

# Chapter 12

## Holistic Approaches for Enhanced Production of Prodigiosin—a Natural Biocolour



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**Abstract** Nowadays, pigments from microbial sources have gained much importance due to its substantial medical and industrial welfares. In this regard, the present book chapter discusses some impactful methodologies regarding the improved production of prodigiosin drug—a promising supplement of synthetic colour. Prodigiosin is a vivid red colour pigment mainly synthesized by bacteria *Serratia marcescens*. Other than its significant contribution towards the cosmetic and food industry, it also poses antifungal, antibacterial, immunosuppressive and antiproliferative activities. Optimization of media components, scale-up study and biokinetic analysis from wild type and recombinant sources have been thoroughly discussed in this chapter. Particular emphasis was put forward on its nanotechnological synthesis and drug delivery for various diseases. Efficient extraction practice, estimation by different analytical instruments and purification of the final product were also highlighted in a best possible way.

**Keywords** Prodigiosin · Bioengineering · Optimization · *Serratia marcescens* · Enhance

### 12.1 Introduction

Supplementation of natural ingredients in the food industry is a need of the hour due to excessive toxicity of synthetic pigments and organic dyes. The biocolour which are readily accessible from plants have numerous shortcomings due to light sensitivity or unsteadiness, temperature or adverse pH, insoluble or minimal water solubility and most importantly unavailability all over the year (Gulani et al. 2012). Less water solubility is sometimes responsible for application limitation in

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the textile dye industry (Shen and Yang 2013). The microbial pigments being idiolites having a differentiated composition according to their assembly and perhaps accredited to the environmentally harmless compounds in the chemical industry. Creation of an innovative product by developing a unique chromophore is a patentable and novel aspect in biotechnology. Considering all the inadequacies of synthetic, organic and plant-based pigments available in the market, researchers are now focusing on microbial-based biocolour, which could have a broad spectrum of activities. Prodigiosin ( $C_{20}H_{25}N_3O$ ) is one of them. It is a membrane linked red pigment as well as a bioactive secondary metabolite, synthesized by both Gram positive and Gram negative bacteria like *Serratia marcescens*, *Streptomyces coelicolor*, *S. lividans*, *Pseudomonas magnesorubra*, *Vibrio psychroerythrous*, etc. (Khanafari et al. 2006; Shahitha and Poornima 2012; Kurbanoglu et al. 2015). *Serratia marcescens* is the largest producer of this vital pigment worldwide. Being an opportunistic human pathogen, frequency of spreading infection by pigmented *S. marcescens* is much lesser than non-pigmented one (Elkenawy et al. 2017). Prodigiosin is commercially exploited for several industrial applications such as antimicrobial, anti-oxidant, anti-malarial, anti-cancer, anti-protozoal, anti-neoplastic, anti-diabetic, non-steroidal, immunosuppressive, UV protective and antiproliferative activity (Khanafari et al. 2006; Borić et al. 2011; Lee et al. 2011; Montaner and Pérez-Tomás 2001; Park et al. 2012; Soto-Cerrato et al. 2004; Gulani et al. 2012). The same can also act as a bacteriostatic agent against *E. coli* (Danevčič et al. 2016). There are some isoforms of prodigiosin available in the literature, i.e. undecylprodigiosin, metacycloprodigiosin, nonylprodigiosin, norprodigiosin having the same clinical applications (Zang et al. 2014). It also plays a vital role in bacterial cells by acting as a protective cover during stress by reducing the fabrication of reactive oxygen species (ROS) (Gul et al. 2020).

With the growing solicitation of prodigiosin, reducing its manufacturing cost has become one of the most significant targets. It is very crucial to note that several aspects are still needed to be concerned about the large-scale production of prodigiosin. Moreover, the application of high-throughput technology and complex instrumentations with higher operating costs may, in turn, limit their commercialization and industrialization, especially in the third world countries. To develop efficient techniques for prodigiosin production, significant research has been directed towards strain improvement and the identification of dynamic systems and process conditions.

Improving the yield of prodigiosin cost-effectively is one of the critical challenges for its commercial viability. Recovery of prodigiosin from microorganism is a complex procedure. Therefore, enhancing the downstream recovery of the product with low processing cost is another challenge for the bioengineers (Acuña-Argüelles et al. 1995). Microbial prodigiosin production is inducible, and hence the yield could be considerably enhanced with the addition of inducers.

