CLONING AND CHARACTERIZATION OF *swrW* GENE INVOLVED SERRAWETTIN BIOSYNTHESIS OF *Serratia marcescens*

A Master's Thesis Proposal Presented to
The School of Graduate Studies
College of Science and Mathematics
MSU – Iligan Institute of Technology
Iligan City

In Partial Fulfillment of the Requirements
for the Master of Science in Biology

Monabel May Nadayag Apao
May 2010

TABLE OF CONTENTS

CHAPTER 1

Introduction

Background of the Study

Serratia marcescens is a gram-negative bacillus classified as a member of the Enterobacteriaceae. An enteric bacterium recognized as an opportunistic pathogen that has become a serious cause of nosocomial infection, a problem exacerbated by the resistance of many strains to multiple antibiotics (Hejazi & Falkiner, 1997; Aucken & Pitt, 1998). *S. marcescens* produces a range of secreted products, including proteases, nucleases, lipases, chitinases, the biosurfactant serrawettin, and hemolysin (Hejazi. and Falkiner. 1997) many of which are likely to represent virulence factors in human infection (Aucken & Pitt, 1998; Braun *et al.*, 1993; Hines *et al.*, 1988). Swimming and swarming motility and extracellular enzyme activities traits have also been identified that may contribute to pathogenesis.

Biosurfactant production was essential for the population migration behavior of many bacterial species including *S. marcescens* (Matsuyama et al., 1989, 1995; O'Rear et al., 1992; Harris et al., 1998). *S. marcescens* is a dimorphic enteric bacterium capable of undergoing cell differentiation (Harshey, 1994; Liu et al., 2000). Physiological roles of serrawettin have been shown as wetting agent on various surfaces, enhancer of flagellum-independent expansion of bacterial population on agar medium and accelerator of swarming on semi-solid agar medium and antibiotics (Serrawettin 1 has been reported previously as antibiotic serratamolide (Matsuyama et al., 1993). Pigmented *Serratia marcescens* forms a biosurfactant surface active cyclodepsipeptide, serrawettin W1. A single gene *psw*P is responsible for the production of this biosurfactant. Another serrawettin W1 synthetase putative gene *swrW* was identified through genetic analysis of serrawettin-less mutants of *Serratia marcescens* 274.

Bacterial cells can produce and sense signal molecules, allowing the whole popu... initiate a concerted action once a critical concentration of the signal has been reac... a phenomenon known as quorum sensing. *S. marcescens* AS-1 produces *N*-hexanoyl homoserine lactone (C6-HSL) and *N*-(3 oxohexanoyl) homoserine lactone and regulates prodigiosin production, swarming motility, and biofilm formation by AHL-mediated quorum sensing. It has been shown that *Serratia* strains employ quorum sensing for the regulation of genes encoding extracellular virulence factors (Wei and Lai, 2006).

S. marcescens, a temperature-dependent bacterium massively produces serrawettin surfactant. Microbial surfactants like any other biotechnological product have emerged as potential agents in many industrial and environmental processes. Genetically engineered hyper producing organisms giving high yields can bring the real breakthrough in the production process. This is possible only if the genetics of the production is known in details and since genetics of serrawettin synthesis of local strains of *S. marcescens* is not thoroughly studied, cloning and characterization of *swrW* gene particularly is necessary.

Objectives of the Study

The main objective of this study is to clone and characterize the *swrW* gene of the bacteria *Serratia marcescens* that involves in the biosynthesis of prodigiosin. Specifically, it aims to:

1. To determine the sequence of *swrW* gene in local strains of *S. marcescens*;
2. To determine the copy number of the *swrW* homolog in the local strains of *S. marcescens* genome; and
3. To characterize the gene sequence through bioinformatics.

Significance of the Study

The researcher will add a significant knowledge of the genetics of serrawd. n biosynthesis involving *swrW* gene of the local strain *S. marcescens* since there is a limited genetic information of the biosurfactant. Genetically engineered hyper producing organisms giving high yields can bring the real breakthrough in the production process. This is possible only if the genetics of the microbial surfactant production is known in details. Many of these are expected to possess interesting properties as pharmaceuticals and biomedical agents. The knowledge of molecular genetics of microbial surfactant production and its subsequent use to produce hyperproducers will determine the fate of biosurfactant industry.

Characterization of the gene sequence through bioinformatics enables early detection of mis-breeding, genetic drift and enables correlation of genetic information with phenotype. This set of information could serve as a guide in genetic engineering.

