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Strategy for Obtaining Inexpensive Prodigiosin Production by *Serratia Marcescen*

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Abstract. The wild-type of *Serratia marcescens* which could produce prodigiosin was isolated by our laboratory. With utilizing the kitchen waste by solid fermentation method, the strategy for obtaining inexpensive prodigiosin was established. Optimum prodigiosin production was achieved when the moisture level was 200%; the ratio of biomass of rice husk to kichen waste material was 1.2 and adding 1.5% level proline to the substrate. The physiological fermentation factors such as incubation time (60h), pH of the medium (pH 8.0), and fermentation parameter such as temperature (28℃), inoculum level (1%) also have impact on the prodigiosin production. After modifying the fermentation contions, the maximum prodigiosin yield was more than 4155 milligram per kilo kitchen waste at 60h, which was two times more than initial production yield.

Keywords: prodigiosin production, kitchen waste, solid fermentation, waste recycling, *Serratia marcescens*

1. Introduction

Prodigiosin is one kind of secondary metabolite produced by *Serratia marcescens* and other bacteria (1). Nowadays, it is clear that "prodigiosin" has a series of close relatives bearing the same pyrolylpyrromethene core with different alky1 substituent (2), and these relatives have caused wide concern due to their ability to induce apoptosis of several cancer cell lines (3,4). Many prodigiosin-derivatives with lower toxicity like GX15-070 have been used in clinic. Because of its low fermentation yield and high production cost, the price of it is up to $500/mg (Santa Cruz Biotechnology, Inc., U.S.A., 2010). In light of its potential commercial values, it is worth finding a strategy for getting inexpensive prodigiosin.

In our study, the first step to decrease the production cost of prodigiosin is finding an inexpensive, replaceable substrate. In all kinds of industrial and agricultural waste, kitchen waste is available substrate material for producing prodigiosin. Kitchen waste has rich carbon source, moderate nitrogen source and low toxic material. Only needing some modification, the kitchen waste could become the ideal substrate for *Serratia marcescen* to grow and produce prodigiosin.

Recently, solid-state fermentation has gained renewed interest and fresh attention form researchers owing to its importance in recent developments in biomass energy conservation. Compared with normal fermentation, solid-state fermentation reveals many advantages including higher production yield, more effectiveness in labor and more friendly to the environmental (5,6). Although many produces have gained form solid-state fermentation, there is no effort to produce prodigiosin through that method. In this study, we aimed to using kitchen waste to producing prodigiosin by *Serratia marcescen* in solid-state fermentation.

2. Materials and Methods

2.1. Microorganism

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This red wild-type strain was insolated in our laboratory form kitchen waste in nature and was characterized as *Serratia marcescen* through 16S rDNA analysis. Its logging number of GenBank was DQ832185. The strain produces a red pigment that has been identified as prodigiosin by Nuclear Magnetic Resonance and mass spectrometry. Normally, the strain was maintained on the medium which contains (%) peptone 1.0, beef extract 0.5, NaCl 0.5, agar 2%, stored at 4℃ and sub-culture at one month intervals.

2.2. Substrate

Kitchen waste material was collected form the local kitchen waste handle factory, and then the waste was dehydrated and shattered. The mean particle size of the substrate was 1.4-1.0 mm and the main composition of waste was analyzed.

Table.1 Characteristic of the kitchen garbage used in

	%
C	51.84
N	3.41
Total sugar	47.12
Starch	38.12
Crude protein	20.64
Crude fat	24.16
Crude fiber	5.96
NaCl	1.53
pH	4.16

2.3. Solid-state fermentation

Ten grams of kitchen waste was taken in 250ml flasks and predetermined quantity of water and rice husk were added. Then the substrate was mixed thoroughly and autoclaved at 121 for 20 min at $1kgf/cm^2$. When the flasks were cooled, they were inoculated with 1ml of 12-h grown culture broth under sterile conditions. After mixing the contents of the flasks, these flasks were incubated in standing-temperature cultivator at different temperate and predetermined time period. For optimizing the culture medium, different carbon and nitrogen sources were added individually at 2% level (compared with kitchen waste) to the medium. And in order to study the pH influence, the medium was adjusted into different pH by adding NaOH solution. Also in order to study the effect of inductor, predetermined quantity proline solution was added into medium through 0.22um bacterial filter after the medium was autoclaved. In case of incubation time, flasks contents at every 12h were used for extraction and estimation of prodigiosin yield. Results reported in this study are averages of triplicate findings.

2.4. Prodigiosin extract and content measure

Prodigiosin in the flasks was extract by acidic methanol (pH 3.0). In this 1 ml of the flask content was added to 9 ml acidic methanol and shake at 150 rpm for 30 min at 30℃. Debris was removed by centrifugation at 10,000 x g for 20 min at 4℃, and absorbance of appropriately diluted samples was measured in UV–Visible spectrophotometer at 535 nm. The prodigiosin content was calculated using prodigiosin standard curve.