Pilot-scale production and solicitations of prodigiosin in developing countries do not seem to flourish dramatically due to excessive capital investment for industrial applications. Considering the current market demand, implementation of this potent biocolour is projected to increase logarithmically within the next few decades.

Considering the current mandate, usefulness and cost-effectiveness of the prodigiosin in the industry, it is imperative to focus on its enhanced yield using several existing tools of biotechnology. It will surely amplify the applications of prodigiosin in several industries, manufacturing processes and services for economic mobility through biotechnology and bioengineering.

The present book chapter attempts to address the knowledge gap surrounding efficient prodigiosin production by fermentation, nanobiotechnology and other essential methods for its effective commercialization. Bioengineering aspects that have been taken into consideration for its enhanced yield will also be covered in this chapter.

## 12.2 Natural Occurrence and Recombinant Sources of Prodigiosin

Prodigiosin is a naturally occurring pigment that can be obtained from both plants as well as microorganisms. Terrestrial plants are the primary sources of this pigment. There are numerous advantages for selecting microorganisms over plant sources for extracting prodigiosin. Some of the benefits like they are stable, their accessible cultivation technology, can be biosynthesized any time in the lab, a concise doubling time of bacteria thus fast specific growth rate, cost-effective culture media, optimal environmental parameters and their production is independent of weather condition (Chidambaram and Perumalsa 2009; Gulani et al. 2012). The pigment-producing microorganisms are found everywhere like air, water, soil, sewage and even in some household animals (Su et al. 2011). Nguyen et al. (2020) recently discovered that prodigiosin could also be synthesized by marine chitin (Nguyen et al. 2020). Among different chitin molecules,  $\alpha$  chitin is the potent producer of prodigiosin by fermentation. However, *Serratia marcescens* is the dominant producer of this pigment to date; its large-scale production can be trimmed due to its durable infectious nature. On the contrary, the actinomycetes group of organisms, viz. *Streptomyces* spp. are on the safer side. Optimization and large-scale production can be easily carried out by these species (Luti and Mavituna 2011). Among the *Streptomyces* sp. *Streptomyces coelicolor* A3(2) have been reported to be capable of producing prodigiosin pigment (Hobbs et al. 1990). Besides *S. marcescens*, other extremophiles like *Hahellacea* and *Pseudoalteromonas* are also capable of synthesizing prodigiosin (Kim et al. 2007; Schloss et al. 2010).

Massive bioengineering studies were attempted to amplify the yield of prodigiosin by modifying the parent strain. Researchers have used classical mutational techniques along with molecular biology approaches to enhance the yield of this antibiotic. Prodigiosin biosynthesis genes from *S. marcescens* were randomly integrated into *Pseudomonas putida* KT2440 for making a potent strain (Domröse et al. 2015). Often, an isogenic mutant strain of *S. marcescens* SS-1 was also used for producing prodigiosin resembling pigments (Wei and Chen 2005).

## 12.3 Nanotechnological Aspects of Prodigiosin Synthesis

Nanobiotechnology is a rapidly growing field in medical science for its unique and promising features. 'Nano' ( $10^{-9}$ ) is the smallest size that exists in the world having the capability of reflecting light. Nanoparticle or nanomaterials consist of a cluster of atoms in the range of 1–100 nm (Karthika et al. 2015; Al-Shabib et al. 2018; Prasad et al. 2016). They are mainly of two types, i.e. organic and inorganic. Inorganic nanoparticles have vast applications in the medical devices like biosensors; in environmental sample purification, in the pharmaceutical industry and also they can act as antimicrobials which can be integrated into optical fibres (Karthika et al. 2015; Prasad et al. 2017a,b, 2018a,b, 2020). Their potential biocidal activity is due to tiny size and relatively higher surface to volume ratio (Al-Shabib et al. 2018; Morones et al. 2005). Among inorganic nanoparticles, gold (AuNPs) and silver nanoparticles (AgNPs) have higher material property, functional diversity and stable in harsh environmental conditions (Karthika et al. 2015). Metal oxides can also be used as nanomaterial, i.e. Zinc oxides (ZnO). ZnO nanoparticles (ZnO-NPs) exhibit broad-spectrum antibacterial activity (Al-Shabib et al. 2018; Bhuyan et al. 2015). Prodigiosin driven biosynthesis of silver nanoparticles, conjugated gold/prodigiosin synthesis, its characterization and application in breast cancer will be highlighted in this chapter.