Scope and Limitation of the Study

The study will be limited to the local strain of *S. marcescens* from the National Culture Collection, UPLB. Genomic DNA isolation will be done to *S. marcescens* culture and DNA by PCR shall be amplified by following the manufacturer's (QIAGEN PCR Kit) instructions. The DNA fragments generated by PCR will be separated by means of agarose gel electrophoresis. DNA samples will be sent to MACROGEN, Korea for sequencing and sequence analysis will be done using bioinformatics open software (NCBI and EMBL).

CHAPTER 2

Review of Related Literature

Serratia marcescens

Serratia marcescens, a gram-negative bacillus classified as a member of the Enterobacteriaceae, has been known as a cause of hospital-acquired infection for the last two decades. It is a widely dispersed saprophytic bacterium, and has been found in food, particularly in starchy variants which provide, an excellent growth environment.

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: ***Serratia***

Species: ***S. marcescens***

Figure 1.Taxonomic Classification of *Serratia marcescens*

S. marcescens was considered originally to be an innocuous, non-pathogenic saprophytic water organism and was often used as a biological marker because of its simply recognized red colonies. After a review in 1896 of a small number of incidents, Professor Scheurlen of the University of Strasbourg concluded that this organism contributed to more deaths than many pathogenic bacteria. Its ability to cause infection was once thought to be limited to patients with chronic debilitating disorders, but S. *marcescens* has now been implicated as an aetiological agent in every conceivable kind of infection, including respiratory tract infection, urinary tract infection (UTI), septicaemia, and meningitis and wound infections. S. *marcescens* has been

reported to cause infective endocarditis acquired in the community and in hospitals. *S. marcescens* has attained the status of a fully fledged pathogen that causes infections particularly in two disparate groups: heroin addicts and hospitalized patients. The pigmented bacterium is found in various ecological niches, including soil, water, air, plants and animals (Grimont and Grimont, 1984). The ability to form prodigiosin is characteristic of *S. marcescens*, but the function of this red pigment remains unclear because clinical isolates are rarely pigmented.

The general characteristics of Serratia identified were as follows: rod shaped; motile; 0.50.8 - 1.05.0 microns, highly mucoid colonies; facultative anaerobe; gram stain negative; reduce nitrate and indole negative; Vogues-Proskauer positive; and Simmon's citrate positive. *S. marcescens* was also defined by Grimont and Grimont as an oxidase-negative gram-negative bacillus producing DNAase.

Quorum sensing

Bacteria use small molecule signals to communicate with each other. This communication results in a coordinated gene regulation and is generally called quorum sensing. Quorum sensing is an intercellular signalling at high population cell densities and explains many aspects of bacterial physiology observed in single species cultures entering stationary phase in the laboratory. Quorum sensing is used by diverse species to control a multitude of phenotypic traits that often include virulence factors (e.g., exoenzymes) and secondary metabolites (e.g., antibiotics and biosurfactants).

One of the possible quorum sensing-regulated phenotypes is swarming, a flagella-driven movement of differentiated swarmer cells (hyperflagellated, elongated, multinucleated) by which bacteria can spread as a biofilm over a surface. The glycolipid or lipopeptide biosurfactants thereby produced function as wetting agent by reducing the surface tension. Quorum sensing

systems are almost always integrated into other regulatory circuits. This effectively exp range of environmental signals that influence target gene expression beyond population In this review, we first discuss the regulation of AHL-mediated surface migration and involvement of other low-molecular-mass signal molecules (such as the furanosyl borate diester AI-2) in biosurfactant production of different bacteria. In addition, population density-dependent regulation of swarmer cell differentiation is reviewed. Also, several examples of interspecies signalling are reported. Different signal molecules either produced by bacteria (such as other AHLs and diketopiperazines) or excreted by plants (such as furanones, plant signal mimics) might influence the quorum sensingregulated swarming behaviour in bacteria different from the producer. On the other hand, specific bacteria can reduce the local available concentration of signal molecules produced by others. (Daniels et al., 2003)

Serrawettin

S. *marcescens* is exceptional among enteric bacteria in many respects. It secretes extracellular chitinase; several proteases, a nuclease and a lipase (Hines et al., 1988), and produces a wetting agent or surfactant called 'serrawettin' which helps in the colonization of surfaces. In keeping with its varied habitat, *S. marcescens* produces alternate forms of differentially flagellate cells; these display different types of motility depending on whether the growth medium is liquid or solid. Non-flagellate cells of *S. marcescens* can also translocate efficiently over the surface of low-agar media. Matsuyama *et al.* showed that flagellate but non-swarming *dps* mutants of S. *marcescens* with defects in serrawettin production do not swarm on media solidified with Difco agar. The spreading deficiency can be overcome by serrawettin supplied extracellularly. Introduction of *flu* defects into chemotaxis mutants does not affect this mode of surface translocation. (Matsuyama et al., 1985-1992)

