Int.J.Curr.Microbiol.App.Sci (2022) *11*(05): 131-143

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 05 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1105.017

Yeast Extract Inhibits Prodigiosin Production and Promotes Serrawettins Production in *Serratia marcescens*

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Keywords

Serratia marcescens, Prodigiosin, Serrawettins, Yeast extract

Article Info

Received:
05 April 2022
Accepted:
28 April 2022
Available Online:
10 May 2022

A B S T R A C T

Serratia marcescens is a gram-negative bacterium, which can produce many metabolites, among which the study of prodigiosin is the most extensive. This experiment explored the effect of yeast extract on intracellular metabolic pathway in *S. marcescens.* The experimental results showed that yeast extract could inhibit the production of prodigiosin in *S. marcescens* to a considerable extent. It was verified by RT-qPCR that yeast extract inhibited the expression of genes in the synthesis pathway of prodigiosin. However, although there was no production of prodigiosin in the fermentation broth added with yeast extract, a large number of lipopeptide serrawettins was produced through preliminary detection and identification. Serrawettins have surface activity, antibacterial and antitumor activity. Therefore, the addition of yeast extract can be used to promote the production of serrawettins by *S. marcescens*. There was no report on the effect of yeast extract on intracellular metabolic pathway in *S. marcescens* in other literatures at present.

Introduction

S. marcescens is a Gram-negative bacterium widely existing in water, air, soil, animals and plants (Sharmist and John *et al.,* 2019). Varieties of substances such as prodigiosin, 2-keto-D-gluconic acid, lipopeptides, carbamonems, chitinases etc. can be produced by *S. marcescens* (Bai and Liu *et al.,* 2017). Prodigiosin is a microbial secondary metabolite with three pyrrole rings. Its molecular formula is $C_{20}H_{25}N_3O$, and molecular weight is 323.42 Da (Lin *et al.,* 2020). Full name of prodigiosin is 4-methoxy-2,2'-dipyrrole-5-methylpentyl pyrrole, and the three pyrrole rings of prodigiosin are usually named A, B and C rings (Stankovic *et al.,* 2014; Hu *et al.,* 2016; Sakai-Kawada *et al.,* 2019). Prodigiosin is a dark red powder and subordinate to lipid-soluble pigment,

which is insoluble in water and easily soluble in organic solvents such as methanol, ethanol, acetone and dimethyl sulfoxide (Darshan and Manonmani *et al.,* 2015; Paul *et al.,* 2020). Prodigiosin is so sensitive to temperature, light, pH and metal ions, it is easy to decompose when in high temperature, strong light and alkaline environment (Liu *et al.,* 2007; Stankovic *et al.,* 2014; Yip *et al.,* 2019).

S. marcescens can produce many products such as 2-keto-D-gluconic acid, Serrawettins, Carbapenems and chitinases. 2-keto-D-gluconic acid (2-KGA) is an organic acid, it is mainly used in the synthesis of D-Isoascorbic acid and D-Ascorbate acid and is used as food antioxidant in food industry (Li *et al.,* 2018). *Pseudomonas*, *Erwinia*, *Acetobacter* and *Serratia* can produce 2-KGA in nature (Luo *et al.,* 2019). Lipopeptides Serrawettins include Serrawettin W1, Serrawettin W2 and Serrawettin W3. So far, only *S. marcescens*is known to produce serrawettins among all reported strains. The Synthetic mechanism of serrawettin W1 is clearer compare to the synthetic mechanism of serrawettin W2 or serrawettin W3 up to now (Bai and Liu *et al.,* 2017). Carbapenems have the characteristics of wide antibacterial range, strong antibacterial activity and low toxicity, and have become one of the most important antibiotics in the treatment of bacterial infections (Xie *et al.,* 2011; Zhang *et al.,* 2004; Liu *et al.,* 2013). Besides, *S. marcescens* can produces two important chitinases, ChiA and chiB which have antibacterial activity (Wang and Liu *et al.,* 2006). Prodigiosin, lipopeptides, carbapenems and chitinases as mentioned above produced by *S. marcescens* all have antibacterial activity.

In the previous experiments, it was found that yeast extract could inhibit the production of prodigiosin in *S. marcescens*, and peptone was conducive to the production of prodigiosin by *S. marcescens*. Therefore, our research focuses on the inhibitory effect of yeast extract on prodigiosin and the trend of its products after inhibition. The prodigiosin synthesis pathway which is controlled by *pig* gene cluster, the pathway can be divided into MAP synthesis pathway (2-methey-3-pentylpyrrole synthesis pathway) and MBC synthesis pathway (4-methoxy-2-2'-bipyrole-5-carbaldehyde synthesis pathway), then MAP and MBC condense to form prodigiosin in *S. marcescens* (Hu *et al.,* 2016; Sakai-Kawada *et al.,* 2019). Then, RT-qPCR was used to detect the expression of the gens on the prodigiosin synthesis pathway in *S. marcescens*. Generally, this study revolove around the effect of yeast extract on the metabolic pathway in *S. marcescens*, hope to provide new research ideas for the metabolic mechanism and development and application of *S. marcescens*.