### 12.3.1 Prodigiosin Mediated Biosynthesis of Silver Nanoparticles (AgNPs)

Karthika et al. (2015) synthesized silver nanoparticles using culture supernatant containing prodigiosin from the bacterial strain of *S. marcescens* (Karthika et al. 2015). In brief, culture supernatant and 0.1 (M)  $\text{AgNO}_3$  solution (equal volume) were mixed and incubated at  $60^\circ\text{C}$  until the colour change was observed from pink to black. Reduction of  $\text{Ag}^+$  was confirmed by the colour change and subsequently indicated the synthesis of silver nanoparticle. The resulting mixture was separated, followed by repeated washing and centrifugation at  $10000\times g$  for 15 mins. The final suspension was dried and ready to use for further study. AgNPs were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), scanning and transmission electron microscopy (SEM and TEM). Antibacterial activity against *Staphylococcus* sp., *E. coli* and *Pseudomonas* sp. was examined and observed by disc diffusion method. TEM images confirmed the spherical shape of AgNP and size less than 100 nm. Due to their tiny shape and size, AgNPs can easily reach to the genomic content of the bacteria, have the accessibility of the large surface area and subsequently destroy them.

### 12.3.2 *Biosynthesis of Gold/prodigiosin Nanoparticles (AuNPs) and Its Application*

Gold nanoparticles also have prospective applications in drug delivery, tissue analysis and in gene transfer (Dozie-Nwachukwu et al. 2017b). Dozie-Nwachukwu et al. (2017) biosynthesized gold/prodigiosin nanoparticles (AuNPs) from *Serratia marcescens* strain to combat triple-negative breast cancer (MDA-MB-231) cells (Dozie-Nwachukwu et al. 2017b; Dozie-Nwachukwu et al. 2017a). To target a specific cancer cell, AuNPs/PG drug was prepared under diverse pH environments.

Prodigiosin from *S. marcescens* was extracted and purified using size exclusion chromatography before nanomaterial synthesis. Unlike silver nanoparticle, AuNP/PG conjugate was prepared through physisorption, where particles were held together by weak Vander-walls force (Dozie-Nwachukwu et al. 2017a). Here, 1 mg of solid prodigiosin was dissolved in 2 ml of methanol and mixed to the gold nanoparticles conjugate. The resulting mixture was agitated at 500 rpm at 4°C for 30 min. AuNPs were characterized by UV-spectrophotometry, electron diffraction (ED), dynamic light scattering (DLS) and energy-dispersive X-ray spectroscopy (EDS). The adhesion between luteinizing hormone-releasing hormone (LHRH)-conjugated AuNP/PG drug and MDA-MB-231 breast cancer cells (nanocluster) was studied by atomic force microscopy (AFM) technique. It was observed that the adhesion force was higher in nanocluster than healthy breast cells. Greater adhesion leads to 5 fold increase overexpression/interaction between ligand and receptors on the membrane surface, which is very important to treat a specific target of triple-negative breast cancer.

In another study, cell-free extracts of prodigiosin solution was allowed to react with 2.5 mM  $\text{HAuCl}_4$  (tetrachloroauric acid) to prepare gold/prodigiosin nanoparticle (Dozie-Nwachukwu et al. 2017b). The formation of nanoparticle was confirmed by colour change (bright red to pale pink). Such colour change occurred due to reduction of  $\text{HAuCl}_4$  to  $\text{Au}^0$  and the possible reaction is as follows,  $\text{HAuCl}_4 + 3e^- \rightarrow \text{Au} + 4\text{Cl}^- + \text{H}^+$ . However, the total colour change was observed after 6<sup>th</sup> day of incubation. Reduction of  $\text{HAuCl}_4$  to  $\text{Au}^0$  results from supersaturation solution and nucleation. Different analytical instruments were used to characterize gold nanoparticles. The reduction of  $\text{Au}^{3+}$  to  $\text{Au}^0$  was observed using UV–vis spectrophotometer. The size and shape of nanoparticle was determined by TEM, higher resolution image of particle surface was taken with the help of Helium Ion Microscopy (HIM), and the lower concentration of AuNPs was detected with the help of DLS (Dozie-Nwachukwu et al. 2017b). Both biomass and cell-free extracts of prodigiosin were used for nanoparticle synthesis. Still, the cell-free extract is a better choice for lower reaction time. Final nanoparticle size was around 40–60 nm which is very efficient for cancer detection and treatment.