Genes involved in regulating the biosynthesis of serrawettin W1 are *pswP* and *swrW*. The *pswP* gene is indispensable for the synthesis of prodigiosin and serrawettin W1 has been shown to be the gene encoding phosphopantetheinyl transferase (Sunaga S et al., 2004). The *swrW* of *Serratia marcescens* has been shown to encode serrawettin I synthetase having an enzymatic multi-domain array characteristic of nonribosomal peptide synthetase (Li H et al., 2005).

In Sunaga et al. result, Serratia marcescens mutants defective in production of the red pigment prodigiosin and the biosurfactant serrawettin W1 in parallel were isolated by transposon mutagenesis of strain 274. Cloning of the DNA fragment required for production of these secondary metabolites with different chemical structures pointed out a novel open reading frame (ORF) named pswP. The putative product *pswP* (230 aa) has the distinct signature sequence consensus among members of phosphopantetheinyl transferase (PPTase) which phosphopantetheinylates peptidyl carrier protein (PCP) mostly integrated in the nonribosomal peptide synthetases (NRPSs) system. Since serrawettin W1 belongs to the cyclodepsipeptides, which are biosynthesized through the NRPSs system, and one pyrrole ring in prodigiosin has been reported as a derivative of L -proline tethered to phosphopantetheinylated PCP, the mutation in the single gene pswP seems responsible for parallel failure in production of prodigiosin and serrawettin W1.

Li et al. showed through the genetic analyses of serrawettin-less mutants of S. marcescens 274, the *swrW* gene encoding putative serrawettin W1 synthetase was identified. Homology analysis of the putative *swrW* demonstrated the presence of condensation, adenylation, thiolation, and thioesterase domains which are characteristic for nonribosomal peptide synthetase (NRPS). NRPSs have been known as multi-modular enzymes. Linear alignment of these modules specifying respective amino acids will enable peptide bond

formation resulting in a specific amino acid sequence. Putative in W1 area uni-modular NRPS specifying only L-serine. Possible steps in this simple unimodular NRPS for biosynthesis of serrawettin W1 [cyclo-(D-3-hydroxydecanoyl-L-seryl) (2)] were predicted by referring to the ingenious enzymatic activity of gramicidin S synthetase (multi-modular NRPS) of Brevibacillus brevis.

Serratia marcescens has been known as a temperature-dependent producer of two chemically different exolipids namely prodigiosin and serrawettin W1 in parallel. In Tanikawa et al., during genetic investigation of such control mechanisms, mini-Tn5 insertional mutant Tan1 overproducing these exolipids was isolated. The gene concerning such disregulation was identified as *hexS* by DNA cloning followed by sequencing and homology analysis of the presumed product with 314 amino acids. The product HexS was the homologue of HexA of *Erwinia carotovora* ssp. *carotovora* and classified as a transcriptional regulator belongning to LysR family. By RT-PCR analysis, the *hexS* mutant was shown to over-transcribe the *pigA* gene (the first gene of the *pig* cluster involved in prodigiosin synthesis) and the *swrW* gene encoding serrawettin W1 synthetase belonging to the nonribosomal peptide synthetase family. In contrast, transcription of the *pswP* gene encoding phosphopantetheinyl transferase in Tan1 was in the level of parent strain 274. Purified protein encoded in *his$_6$-hexS* demonstrated binding activity to DNA fragments of the upstream region of *pigA* and *swrR* genes and not to that of the *pswP* gene. *S. marcescens* strain 274 transformed with a low-copy plasmid carrying *hexS* demonstrated reduced production of prodigiosin and serrawettin W1, and reduced activity of exoenzymes except phospholipase C.

CHAPTER 3

Materials and Methods

Local strains of *S. marcescens* will be obtained from the National Culture Collection, UPLB and local isolates maintained in the Department of Biological Sciences, MSU-IIT.

DNA manipulations and related techniques will be carried out according to standard methods (Maniatis, et.al., 1989).