Materials and Methods

Specific components in yeast extract inhibiting prodigiosin synthesis

In order to explore the substances that inhibit the production of prodigiosin in yeast extract, experiments were carried out from the different components of yeast extract and peptone. Compared with peptone, yeast extract contains higher levels of Vitamin B2 (VB2), sugars (polysaccharides composed of glucose), Zn and other trace elements such as Cu, Fe, Mn according to the composition as shown in Table I . Therefore, the effect of VB2, glucose and the salts of each trace element on the production of prodigiosin were explored. The salts used in this experiment were $CuSO_4$, $ZnCl_2$, $MnCl_2$, $FeCl_3 \cdot 6H_2O$ respectivily. In this experiment, the contral group and experimental group were set up, the fermentation medium of the contral group was: 15 g/l glycerol, 12 g/l peptone and 3 g/l calcium chloride. Glucose, VB2 and the salts of Cu, Fe, Zn, Mn were converted into the concentration contained in yeast extract, the concentration of each substance after conversed was as follows: glucose: 1.1436 g/l, VB2: 0.4992 mg/l, Cu: 0.01752 mg/l, Fe: 0.71136 mg/l, Zn: 1.91628 mg/l, Mn: 0.10548 mg/l, they were added to the contral fermentation medium respectively as the experimental group. After the fermentation, whether the substances inhibit the production of prodigiosin was decided by OD_{535} value compare with the control group. Then, the gradient addition experiment of glucose, VB2 and

Zn were carried out to determine their minimum inhibitory concentration on production of prodigiosin. The equipments used in this experiment were centrifuge (centrifuge 5430, Eppendorf China Ltd), shaker (ZWYR-D2403, Zhicheng, China), sterile operating Table (AlphaClean 1300, Heal Force, China), UV spectrophotometer (UV-9000, Yuanxi Co., Ltd, Shanghai, China), 250 ml sterile Erlenmeyer flask respectively.

RT-qPCR detect gene expression on prodigiosin synthesis pathway

Sample preparation

Firstly, RT-qPCR was used to detect the expression of genes on prodigiosin biosynthesis pathway when yeast extract was used as nitrogen source, so that explore the concrete inhibition position of yeast extract on prodigiosin synthesis pathway. The control group and the experimental group were set up respectively. The fermentation medium of the control group included 15 g/l glycerol, 12 g/l peptone and 3 g/l $CaCl_2$, while the fermentation medium of the experimental group included 15 g/l glycerol, 12 g/l yeast extract, 3 g/l $CaCl_2$.

Then the quantitative expression of genes in prodigiosin synthesis pathway in the experimental group and the control group was detected. The genes *pigD* and *pigE* in MAP synthesis pathway, the genes *pigA* and *pigI* in MBC synthesis pathway and the gene *pigC* which finally controls the condensation reaction between MAP and MBC were selected in this experiment.

Primer synthesis

The primers were synthesized according to the sequence presented in Table Ⅱ, and the sequence of *16S rRNA* primers was referred to Xu *et al.,* (Xu *et al.,* 2014), the sequence of *pigD* and *pigE* were referred to Liu *et al.,* (Liu *et al.,* 2021), the sequence of *pigA*, *pigI* and *pigC* were referred to the complete gene sequence of prodigiosin synthesized by *S. marcescens* (You *et al.,* 2018).

RNA extraction, reverse transcription and RT-qPCR

Total RNA was extracted using the SparkZol Reagent (SparkJade, Shandong, China). Took an appropriate volume RNA, 1% agarose, 120 V, 20 min was used for agarose gel electrophoresis. Reverse transcription was conducted using SPARKscript Ⅱ RT Plus Kit（With gDNA Eraser (SparkJade, Shandong, China). According to sparkscript Ⅱ RT plus kit, compounded 10 μl reverse transcription reaction system, Joined 8 μl total RNA and 1 μl gDNA Eraser firstly, then RNase free H_2O was added to 10 μl. The reaction was carried out at 42 ℃ for 5 min. Lastly joined 10 μl 2× Sparkscript II RT Plus Master Mix to carry out reverse transcription, reverse transcription reaction program was 50 ℃ 15 min, 85 ℃ 5 min. RT-qPCR was performed using 2×SYBR Green qPCR Mix (With ROX). According to the requirements of qPCR kit, compound 20 μl SYBR Green Ⅰ PCR system, including 10 μl 2×SYBR Green qPCR Mix, 0.4 μl Primer F (10 μM), 0.4 μL Primer R (10 μM), 1 μl cDNA, RNase free H_2O was added to 20 μl finally. Used 16S rRNA as internal reference, the amplification conditions were 94 ℃ 15 s, 60 ℃ 15 s, 72 ℃ 20 s, 45 cycles. The equipments used in this experiment were velocity 18R high speed freezing centrifuge (Dynamica), powerpac basic electrophoresis apparatus (Bio-Rad), JY300C electrophoresis tank (Junyi electrophoresis Co., Ltd, Beijing, China), tanon 1600 Gel imager (Tianneng Technology Co., Ltd, Shanghai, China), nano-300 Micro spectrophotometer (Allsheng Instruments Co., Ltd, Hangzhou, China), roche lightcycler 480 Real time quantitative PCR instrument (Roche) respectively.