2.5. Measurement of biomass

The bacterial growth was estimated by measuring the cell dry weight. 1g of the flask content was added to appropriate water then through 100 um filter and absorbance of diluted samples was measured in UV–Visible spectrophotometer at 660 nm. The biomass was calculated using OD660 of broth versus dry cell weight standard curve.

3. Results and Discussion

3.1. Evaluation of kitchen waste material for prodigiosin production

In nature, *Serratia marcescens* could produce prodigiosin on the medium containing proper carbon and nitrogen source. Tao got the maximum prodigiosin production as 583 mg/l (7), and Wei got 790mg/l when the LB medium was added oil (8). In our study, kitchen waste was proven as an ideal replaceable for prodigiosin production which contains abound nutrition. With some modification, the prodigiosin yield of our study could be up to 4155 milligram per kilo kitchen waste. Moreover, solid fermentation methods could save the cost of maintaining and operating normal fermentation equipments and shorten the refining process.

3.2. Influence of incubation time

Using initial medium (10g kitchen waste and 4g rice husk in 250ml flasks, moisture content 200%, pH 8.0) and on the initial fermentation condition (28℃, 1% inoculum level), results of our study showed that prodigiosin production increased with incubation time at the beginning of 60h and further incubation showed reduction in its production. Maximum prodigiosin production of per kilo kitchen waste was 1290mg at 60h. Cell growth is in accordance with prodigiosin production and got its peak, 24.5g per kilo substrate at 36h, which means that prodigiosin production associates with cell growth in nature.

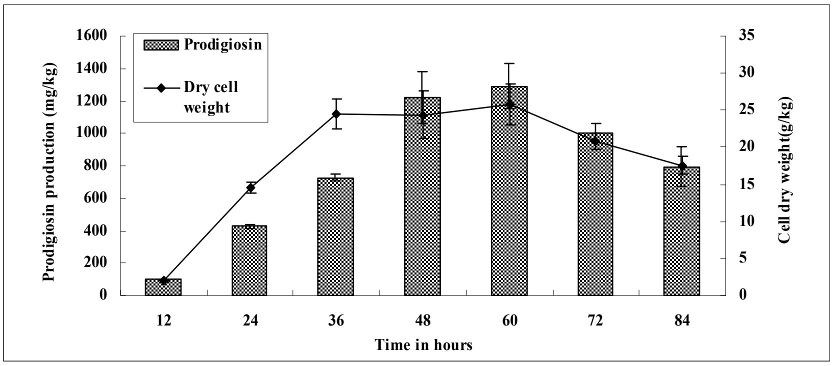

Fig. 1 Influence of incubation time on *Serratia marcescens* growth and prodigiosin production under solid-state fermentation with kitchen waste substrate

3.3. Influence of moisture content and substrate support material

Different form submerged fermentation, microbial growth and product formation of solid fermentation occurs at or near the surface of the surface of the solid substrate particle having low moisture contents. Thus, it is crucial to provide optimized water level that controls moisture content of the fermenting substrate for achieving maximum product. In order to know optimum water level，experiments were conducted with increase in water adding. The solid material was provided with calculated amount of water with respect to solid material (for example for 100% moisturization, 10ml of water was added to 10g of solid material). Our study showed that maximum prodigiosin production was got with 200% moisture content and the percent reduction of that form either side of the optimum moisture level (200%), varied. At the same time, as ideal substrate support material, rice husk could increase the ratio of the surface area to the weight of substrate sharply, and also improve the air flow of the substrate. Bernard Heinemann reported that production of prodigiosin required supply of enough oxygen (9). In present study, the maximum yield of prodigiosin 2830mg/kg was observed when we adding rice husk into the kitchen waste substrate at 120% level compared with dry weight of kitchen waste.

3.4. Influence of fermentation parameters

In our study, many fermentation parameters including temperature, inoculum level, and pH have impacts on yield of prodigiosin. When the temperature was between 20℃ and 30℃, there was a mild fluctuation on prodigiosin production. Also due to quick growth of strain, inoculum level influenced the final prodigiosin output weakly. Different form other microorganism, *Serratia marcescens* does not require the initial pH of medium strictly due to its strong buffering capacity (10). As long as the initial pH of the medium is between 7 and 9, *Serratia marcescens* could produce prodigiosin normally, however, the maximum yield was obtained at initial pH 8, 28℃ and 1% inoculum level.

3.5. Role of different carbon sources

Different carbon sources such as starch, vegetable oil, sucrose, fructose, lactose, glycerol, glucose were added into initial medium at 2% level for studying optimum concentration of carbon sources. The results showed that the carbon sources in kitchen waste were enough for the prodigiosin production by *Serratia marcescens* and excess carbon sources even lead a reduction of prodigiosin yield. The negative effect was obvious when monosaccharide was added into the medium.