### 12.3.3 *Biofabrication of Zinc Oxide Nanoparticle*

Al-Shabib et al. (2018) synthesized zinc nanoparticles (ZnNPs) from leaf extract of *Ochradenus baccatus* (Al-Shabib et al. 2018). Zinc metal oxides (ZnO-NPs) were the nanoparticle of choice due to their broad-spectrum bactericidal activity, protein binding study, etc. 0.05 (M) Zinc nitrate solution and *Ochradenus baccatus* leaf extract were mixed and microwaved for 20 min. After cooling down, the precipitate was parted by centrifugation, followed by washing with de-ionized water and 100% C<sub>2</sub>H<sub>5</sub>OH. After that, they were allowed to dry at 80<sup>0</sup>C for 24 hr. Lastly, the product was calcined at 800<sup>0</sup>C for 2 hr. X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and EDX were used for ZnO-NPs characterization.

Effect of zinc oxide nanoparticle was analysed against prodigiosin synthesized by *S. marcescens* culture. Decrease of prodigiosin yield was documented at sub-MIC level ranging between 6.25 µg/ml and 50 µg/ml. It was thought that inhibition of prodigiosin would decrease the pathogenicity and virulence characteristics of *S. marcescens*.

Not only nanoparticles, nanogel is also an excellent choice to treat breast cancer cells (Obayemi et al. 2016b). Crosslinking between dextran and poly(lactide) was proved to be an effective conventional technique for preparing nanogel. It is injectable, multifunctional and biodegradable. The same was loaded with prodigiosin and was very effective against breast cancer cells.

## 12.4 Analytical Methods for Extraction, Estimation and Purification of Prodigiosin

Prodigiosin is principally an intracellular metabolite. It is bound to the cell membrane. During fermentation, only 17% of the total prodigiosin is released into the broth (Wang et al. 2004). Hence, an effective extraction method is necessary to recover the product. Well-defined separation and purification techniques are hugely recommended for its clinical application too. In this chapter, various analytical methods, i.e. extraction, estimation and purification of prodigiosin, will be discussed.

### 12.4.1 *Extraction of Prodigiosin*

More or less similar methods were adopted by the researchers to extract the prodigiosin from the culture broth. The two-step extraction procedure was followed for the efficient recovery of prodigiosin (Kurbanoglu et al. 2015; Williams 1973; Lin et al. 2019). For this purpose, culture broth (5 to 10 ml) from the stationary phase

was taken in a test tube and centrifuged at 10000 rpm for 10 minutes to collect the cell pellet. After that, the supernatant was decanted, and acidified methanol was poured to the cell suspension. The mixture is vortexed and centrifuged under the same condition. Finally, the concentration of the prodigiosin was measured by spectrophotometer at 535 nm (Venil and Lakshmanaperumalsamy 2009; Kurbanoglu et al. 2015). Some researchers have used acetone in place of MeOH for its active recovery (Shahitha and Poornima 2012). Su et al. (2011) used de-ionized water for washing the cell pellet prior to adding acetone (Su et al. 2011). The crude pigment was obtained by concentrating it using a rotary evaporator (Lin et al. 2019). Wei and Chen (2005) extracted prodigiosin like pigment from the recombinant strain of *Serratia marcescens* SMΔR. They have used 3 (M) Chloroform to extract the pigment (Wei and Chen 2005). Park et al. (2012) opted nine organic solvents to find out the optimal and best solvent on the basis of extraction efficiency (Park et al. 2012). They found ethanol as the best solvent, whereas acetone was optimum for prodigiosin extraction from fermentation broth. However, by using acetone, the extraction process was 140 times faster than ethanol under the same conditions. Sometimes, a proper surfactant (Tween 80; 0.1% w/v) is useful for extracting prodigiosin from the bacterial cell envelope (Wang et al. 2004). Wang et al. (2004) have developed an efficient technique for in-situ recovery of prodigiosin from direct fermentation broth (Wang et al. 2004).