Identification of substance in the fermentation added with yeast extract

Sample preparation

Experimental group medium: 15 g/l glycerol, 12 g/l yeast extract, 3 g/l $CaCl_2$; Control group medium: 15 g/l glycerol, 12 g/l peptone, 3 g/l $CaCl_2$. *S.*

marcescens in experimental group and control group were cultured in a shaker with 30 ml fermentation medium for 48 h.

UV scanning and thin layer chromatography detection

Referred to other references, products in two kinds of fermentation broth as mentioned above were extracted with ethyl acetate (Lindum *et al.,* 1998; Shanls *et al.,* 2013). The extracted substances were analyzed and identified by UV scanning, thin layer chromatography (TLC) and bacteriostatic test respectively. Firstly, thin layer chromatography was carried out, chloroform was used as the developing agent for spread. After 15 min, ninhydrin was evenly sprayed on the thin layer and dried (Matsuyama *et al.,* 1992; Yamashita and Silica *et al.,* 2001; Yamashita *et al.,* 2001). Observe if there was pink appears. Then, the extracted substances by ethyl acetate was analyzed by the UV scanning spectrum at 190~1100 nm (Su *et al.,* 2016). Analyze whether there is a specific maximum absorption peak in the scan chromatogram.

Antibacterial activity detection

When carrying out antibacterial activity detection, the extracted substances and ethyl acetate (as the control) were respectively poured into the pre drilled holes in the culture dish containing agar medium, and cultured in the 34 ° incubator for 24 hours to observe whether there was obvious inhibition zone, if a transparent ring appears further confirm that the extracted substances had the antibacteiral activity. The bacteria used in the antibacterial experiment was from the single colony picked out from the air. Finally, the product was determined by combining the results of the three analytical methods.

Results and Discussion

Specific components in yeast extract inhibiting prodigiosin synthesis

In this experiment, there were extremely unlike products of *S. marcescens* with different nitrogen sources in the optimization of fermentation conditions of prodigiosin. When yeast extract was used as a nitrogen source, prodigiosin synthesis was completely inhibited, however, prodigiosin production was reached the highest while peptone was used as a nitrogen source. Yet some literatures showed that considerable prodigiosin production could be obtained by using yeast extract as a nitrogen source (Gulani *et al.,* 2012; Kurbanoglu *et al.,* 2015; Gondi *et al.,* 2017). Therefore, in order to explore the main substances in yeast extract that affect the production of prodigiosin, a comparative experiment was designed according to the composition differences between yeast extarct and peptone. According to Fig.1. A, glucose, VB2 and trace elements Cu, Fe, Zn and Mn in yeast extract had different degrees of inhibition on the synthesis of prodigiosin. Among them, glucose and VB2 in yeast extract had a weak inhibitory effect, and each microelement had a strong inhibition effect, among which, the trace element Zn affected prodigiosin in the greatest extent, the production of prodigiosin was almost completely inhibited, so that the addition of Cu, Fe, Zn and Mn simultaneously produce few prodigiosin similarly. However, there was report shown that the addition of Fe can promote the synthesis of prodigiosin (Silverman and Munoz *et al.,* 1973). Then, glucose, VB2 and the representative trace elements substance Zn were selected for the gradient addition experiment.

According to Fig. 1. B, the production of prodigiosin decreased gradually with the increase of glucose concentration. The production of prodigiosin in fermentation liquid was extremely low when the concentration of glucose is more than 8 g/l. And there had not prodigiosin at all while the glucose concentration reached 10.2924 g/l, which was consistent with most literature conclusions, that is, glucose inhibited the production of prodigiosin (Bunting *et al.,* 1949; Fender *et al.,* 2012). It can be known from Fig. 1. C that VB2 does had a certain degree of inhibition on the synthesis of prodigiosin, but there was no linear relationship between its inhibition and its added concentration. The production of prodigiosin has not decreaseed

significantly and keep constant with the increase of VB2 concentration basically. Therefore, it is concluded that VB2 will not completely inhibit the production of prodigiosin. As shown in Fig. 1. D, Chloride salt $ZnCl_2$ of trace element Zn had a strong inhibition on the production of prodigiosin. The production of prodigiosin was suppressed completely when the concentration of $ZnCl_2$ was 19.16 mg/l, while added 1.916 mg/l $ZnCl_2$ the yield of prodigiosin could be ignored. The obvious inhibition of trace element Zn on the synthesis of prodigiosin can be attributed to the relationship between Zn and metabolism of many enzymes in microorganisms. We infer that the addition of Zn enhanced the activities of related enzymes in the primary metabolic pathway, which made microorganisms absorb a large number of nutrients from the outside environment. Through catabolism and anabolism, it produced materials and energy needed for life-sustaining activities, such as nucleic acid, protein, carbohydrate and other primary metabolites, which promoted the primary metabolic process of microorganisms in the logarithmic phase. A large amount of carbon and nitrogen sources was consumed in the initial logarithmic period of microbial growth, then the synthesis of secondary metabolite prodigiosin was blocked due to the lack of carbon and nitrogen sources at the end of the logarithmic period.

