mM ammonium acetate, pH 7.0, B: 100 % acetonitrile). Prodigiosin was purified from S. marcescens 2170 by methanol/HCl extraction followed by silica gel chromatography and semipreparative reverse-phase HPLC. ESI-MS gave a molecular weight of 323.4 Da, consistent with the expected value for prodigiosin (C$_{20}$H$_{25}$N$_3$O). The structure of prodigiosin was further confirmed by high-field $^1$H-NMR spectroscopy (Montaner et al., 2000).

The pigment produced by Serratia marcescens SMAR (an SpnR-defective isogenic mutant of S. marcescens SS-1 (Horng et al., 2002) was purified and characterized by NMR and mass spectrometry to determine its chemical structure (Wei and Chen, 2005). The pigment was extracted from the fermentation broth.
with methanol and then purified by using a hexane-balanced silica gel column to trap the target product within the column (Cang et al., 2000; Montaner et al., 2000). The loaded column was eluted with 10 M ethyl acetate to liberate the adsorbed product. The orange eluate was harvested and dried in a vacuum drier at 45°C to obtain the purified product (red powder). The pigment obtained in their study, reported as undecylprodigiosin based on the NMR and MS analyses, also known as prodigiosin 25-C, is one of the red pigment produced by Serratia sp. and Streptomyces sp. It possesses immunosuppressive and apoptosis-inducing activities similarly to prodigiosin, but has been less studied (Tomas et al., 2003).

2.7. Molecular biology and genetics

The genes for prodigiosin biosynthesis in Serratia lie in a large operon. The organization of the prodigiosin biosynthetic gene (pig) clusters in Serratia 39006 and in an S. marcescens strain, ATCC 274 (Sma 274) was reported (Cerdeno et al., 2001).

The Serratia 39006 pig gene cluster contains an additional gene, tentatively designated pigO. RT-PCR and primer extension has confirmed that there is transcriptional read through between pigN and pigO, consistent with pigO being part of the pig operon in that strain (Slater et al., 2003).

Many strains of Serratia marcescens produce pigment via a bifurcated pathway in which 2-methyl-3-amylpyrrole (MAP) and 4-methoxy-2, 2'-bipyrrrole-5-carboxyaldehyde (MBC) are enzymatically condensed into 2-methyl-3-amyl-6-methoxypyrroleprodigiosene, or prodigiosin. Several mutants of S. marcescens have been identified as being blocked in either the MAP or MBC pathway. However, little is known about the precursors accumulated by these mutants, and nothing is known about the enzymes or gene products involved (Williams and Qadri, 1980).
Dauenhauer et al., (1984) isolated a *Serratia marcescens* genomic clone capable of condensing the two prodigiosin precursors, MAP and MBC, to form prodigiosin. However, no sequence data were reported for this clone. The pig gene cluster from *Serratia* sp. ATCC 39006 (*Serratia 39006*) was expressed in *Erwinia carotovora* subsp. *carotovora* (Ecc; 25 out of 36 strains tested), though it was not expressed in several other members of the Enterobacteriaceae, including *E. coli* (Thomson et al., 2000). In *Serratia 39006* the production of prodigiosin is regulated by multiple factors, including a quorum-sensing system, via the LuxIR homologues, SmaI and SmaR (Slater et al., 2003; Thomson et al., 2000). Interestingly, prodigiosin production became N-(3-oxohexanoyl)-L-homoserine lactone (OHHL) dependent when expressed in Ecc, despite the latter producing a different signaling molecule from that made by *Serratia 39006* (Thomson et al., 2000).

Prodigiosin is an easily assayed secondary metabolite of *S. marcescens* and may be useful as a model system to study the mechanism of expression of secondary metabolites in bacteria. Isolation of recombinant molecules encoding the prodigiosin biosynthetic pathway would provide an approach to identifying gene products and understanding the enzymology and the genetics of prodigiosin biosynthesis. The isolation of DNA sequences encoding part of the prodigiosin biosynthetic pathway by use of a cosmid vector-*Escherichia coli* cloning system was reported (Dauenhauer et al., 1984).

A cosmid containing <35 kb of the *Serratia* chromosome encodes synthesis of the pigment in the heterologous host, *Erwinia carotovora*, demonstrating, for the first time, that the complete prodigiosin biosynthetic gene cluster had been cloned and functionally expressed (Thomson et al., 2000). Prior to this study the biosynthetic cluster of the related pigment undecylprodigiosin (Red),
produced by *Streptomyces coelicolor* A3(2), had also been cloned (Tsao *et al.*, 1985). Because *S. coelicolor* A3(2) Red2 mutants were able to cross-feed defined *S. marcescens* prodigiosin mutants (Feitelson and Hopwood, 1983), undecylprodigiosin is thought to be produced along a similar biosynthetic route to that proposed for the biosynthesis of prodigiosin by *S. marcescens*. The red biosynthetic cluster of *S. coelicolor* A3(2) was cloned from a cosmid based chromosomal library on a 35.7 kb chromosomal insert (Malpartida *et al.*, 1990) and is thought to comprise at least 18 genes (Coco *et al.*, 1991; Narva and Feitelson, 1990).