### 12.4.2 Estimation of Prodigiosin

There are different methods available for estimating prodigiosin after extraction from fermentation broth. Its concentration can be determined spectrophotometrically as well as by Liquid Chromatography (LC) technique.  $\lambda_{\max}$  for prodigiosin is around 535 nm (Elkenawy et al. 2017). Its concentration can be calculated by taking its specific absorbancy of  $51.5 \times 10^3$  litre per g per cm (Williams 1973). Haddix and Werner (2000) developed one formula to quantify the prodigiosin yield (Haddix and Werner 2000). The same has become one of the most useful equation to estimate prodigiosin concentration. It is shown below: Prodigiosin unit/cell =  $\frac{[(OD_{499} - (1.381 \times OD_{620}))] \times 1000}{OD_{620}}$ ; whereas  $OD_{499}$  = is prodigiosin absorption,  $OD_{620}$  = bacterial culture absorption and 1.381 is  $\frac{OD_{499}}{OD_{620}}$  quotient and taken as a constant. Williams et al. (1961) and Chen et al. (1993) added an alternative recipe to calculate the total prodigiosin (TP) yield, i.e. Total prodigiosin (mg/L) =  $\frac{ADV_1}{7.07 \times 10^4 V_2}$ ; whereas A = Absorbance at 535 nm, D = Dilution ratio,  $V_1$  = volume of methanol added,  $V_2$  = amount of fermentative culture and  $7.07 \times 10^4$  is extinction coefficient of the antibiotic (Williams et al. 1961; Chen and Johns 1993). Lee et al. (2011) assayed the bioactivity of prodigiosin and cycloprodigiosin by *Agar Disc Diffusion* (ADD) method (Lee et al. 2011). They have checked the antimicrobial activity against different microorganisms like *Bacillus subtilis* KCTC 1914, *Escherichia coli* KCTC 1924, *Salmonella enterica* serovar Typhimurium KCTC 1926,

*Staphylococcus aureus* KCTC 1916 and *Candida albicans* KCTC 1940. Filter discs were poured with pure prodigiosin extract, and inhibition zones were recorded against test organisms. Finally, the concentration of the antibiotic was measured by measuring the zone diameter. ADD is a very primitive method for analysing the unknown concentration of many antibiotics, i.e. rapamycin, cyclosporine, etc. (Kojima et al. 1995; Lee et al. 1997; Dutta et al. 2014; Dutta et al. 2017).

### 12.4.3 Purification and Characterization

Many scientists are working on a laboratory scale for the production, purification and characterization of prodigiosin. However, the research terminates at the lab scale with a few progressing beyond it. This is due to problems associated with scale-up and downstream of the production process. LC-MS, thin-layer chromatography (TLC), HPLC, NMR, FTIR, etc., are the methods used for prodigiosin stability, purification and successive characterization study (Aruldass et al. 2014; Park et al. 2012; Wei and Chen 2005; Faraag et al. 2017; Rakh et al. 2017). Crude prodigiosin is purified by a silica gel column (Lin et al. 2019). In brief, crude prodigiosin extract was dissolved in methanol, and the subsequent mixture was allowed to pass through a hexane balanced silica gel packed column. The absorbed product was then eluted out from the column with the help of ethyl acetate. Finally, the orange colour eluate was dried in a rotary evaporator to obtain the red prodigiosin powder (Wei and Chen 2005). Wang et al. (2004) have developed an efficient technique by combining both static and column adsorption process for prodigiosin purification (Wang et al. 2004). They finally obtained 83% higher yield than conventional silica gel method.

Darshan and Manonmani (2013) determined the molecular mass of prodigiosin by LC-Mass spectroscopy (MS) technique as 324 D (Darshan and Manonmani 2013) and the structure was further confirmed by <sup>1</sup>H-NMR spectroscopy. Rakh et al. (2017) also characterized the chemical structure of purified prodigiosin by FTIR method (Rakh et al. 2017). Fourier transform mass spectrometry (FT-MS) technique was used for structural elucidation of prodigiosin like pigments produced by *Vibrio* sp. (Alihosseini et al. 2008). Characterization of prodigiosin by *Zooshikella rubidus* S1-1 was analysed by tandem mass spectroscopy (LC-MS/MS) (Lee et al. 2011). Researchers have identified two major peaks with 'm/z' value of 322 and 324, respectively. Later they have concluded that two peaks were of cycloprodigiosin and prodigiosin.