RT-qPCR detect gene expression on prodigiosin synthesis pathway

RNA extraction and primer synthesis

Extraction and purity analysis of RNA: extract the total RNA of *S. marcescens* and carry out 1% agarose gel electrophoresis. As Fig. 2. A shown, the 28S, 18S and 5S bands were clear, and there was no tail phenomenon between the strips. It shown that RNA had no degradation and good integrity.

The ratio of OD_{260}/OD_{280} detected by the microspectrophotometer is 1.92, indicating that RNA had better purity and met the requirements of RT-qPCR.

Primer synthesis: as shown in Fig. 2. B, the synthetic products of five experimental genes and 16S rRNA were relatively specific and met the requirements of RT-qPCR.

RT-qPCR

The transcription levels of *pigD*, *pigE*, *pigA*, *pigI*, *pigC* genes are shown in Fig. 3. and Table Ⅲ. The transcription levels of the five genes in the experiment group and control group were analyzed by TR-qPCR with *16S rRNA* as the internal reference gene. The results showed that, calculating by method $2^{-\Delta\Delta CT}$, and appointing the $2^{-\Delta\Delta CT}$ of control group was 1, the $2^{-\Delta\Delta CT}$ of experiment group was close to zero, According to hypothesis, if all these genes are not expressed, it indicates that yeast extract may completely inhibit all genes in the process of prodigiosin synthesis or the precursors required for prodigiosin synthesis, if all or part of these genes are expressed, it is inferred that yeast extract inhibits some gene of prodigiosin synthesis, thereby inhibit the synthesis of prodigiosin.

There was almost no expression of the five genes in the experiment group, so it can be determined that the addition of yeast extract inhibited the expression of the genes in the MAP pathway and the MBC pathway, at the same time, the final condensing enzyme gene *pigC* which controlling the condensation reaction of MAP and MBC also was inhibited. In other words, yeast extract inhibited the whole process of prodigiosin synthesis include MAP pathway, MBC pathway and the final condensation reaction, or inhibited the precursors required for prodigiosin synthesis absolutely.

Combined with the previous analysis results, it can be inferred that a large number of nutrients were consumed in the early logarithmic phase of microbial growth, while in the late logarithmic phase of the synthesis of secondary metabolites, the lack of nutrients led to the failure to synthesize prodigiosin or the precursors required for the synthesis of prodigiosin.

Identification of substance in the fermentation added with yeast extract

UV scanning and thin layer chromatography detection

Experiments were carried out according to the experimental method as mentioned above, when the ratio of ethyl acetate: fermentation fluid =1:2 was used to extract the substances in the fermentation liquid, the layer of flocculent substances would appear. While for the same volume of the fermentation fluid, the flocculent substance in control group fermentation liquid was significantly less than the flocculent substance in experiment group fermentation liquid (as shown in Fig. 4. A. In Fig. 4. A, the left side is the stratification of ethyl acetate extract of control group fermentation fluid, and the right side is the stratification of ethyl acetate extract of experiment group fermentation fluid. After ethyl acetate extraction, the control group fermentation fluid was obviously divided into three layers, the top layer was the ethyl acetate solution of prodigiosin, and the middle layer was the flocculent material, the lowest layer was the remaining fermentation liquid. The fermentation fluid of experiment group was also divided into three layers after being extracted with ethyl acetate, the upper layer was ethyl acetate, the middle layer was flocculent material, and the lower layer was the remaining fermentation liquid. The middle layer of fermentation liquid in experiment group was analyzed by TLC. Chloroform was used as the developing agent. After 15 minutes, ninhydrin was sprayed evenly on the TLC plate, then the TLC plate was dried. Observe the TLC plate, If the color was developed, it could be preliminarily determined as lipopeptides or chitinases. As shown in Fig. 4. C, the sample results red, therefore, it proves that lipopeptides or chitinases were produced in the fermentation liquid of experiment group preliminary (Yamashita and Silica *et al.,* 1992; Matsuyama *et al.,* 2001; Su *et al.,* 2016). UV spectrum scanning at 190~1100nm was carried out on pure ethanol, pure ethanol solute of the middle flocculent substance of fermentation liquid in experiment group extracted by ethyl acetate, and pure ethanol solute of the middle flocculent substance of fermentation liquid in control group extracted by ethyl acetate. It has been reported that *S. marcescens* can produce lipopeptide active substances serrawettins. Serrawettins includes serrawettin W1, serrawettin W2 and serrawettin W3. Serrawettins W1 and serrawettin W2 were related to the ability of colony expansion, and serrawettins W2 was similar to surfactin, which had an absorption peak at 215 nm (Matsuyama *et al.,* 1992; Mastuyama and Nakagawa *et al.,* 1996; Zhang *et al.,* 2021).As shown in the Fig. 5, both the middle flocculent substances of experiment group fermentation liquid and control group fermentation liquid extracted by ethyl acetate have obvious maximum absorption peaks at 215 nm, but the solvent pure ethanol had no absorption peak at 215nm. Therefore, according to the analysis results of UV spectral scanning it can be further infer that the possible products was lipopeptide active substances serrawettins W2 (Matsuyama *et al.,* 1992; Shanls *et al.,* 2013). In addition, the pure ethanol solute of the middle flocculent material of control group fermentation broth extracted by ethyl acetate also contained the maximum absorption peak at 535 nm which is characteristic of prodigiosin (Lin *et al.,* 2021).