### 2.8. Application

Prodigiosin has several biological activities such as immunomodulatory, antibacterial, antifungal and antimalarial activities and so on (Lazaro *et al.*, 2002; Pandey *et al.*, 2003). It has been reported that prodigiosin could induce apoptosis in various kinds of cancer cells, such as haematopoietic, colorectal and gastric cancer cells. (Diaz-Ruiz *et al.*, 2001; Montaner *et al.*, 2000; Montaner and Perez-Tomas, 2001). However, the inhibitory effects of prodigiosin on metastasis and invasion, and the underlying mechanism have not been elucidated. The effects of prodigiosin isolated from *Hahella chejuensis* on the production of inflammatory cytokines and nitric oxide (NO) in lipopolysaccharide (LPS)-activated murine macrophage was studied (Huh *et al.*, 2007).

The cytotoxic properties of prodigiosins, tripyrrole red pigments, have been recognised for some times. Fullan *et al.*, (1977) observed the antitumour activity of prodigiosin in mice. Although apoptotic mechanisms of prodigiosins are still to be fully determined (additional *in vivo* assays are necessary), it is clear that prodigiosin are a new class of anticancer drugs, which hold out considerable
promise as a therapeutic agent (Tomas et al., 2003). List of some patents related to prodigiosin is given in Table 2.3.

Prodigiosins also possess anti-cancer properties (Perez-Tomas et al., 2003), with the methoxy group playing a critical role (Boger and Patel, 1988) and their anti-cancer effects have been observed in several human cancer cell lines *in vitro* (Campas et al., 2003; Diaz-Ruiz et al., 2001; Kawauchi et al., 1997; Montaner et al., 2000; Montaner and Perez-Tomas, 2001; Yamamoto et al., 2000a; Yamamoto et al., 2000b) and in human primary cancer cells (Campas et al., 2003) with no marked toxicity toward non-malignant cell lines (Montaner et al., 2000; Montaner and Perez-Tomas, 2001).

Prodigiosin induces apoptosis in certain cancer cells (Montaner et al., 2000; Montaner and Perez-Tomas, 2001; Perez-Tomas et al., 2003). Owing to these characteristics, prodigiosin may have potential for medical applications, for instance, it may be used to develop antitumor drugs (Furstner, 2003; Perez-Tomas et al., 2003). In light of its potential commercial values, there is a demand to develop high-throughput and cost effective bioprocesses for prodigiosin production. The molecular mechanisms of the antitumorigenic potential of prodigiosin are affected by multiple events, giving rise to apoptosis (Soto-Cerrato et al., 2007a).

Prodigiosins and synthetic derivatives have been shown to have potent and specific immunosuppressive activity, with novel targets clearly distinct from other drugs (D’Alessio et al., 2000; Mortellaro et al., 1999; Tsuji et al., 1990). Montaner et al. (2000) showed that prodigiosin extracted from *S. marcescens* could induce apoptosis in haematopoietic cancer cell lines including acute T-cell leukaemia, myeloma and Burkitt’s lymphoma, with little effect on non-malignant cell lines. Prodigiosin has also been shown to induce apoptosis in human primary cancer cells.
in the case of B and T cells in B-cell chronic lymphocytic leukaemia samples (Campas et al., 2003). The anticancer agent prodigiosin has been shown to act as an efficient immunosuppressant, eliciting cell cycle arrest at non-cytotoxic concentrations, and potent proapoptotic and antimetastatic effects at higher concentrations (Soto-Cerrato et al., 2007b).

The apoptotic effects of prodigiosin have been observed in several human cancer cell lines in tissue culture (Azuma et al., 2000; Yamamoto et al., 2001; Yamamoto et al., 2000a; Yamamoto et al., 2000b), in hepatocellular carcinoma xenografts (Yamamoto et al., 1999). Prodigiosin was found to be a potent anticancer agent which could induce apoptosis of several cancer cell lines in vitro including haematopoietic cancer cells, colon cancer cells, B-cell and chronic lymphocytic leukemia cells (Campas et al., 2003).