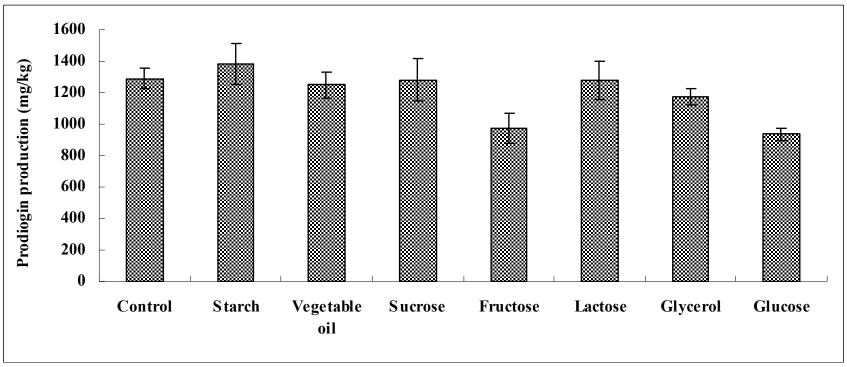

Fig. 2 Influence of adding different carbon sources on prodigiosin production by *Serratia marcescens* under solid-state fermentation with kitchen waste substrate.

3.6. Role of different nitrogen sources

Different nitrogen sources such as yeast extract, peptone, beef extract, fish flour, NH_4Cl, NH_4NO_3, $NaNO_3$ were added into initial medium at 2% level for studying optimum concentration of nitrogen source. The results showed that adding excess nitrogen source supported litter for prodigiosin production. Moreover, under solid-state condition, inorganic nitrogen compounds for example NH_4Cl, NH_4NO_3, $NaNO_3$ are fatal for prodigiosin production.

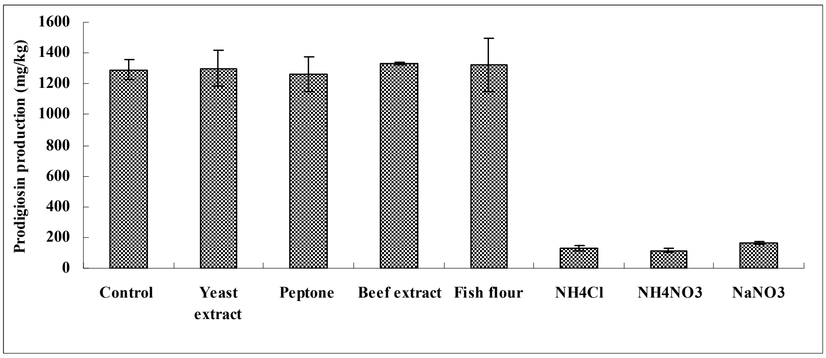

Fig. 3 Influence of adding different nitrogen sources on prodigiosin production by *Serratia marcescens* under solid-state fermentation with kitchen waste substrate.

3.7. Influence of inductor

Many amino acids which contain pyrrole-like structures have inductive effect for prodigiosin production (11). In our present study, proline had the best inductive effect than other amino acids (date not shown). In order to optimize the proline concentration as inductor for the prodigiosin production, experiments were conducted with increase in proline. The results indicated that adding 1.5% level proline could enhance the yield of prodigiosin by 60%, but higher proline concentration had litter further effect.

In the end, experiments were conducted with improved medium and optimized fermentation condition to understand the combinatorial influence of various factors. The results were depicted in Fig 4. It is evident that after improvement, the prodigiosin production was more than 4155 milligram per kilo kitchen waste at 60h, which was two times more than initial production yield.

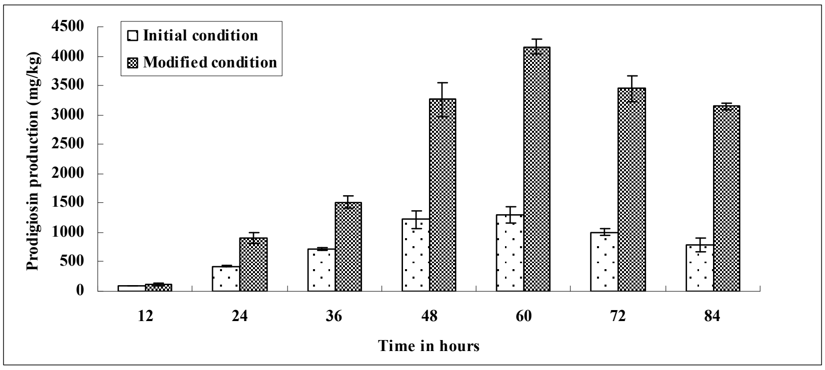

Fig 4 Prodigiosin production by Serratia marcescens on initial and optimum fermentation conditions under solid-state fermentation with kitchen waste material.

4. Acknowledgments

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