Antibacterial activity detection

Finally, the middle layer flocculent material of experiment group and control group was tested for bacteriostasis, as shown in Fig. 4. B. The hole 1 was injected with ethyl acetate as control, the hole 2 was injected with the middle layer flocculent material of experiment group fermentation liquid extracted by ethyl acetate, and the hole 3 was injected with the middle layer flocculent material of control group fermentation liquid extracted by ethyl acetate. The result shown that ethyl acetate had no antibacterial effect, and the flocculent substance in experiment group fermentation liquid has the antibacterial effect, the diameter of its transparent circle was 8 mm. The flocculent substance in control group fermentation liquid had stronger antibacterial effect because it was mixed with prodigiosin.

Table.1 Comparison of yeast extract and peptone components

Substance	Yeast extract	Peptone
VB2 (mg/kg)	41.6	1.2
Glucose (%)	9.53	3.88
Cu (mg/kg)	1.46	Not detected
Fe (mg/kg)	59.28	Not detected
Zn (mg/kg)	159.69	Not detected
Mn (mg/kg)	8.79	Not detected

Fig.1 Inhibitory effect of various substances on prodigiosin. A. Effects of glucose, VB2, trace elements such as Cu, Fe, Zn and Mn on the yield of prodigiosin; B. Effects of glucose concentrations on the yield of prodigiosin; C. Effects of VB2 concentrations on the yield of prodigiosin; D. Effects of $ZnCl_2$ concentrations on the yield of prodigiosin.