## 12.5 Bioengineering Strategies Adopted for the Enhanced Yield of Prodigiosin

It was observed that there are two policies adopted to date for microbial prodigiosin production. It was either produced by wild type culture or recombinant or mutant microbial strain. The critical challenges behind using wild type microorganism include the availability of potent microbial strain and the solicitation of this biocatalyst for industrial-level production. Furthermore, isolating a dominant strain of anticipated product is tiresome and time-consuming. Hence, the robust approach will be to generate a mutant strain having the capability of producing a large amount of prodigiosin with the help of available biotechnological tools.

### 12.5.1 Contribution of Influential Parameters

Ryazantseva and Andreyeva (2014) experimented with *Serratia marcescens* 9986 for production of prodigiosin. It was reported that growth parameters controlled the synthesis of pigment. Prodigiosin yield was found to be 0.2–0.4 mg/L of culture medium in a batch process under aerobic environments. 0.1% of sodium dodecyl sulphate (SDS) was treated in biomass for extraction of prodigiosin (Ryazantseva and Andreyeva 2014). Locally isolated pigment-producing strain *Serratia marcescens* UMT-1 was used by Aruldass et al. (2014) for enhanced prodigiosin yield (Aruldass et al. 2014). They have used brown sugar as a nutrient for the development of bacteria. It was reported that not only culture conditions but also lactose and L-tryptophan supplementation were necessary for higher production of prodigiosin in both shake flask and 5-L bioreactor. The final output of prodigiosin was found to be 8000 mg/L. The study revealed that brown sugar could be used as cheap and potential media constituents for prodigiosin production. In another study, an isolated bacterial culture *Serratia marcescens* N10612 was used for the production of prodigiosin. In this experiment, a mixed culture media consisting of sucrose, peptone and yeast extract was used for maximum prodigiosin production. Media optimization was carried out through statistical experimental designs for maximum yield. The optimum concentrations of medium were yeast extract 5.6 g/L, peptone 6.9 g/L, sucrose 31.6 g/L and NaCl 1.0 g/L (Zang et al. 2014). Nakamura and Kitamura (1981) patented their research work on prodigiosin production from a novel *Serratia marcescens* R-2 strain. A fatty acid having 12 to 18 carbon atoms was employed as a sole carbon source in simulated culture media. It was observed that *Serratia marcescens* could assimilate the carbon and produce prodigiosin (Nakamura and Kitamura 1981). In another patent, production of prodigiosin was carried out in fermentor having media composition of 3% sorbitol, 1.5% soy flour, and 0.125% of  $\text{Mg}(\text{SO}_4)_2$ . The fermentation media was inoculated with a rough, nonmucoid strain of *Serratia marcescens*. The culture was transferred to a sizeable non-agitated fermenter having the capacity of 100 gallons and grown for 16 hours at

28°C. The antifoaming agent used was 0.3% solution of octadecanol in mineral oil (Harned 1953).

In India, most of the works on prodigiosin production have been focused on either submerged fermentation where the pre-formulated synthetic medium was employed. Sundaramoorthy et al. (2009) investigated the production of prodigiosin from *Serratia marcescens*. Prodigiosin was characterized and reported that it has antifungal, immunosuppressive and antiproliferative activity. In this investigation, the factors, viz., temperature, pH, sugar and oil substrate were found to be the key parameters for prodigiosin production. Further, the optimization of influential factors was carried out to enhance the prodigiosin yield. It was also noticed that the maximum amount of prodigiosin was produced at a temperature of 30°C and pH 7.0. Besides, the maltose was found to be most suitable carbon source in the medium and yielded 425±40 mg/L of prodigiosin. It was also found that peanut oil considered to be the most appropriate oil substrate for production of prodigiosin (535±45 mg/L) (Sundaramoorthy et al. 2009).