Furthermore, several pharmaceutically relevant prodigiosins (PGs) such as undecylprodigiosin, metacyclop prodigiosin, roseophilin and nonylprodigiosin, in addition to prodigiosin, are thought to have potential for antibacterial, antimalarial, anticancer, cytotoxic and immunosuppressive activities (D’Alessio et al., 2000; Diaz-Ruiz et al., 2001; Han et al., 1998; Montaner and Perez-Tomas, 2001).
Table 2.3. Prodigiosin patents

<table>
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<tr>
<th>Patent No</th>
<th>Abstract</th>
<th>Inventors</th>
<th>Published date</th>
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<tr>
<td>Patent 6638968</td>
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<tr>
<td>United States</td>
<td>The prodigiosin from <em>Serratia marcescens</em> is useful as an immunosuppressive in various fields, including the treatment of the diseases requiring immunosuppression and the basic research for the diseases, the transplantation of the organs or tissues, and the immune cells. Link: <a href="http://www.freepatentsonline.com/6645962.html">http://www.freepatentsonline.com/6645962.html</a></td>
<td>Kim, Hwanmook (Taejon, KR), Kim, Youngkook (Taejon, KR), Han, Sangbae (Choongcheongbuk-do, KR), Yoo, Sungrak (Seoul, KR)</td>
<td>11/11/2003</td>
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<td>Patent 6645962</td>
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United States Prodigiosin, an antibiotic, is effectively produced by culturing a novel Serratia marcescens R-2 strain. A synthetic culture medium is also provided, which contains a higher fatty acid having 12 to 18 carbon atoms, a salt thereof or an ester thereof as the sole or main source of carbon and in which a strain of Serratia marcescens having the abilities to assimilate the source of carbon and to produce prodigiosin can be cultivated to obtain prodigiosin. Link: http://www.freepatentsonline.com/4266028.html

United States The invention relates to novel use of prodigiosin for the treatment of Rheumatic arthritis and can provide excellent treatment effect to Rheumatic arthritis by treating composition including prodigiosin isolated from Serratia marcescens as an active principle to DBA-1 mouse of collagen-induced Rheumatic arthritis animal model and thereby inhibiting production of internal cytokine which is a important pathogen of Rheumatic arthritis. Link: http://www.freepatentsonline.com/20040127547.html

2.9. Prodigiosin from marine bacteria

The marine microorganisms are yet to be tapped as a source of prodigiosin in spite of few reports. *Serratia marinorubra*, a species producing prodigiosin isolated originally from Pacific Ocean water was described by ZoBell and Upham, (1944). The purple pigment in *Serratia marinorubra* was easily extracted from the bacterial biomass with ethanol (Courington and Goodwin, 1955). In acidified ethyl ether it is purplish-pink, and in alkaline ether yellowish-brown; the absorption spectra of these two forms were identical with those of prodigiosin (Hubbard and Rimington, 1950). The pigment produced by two aerobic, Gram negative, rod-shaped bacteria, was compared morphologically and physiologically with *Serratia* sp., was shown to be similar to prodigiosin, the red pigment of *S. marcescens* (Lewis and Corpe, 1964).

The red pigment of *Vibrio psychroerythrus* (formerly marine psychrophile NRC 1004) was identified as prodigiosin by comparison of its mass spectrum, absorption spectrum in the visible range, and chromatographic behavior with prodigiosin isolated from *Serratia marcescens* (D’ Aoust and Gerber, 1974). Kim et al. (2007) reported that the main red-metabolite of *Hahella chejuensis* KCTC 2396 as prodigiosin. *Serratia rubidaea* N-1, which was isolated from the Ariake Sea, a bay located in the Kyusyu region, Japan, is a producer of red pigment prodigiosin (Yamazaki et al., 2006).

Three strains of red, antibiotic-producing marine bacteria, which are commonly found in Mediterranean coastal waters during the autumn months (Gauthier et al., 1975), were described and named *Alteromonas rubra* (Gauthier, 1976). The absorption spectra at pH 4.0 and 9.0 of ethanolic extracts of the red pigments resembled closely those of a similar *S. marcescens* extract. However, high pressure liquid chromatography of chloroform methanol extracts showed
considerable heterogeneity for each extract, with some similarities and some differences in peak positions. Thus, the marine pigments were judged to be similar, but not identical, to the “prodigiosin” of \textit{S. marcescens} (Gauthier, 1976).

2.10. Indian Scenario

In general, the scenario on research on microbial pigments at national level is limited to very few reports over the years. The role of pigment Xanthomonadin produced by the genus \textit{Xanthomonas} against photodamage was reported (Rajagopal \textit{et al.}, 1997). Barbhaiya and Rao, (1985a) optimized the nutritional requirements for pyoverdine production by \textit{Pseudomonas aeruginosa}. Studies on a new generation biocolours from \textit{Monascus} sp. and related fungi for use as a colourant to the foods was reported (Tamilselvan, 2001). Cellulose acetate membrane was used for the purification and concentration of natural pigments (Chaudhuri \textit{et al.}, 2004). A maroon dye was extracted from the rhizome of \textit{Arnebia nobilis} (Indrayan \textit{et al.}, 2004). Effect of hexoses and di-hexoses on the growth, morphology and pigment synthesis in the transformed root cultures of red beet was studied (Ravishankar, 2004). A novel medium for enhanced cell growth and production of prodigiosin from \textit{Serratia marcescens}, isolated from soil was optimized (Giri \textit{et al.}, 2004).