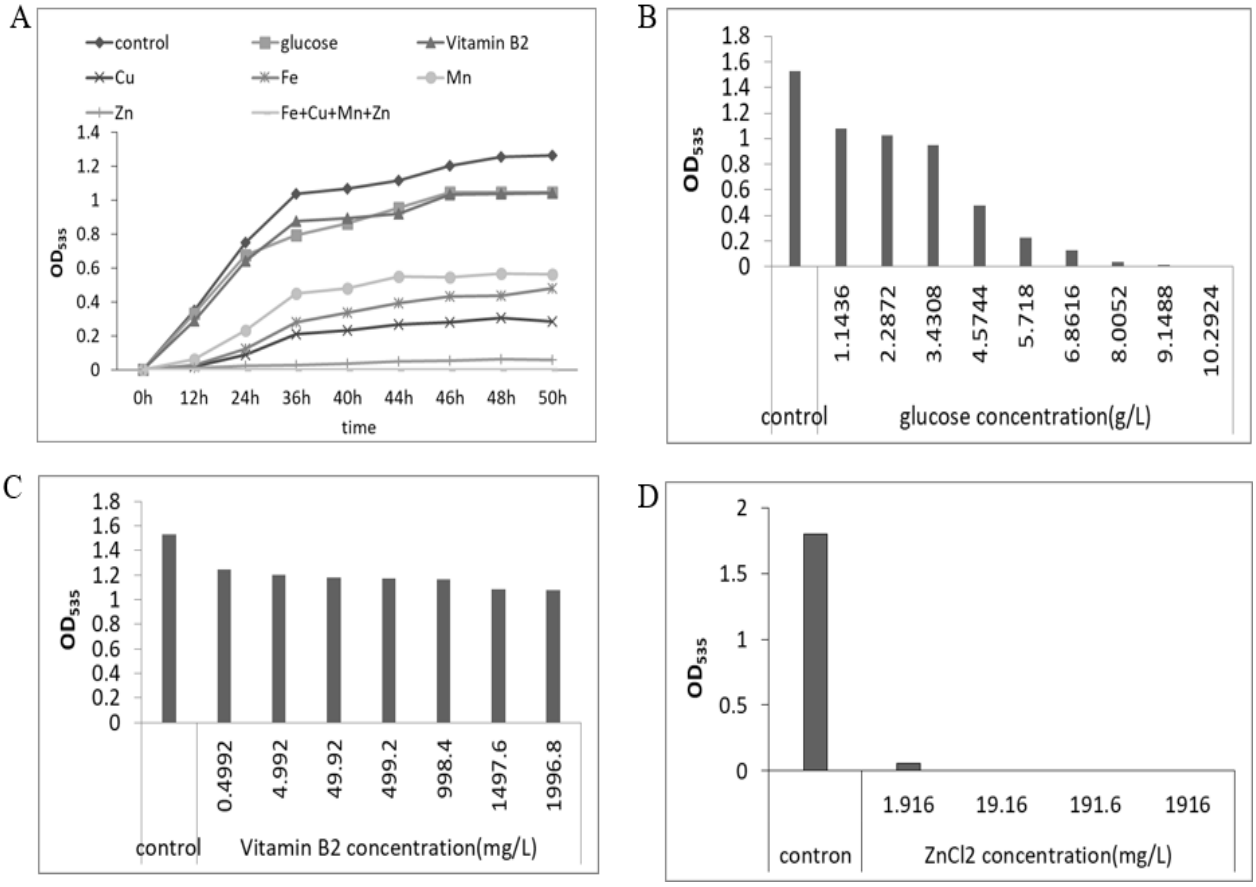


Table.2 Primer synthesis sequence of each gene

Gene	Primer	Sequence(5'-3')
16S rRNA	*16srRNA*-F	GCCCAGGTAAGGTTCTTC
	16srRNA-R	GGTGTAGCGGTGAAATGC
pigD	*pigD*-F	CTGACAGGACAAGCAAGACTC
	pigD-R	TACGCTAAGCCCGGTTTG
pigE	*pigE*-F	CAACTCGCTGACCACCTAC
	pigE-R	TGGAGAGAGAAACCGTGC
pigA	*pigA*-F	CGTCTGGAACAGTGTCGGCTAATG
	pigA-R	ACGGCATATTCGGAAATCAACAGTTTG
pigI	*pigI*-F	GCTGAGCGGCAACCACTATGTC
	pigI-R	CATCGAGATCGGTGCGGTCATC
pigC	*pigC*-F	CTGGATCTGACGACCATGACACATC
	pigC-R	GCGAACGACTGAAGGAAGAAGGG

Notes: Gene reference [Lindum *et al.* 1998; Shanks *et al.* 2013; Paul *et al.* 2020].

Fig.2 RNA quality inspection and fusion curves. A. RNA quality inspection: Loading sequence: From left to right: Spark 2000 DNA marker, experiment group, control group; B. Fusion curves of each gene.

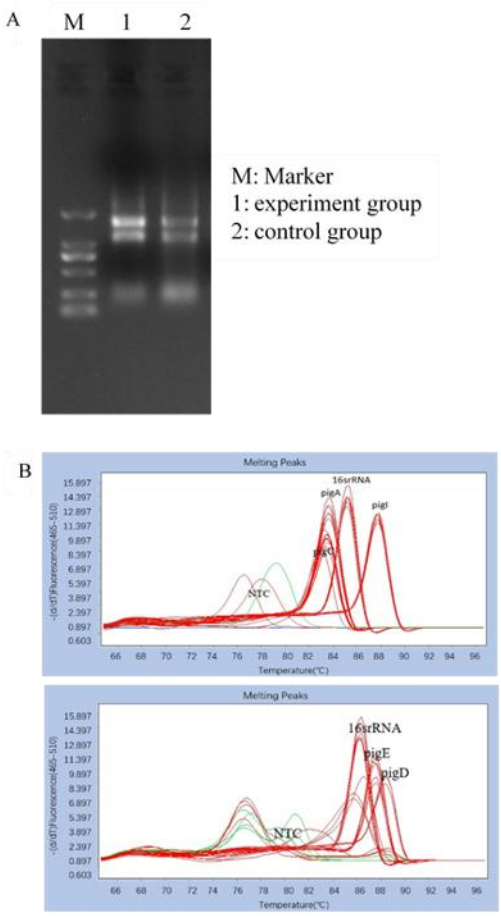


Table.3 Expression of each gene between experimental group and control group

gene	*pigA*	*pigI*	*pigD*	*pigE*	*pigC*
control group	1.00	1.00	1.00	1.00	1.00
Experiment group	0.03	0.01	0.07	0.01	0.02

Note: The number in the table is $2^{-\Delta\Delta Ct}$.

Fig.3 Quantitative analysis histogram of each gene: A. Genes in MAP synthesis pathway; B. Genes in MBC synthesis pathway; C. Final condensation gene *pigC*.