### 12.5.2 Scale-up Study of Prodigiosin

Few scale-up studies were carried out for prodigiosin production by *Serratia marcescens* strain. There is a clear difference between scale-up and large-scale production of any metabolite. The scale-up study requires geometrical similarity, constant  $K_{La}$  (volumetric mass transfer coefficient), constant impeller tip speed ( $N_i$ ) as well as constant H:D (H= height of the tank and D= tank diameter) ratio of the reactor. However, large-scale production of prodigiosin has been achieved by increasing the yield of metabolite through mutation, synthetic biology approach as well as molecular technique obviously from a small shake flask to reactor. Lack of scale-up study leads to higher in vivo experiment expenditure as well as an expensive downstream process. Naik et al. (2012) used peanut oil cake (POC) as a cheap substrate for improved prodigiosin production by *Serratia marcescens* CF-53 strain (Naik et al. 2012). They have also carried out scale-up study in a lab-scale 2 L bioreactor at temperature 30°C, airflow rate of 2 vvm, agitation 200 rpm and pH 7. 40 mg/L prodigiosin yield was finally achieved. Chen et al. (2013) reported that they had increased the prodigiosin yield by 6.78 fold using statistical optimization and porous carrier addition strategy (Chen et al. 2013). *Serratia marcescens* C3 used in the study was a quorum sensing strain. A fractional factorial design was adopted for statistical analysis with four significant factors, i.e.  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 4\text{H}_2\text{O}$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ . After optimization, around 7.05 g/L prodigiosin was obtained. Finally, they carried out immobilization study by adding calcium alginate beads as a porous carrier. Yield significantly increased from 7.05 g/L to 15.6 g/L. Incorporation of physical (UV ray) and chemical (EMS) mutagen for enhanced prodigiosin production was achieved by El-Bialy and El-Nour (2015) (El-Bialy and El-Nour 2015). The potent chemical mutant  $S_{26}$  strain of *Serratia*

*marcescens* produced eightfold higher prodigiosin than that of parent culture. The said mutated culture was stable up to 80°C and at alkaline pH.

### 12.5.3 Biokinetic Study

Prodigiosin is an idiolite. Its production mainly occurs during the transient of exponential phase and throughout the whole stationary period of bacterial growth. Hence, it is a non-growth associated product. A robust and deliberate kinetics study is very much necessary to observe the growth pattern of microorganism with its product formation strategy in the presence of different substrates available (Dutta et al. 2014). It is also very much crucial to develop and establish a proper mathematical model that can describe the kinetic behaviour of the organism easily.

Very few kinetics studies have been carried out by different researchers for prodigiosin production using diverse strains of *Serratia marcescens* in a lab-scale bioreactor (Mohammed and Luti 2020; Casullo de Araújo et al. 2010; Hobbs et al. 1990). Mohammed and Luti (2020) carried out the growth kinetics study of *S. marcescens* in a 7 L bioreactor using optimized media condition (Mohammed and Luti 2020). Preliminary they have optimized the culture media constituents, i.e. C/N ratio, inoculum size, etc., by Response Surface Methodology (RSM) based Central Composite Design (CCD) technique. It is globally used fractional factorial method for optimization studies. Cell growth kinetics calculation was based on dry cell weight (DCW), and product formation kinetics followed the Luedeking–Piret model. All the simulations were carried out by polymath 6 software. Biokinetic parameters were determined from logistic equation model. Stoichiometric and kinetic variables are shown in Table 12.1. Finally, they have achieved the maximum cell mass and prodigiosin yield of 14.4 mg/mL and 594.88 mg/L, respectively. Casullo de Araújo et al. (2010) carried out the kinetics study of prodigiosin production by *Serratia marcescens* UCP1459 using ‘*manipueira*’ (cassava wastewater) supplemented with 2% mannitol as a renewable low-cost substrate (Casullo de Araújo et al. 2010). They were able to produce a very high level of prodigiosin, i.e. 43,000 mg/L in just 48 hr after fermentation. The specific growth rate ( $\mu$ ) and generation time ( $T_d$ ) were found to be 0.36 h<sup>-1</sup> and 1.88 hr. During kinetics study, they observed diauxic pattern of growth at 12 hr of fermentation. Williams (1973) have compared prodigiosin kinetics study from two different strains of *Serratia marcescens*, one is wild type and another is mutant (Williams 1973). He found that maximum prodigiosin production was achieved after 5 days of incubation apparently when the culture is in mid-stationary phase. From this study it has been again proved that prodigiosin is a secondary metabolite. Similar kinetics study was carried out for prodigiosin like pigments, i.e. actinorhodin and undecylprodigiosin by *Streptomyces coelicolor* A3(2) (Hobbs et al. 1990). Unlike prodigiosin, undecylprodigiosin is a growth-associated product, whereas actinorhodin was found to be a non-growth associated.