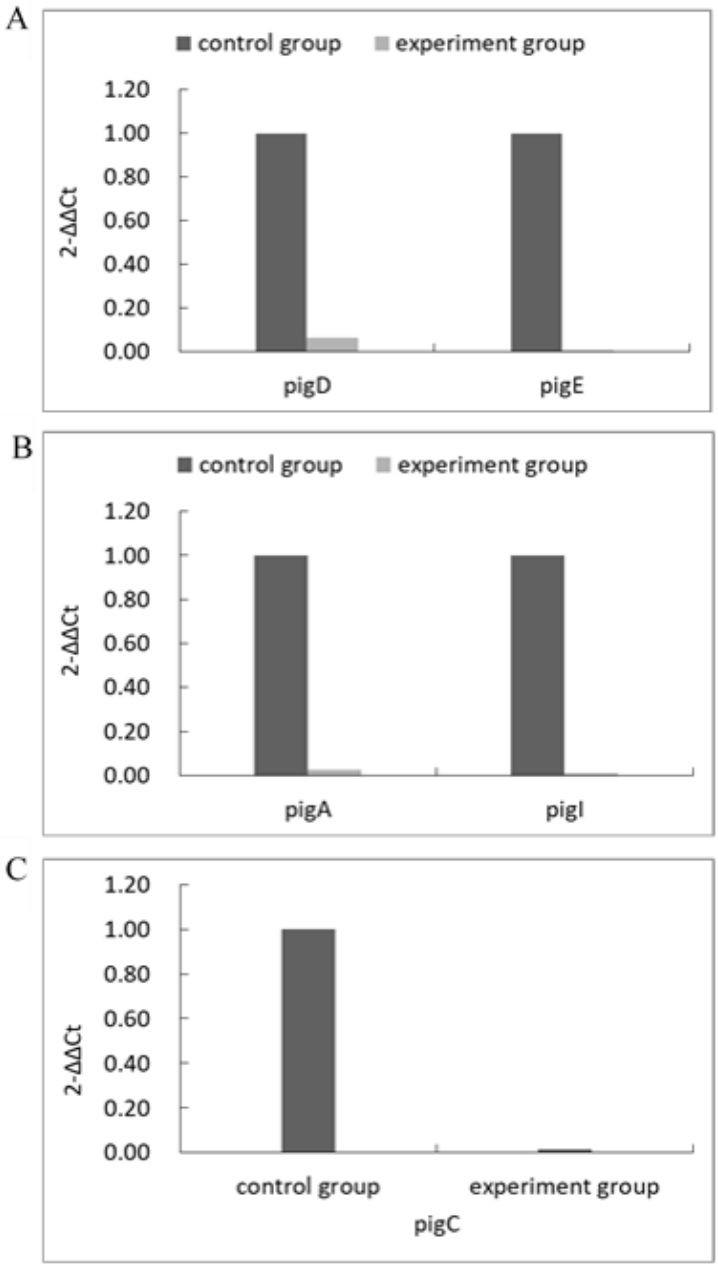


Fig.4 A. Delimitation after ethyl acetate extraction of control group fermentation liquid (left) and experiment group fermentation liquid (right); B. Antibacterial activity detection of ethyl acetate extract; C. Thin layer chromatography of ethyl acetate extract.

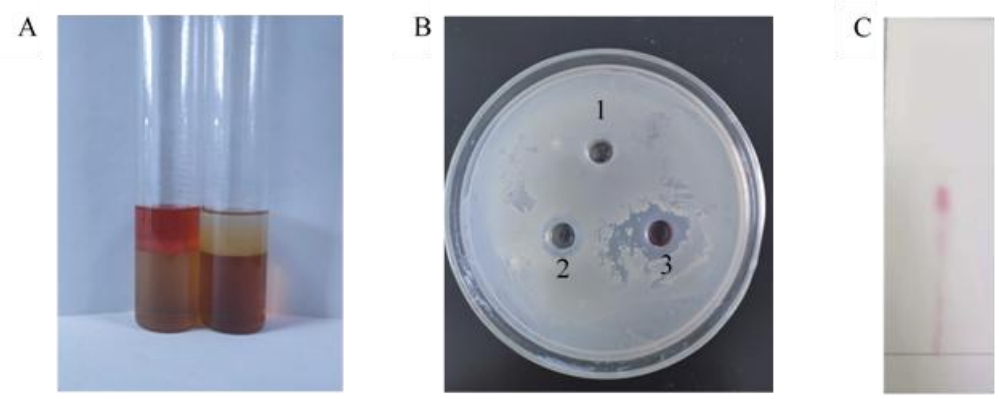
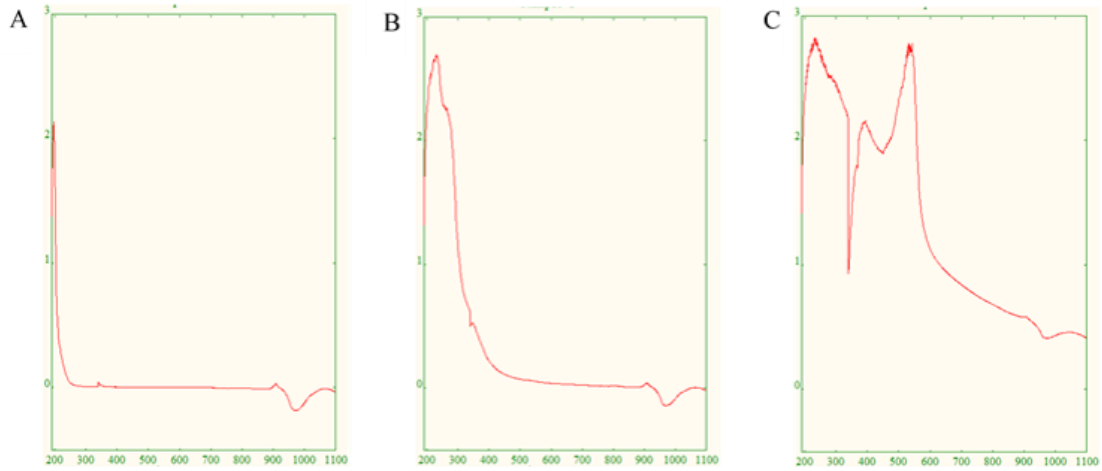


Fig.5 UV scanning spectrum. A. The UV scanning spectrum of pure ethanol; B. The UV scanning spectrum of pure ethanol solution of flocculent substance extracted by ethyl acetatein experiment group fermentation liquid; C. The UV scanning spectrum of pure ethanol solution of flocculent substance extracted by ethyl acetatein control group fermentation liquid.



According to reports, prodigiosin, lipopeptides, carbapenems and chitinases produced by *S. marcences* all have the antibacterial activity besides prodigiosin (Sharmista and John *et al.*, 2019; Liu *et al.*, 2013; Wang and Liu *et al.*, 2006; Zhang *et al.*, 2021). This experiment proved that lipopeptides, carbapenems and chitinases were produced probably in the two kinds of fermentation broth (Bai and Liu *et al.*, 2017; Zhang *et al.*, 2021). Comprehensively consider and analyze the results of three analytical methods, it is inferred that the product in experiment group fermentation liquid was serrawettin W2.

This study focuses on the effect of yeast extract on intracellular metabolic pathway in *S. marcescens*, mainly involving two metabolites of *S. marcescens*, prodigiosin and serrawettins. As microbial natural products with many biological activities, such as anti-cancer, antibacterial and anti malaria, prodigiosin and serrawettins have great application space in the future (Sakai *et al.*, 2019; Yip *et al.*, 2019; Lin *et al.*, 2020). Firstly, the specific substances in yeast extract that inhibit the production of prodigiosin were explored. The results showed that glucose and trace elements had different

inhibitory effects on prodigiosin. It should be noted that the trace element Zn completely inhibited the production of prodigiosin at the concentration of 19.16 mg/l merely. Then, RT-qPCR was used to detect the expression of genes on prodigiosin synthesis pathway in *S. marcescens* between control group and experiment group. The results of RT-qPCR showed that yeast extract inhibited prodigiosin biosynthesis totally. The inhibition mechanism of yeast extract on prodigiosin was inferred that yeast extract greatly promotes the process of microbial primary metabolism and consumes a large amount of nutrients in the logarithmic period. While the secondary metabolite prodigiosin is produced in the late logarithmic period, at this time, a small amount of prodigiosin is synthesized or even not produced due to the lack of carbon and nitrogen source. Finally identified the substance in the fermentation liquid added with yeast extract. According to the UV scanning spectrum, the thin layer chromatography (TLC) and the bacteriostatic test we can infer serrawettin W2, a kind of lipopeptides, was produced in the fermentation liquid added with yeast extract. Serrawettin W2 can be used as a good source of surfactants, potential antibacterial drugs and antitumor drugs. Although yeast extract inhibited the synthesis of prodigiosin, it could promote the synthesis of lipopeptide serrawettins in *S. marcescens*. Therefore, the addition of yeast extract may be used as an optimization technique for the mass production of serrawettins by *S. marcescens*. This is the first time report about the effect of yeast extract on intracellular metabolic pathway in *S. marcescens*. The result of our study provides reference for the industrial production of prodigiosin, lays a foundation for exploring the inhibition mechanism of yeast extract on prodigiosin, and breaks through ideas for the wide application of prodigiosin, serrawettins and many other products produced by *S. marcescens*.

Acknowledgments

This study was financially supported by Sub Project of Science, Education and Industry Integration Innovation Pilot Project of Qilu University of Technology (grant no. 111708) and Qilu University of Technology (Shandong Academy of Sciences) - Industry University Research Collaborative Innovation Fund (grant no.2020-cxy20).

Conflicts of Interests

The authors declare that there is no conflict of interests.

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How to cite this article:

Miao Feng, Yanjun Tian, Jianjun Liu, Yang Liu, Yanhong Huang, Kunfu Zhu, Shanshan Wang, Xingrong Zhang and Hui Xu. 2022. Yeast Extract Inhibits Prodigiosin Production And Promotes Serrawettins Production in *Serratia marcescens*. *Int.J.Curr.Microbiol.App.Sci.* 11(05): 131-143.
doi: https://doi.org/10.20546/ijcmas.2022.1105.017