**Table 12.1** Biokinetic parameters obtained from different study

Kinetic parameters	Microorganism	Reaction vessel	Experimental value	Reference
$X_{\max}$ (mg/ml)	<i>S. marcescens</i>	7 L bioreactor	14.42	(Mohammed and Luti 2020)
$P_{\max}$ (mg/L)	<i>S. marcescens</i>	7 L bioreactor	594.8	(Mohammed and Luti 2020)
$Y_{x/s}$ (g/g)	<i>S. marcescens</i>	7 L bioreactor	2.8	(Mohammed and Luti 2020)
$Y_{p/s}$ (mg/g)	<i>S. marcescens</i>	7 L bioreactor	129.1	(Mohammed and Luti 2020)
$Y_{p/x}$ (mg/g)	<i>S. marcescens</i>	7 L bioreactor	45.7	(Mohammed and Luti 2020)
$q_p$ (mg <sub>prod</sub> /g <sub>cell</sub> .h)	<i>S. marcescens</i>	7 L bioreactor	0.79	(Mohammed and Luti 2020)
$\mu_{\max}$ (h <sup>-1</sup> )	<i>S. marcescens</i>	7 L bioreactor	0.0605	(Mohammed and Luti 2020)
	<i>S. marcescens</i> UCP1459	Shake flask	0.36	(Casullo de Araújo et al. 2010)
Td (hr.)	<i>S. marcescens</i>	7 L bioreactor	11.45	(Mohammed and Luti 2020)
	<i>S. marcescens</i> UCP1459	Shake flask	1.88	(Casullo de Araújo et al. 2010)

In another study, it was found that prodigiosin production is related to ATP synthesis (Haddix and Shanks 2018; Haddix et al. 2008). When the quantity of ATP within a cell is minimum, the cell population arrives at high-density exponential phase. Similarly, when the growth rate ceases, prodigiosin synthesis begins. The parallel result was also found during the kinetics analysis of prodigiosin production by *S. marcescens* Nima strain in batch culture (Haddix et al. 2008). Haddix et al. (2008) also suggested that prodigiosin production and ATP synthesis have an inverse relationship (Haddix et al. 2008). In the latest research published by Obayemi et al. (2016b) revealed the release kinetics of anti-cancer drug prodigiosin obtained from *Serratia marcescens* subsp. *Marcscens* (Obayemi et al. 2016a). They have analysed the drug release kinetics using the following model developed by Korsmeyer and Peppas, i.e.  $F = K_p t^n$ , where F is the amount of drug release at time t;  $K_p$  is the Peppas release rate constant and n is the release exponent. The release constant (k) and release coefficient (n) were found to be 0.93 min<sup>-1</sup> and 0.85.

Dyeing kinetics of prodigiosin pigment and thermodynamical aspects of polyester fibre were carried out to get an overview to develop a suitable pigment dyeing technology (Shen and Yang 2013). Shen and Yang (2013) calculated the enthalpy and entropy change during dyeing kinetics study. Negative enthalpy and positive entropy value indicate that dyeing of prodigiosin is an exothermic process. The dyeing temperature was kept below 120°C.

## 12.6 Conclusion

The present chapter has summarized more or less critical and holistic approaches that can be taken into consideration for enhanced prodigiosin yield. Some recent advancements on the nanotechnological synthesis, bioengineering aspects and relevant analytical techniques were concentrated. A lot of research has been conducted and is going on various parts of the globe on prodigiosin. However, in a country like India, researchers are still in their initial stages in the field of biocolour. The technological gaps reside on its efficient downstream processing and extraction hinders the solicitation of large scale prodigiosin in the food industry. Optimization of production media, incorporation of the robust mathematical model with the suitable molecular technique will help to trim the production cost of this potential multitasking component. After compelling purification study, the prodigiosin can assist as a promising drug for cancer treatment. This book chapter will serve as a bridge between the industry and the scientific institutes by helping them to develop a bench-scale technology that can be transferred to the industry and will further improve the country's economics.

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