3.1 Genomic DNA Isolation

An overnight *S. marcescens* strains culture (grown in LB broth in 1.5 ml eppendorf tubes at 37°C with shaking) will be centrifuged at 14,000 rpm for 15 seconds. 310 µl THE buffer will be added to it and the cell pellet will be resuspended by flicking the tube. 350 µl 2% sarcosyl in THE buffer is added and mixing is done by inversion. 5 µl RNase is added and will be incubated at 37°C for 15mins. 35 µl of pronase (or protienase K) is added and will be heated at 50°C until lysis is complete (about 30-90mins). To shear the DNA, the lysate will be vortexed at maximum speed for two minutes. An equal volume of buffered phenol is added and will be mixed by inversion, and will be centrifuged at 14,000 rpm for 5mins at room temperature. The aqueous phase will be removed with a micropipette and will be transferred to new sterile eppendorf. Tubes will be labeled properly. The organic phase will be discarded into the designated container. ½ volume of phenol and ½ volume of chloroform:isoamyl alcohol (24:1, v/v) is added and will be mixed by inversion, and will be centrifuge at 14,000 rpm for 5 minutes at room temperature. The aqueous phase will be transferred to another clean and sterile eppendorf. This step will be repeated until a clean interphase is seen. One volume of chloroform:isoamyl alcohol

is added and will be mixed by inversion and will be centrifuged as above. The aqueous phase will be transfered once again to a sterile eppendorf. 1/10 volume 0.3 M sodium acetate and 2.5 volume cold 100% ethanol will be added, mixed by inversion and placed in the freezer for at least one hour. The tubes will be centrifuged at 14,000 rpm for 30 minutes using the refrigerated centrifuge and will be decanted. One volume of 70% ethanol is added for washing and will be centrifuged at 14,000 rpm for five minutes at room temperature. The ethanol will be decanted and by using a micropipette, any visible alcohol remaining will be removed carefully. In the absence of a vacuum dryer, the open tubes will be allowed to stand in a clean area in the laboratory for a few minutes for drying. The precipitated DNA will be dissolved in 50 µl TE buffer and shall be placed at room temperature for a few minutes to complete dissolution and store in the refrigerator. To check the concentration, 2.0 µl shall be run in agarose gel electrophoresis.

3.2 PCR Amplification

Genomic DNA by PCR shall be amplified by following the manufacturer's (QIAGEN PCR Kit) instructions.

To amplify the swrW fragment from *S. marcescens*, a nested manufacturer's (QIAGEN PCR Kit) PCR protocol shall be followed.

The primers that will be used for PCR amplification of swrW gene are the primers complementary to the conserved published swrW gene sequences of various *S. marcescens* strains downloaded from GenBank.

3.3 Agarose Gel Electrophoresis

The DNA fragments which have been generated by PCR will be separated by means of agarose gel electrophoresis. In preparing the gel, electrophoresis buffer (1x TAE) and electrophoresis-grade agarose (0.8%) will be used by melting in a microwave oven, mixing, cooling at 50°C, and then pouring into a sealed gel casting platform, and inserting the gel comb. After the gel has solidified, the seal from the gel casting platform and the gel comb will be removed. The gel will be placed into an electrophoresis cell containing sufficient electrophoresis buffer (1x TAE) to cover the gel. DNA samples (10 µl) will be loaded into the wells mixed with loading buffer using a micropipettor. Once all the samples have been loaded, electrophoresis (80 V for 2-4 hours) is then followed. The gel is then stained with ethidium bromide for 30 minutes to visualize how the DNA molecules resolved into bands along the gel and it will be viewed under UV using a transilluminator.

3.4 DNA Sequencing and DNA Analysis

DNA samples will be sent to MACROGEN, Korea for sequencing because this method is not available in the Philippines. Sequence analysis will be done using bioinformatics open software (NCBI and EMBL).

Anoop, P.K., Gautham, P., Oommen, V. (2006). Analysis of Serratia marcescens
Identifying the Biosynthetic Pathway of the Pigment Prodigiosin, Iden
Alternative Pathway. Centre for Bioinformatics, University of
Thiruvananthapuram.

Aucken, H.M., Merkouroglou My Miller AW, Galbraith L, Wilkinson SG. (1995)Structu
serological studies of lipopolysaccharides from proposed new serotypes (025 and 0
Sermtia marcescens. FEMS Microbiol Lett; 130

Bolotin, A., P. Wincker, S. Mauger, O. Jaillon, K. Malarme, J. Weissenbach, S. D. Ehrlich, a
S. Sorokin. 2001. The Complete Genome Sequence of the Lactic Acid Bacteriu
Lactococcus lactis ssp. lactis IL1403. Genome Res. 11:731-753.

Braun, V. & Schmitz, G. (1980). Excretion of a Protease By Serratia marcescens. Arch
Microbiol 124, 55–61.

Clegg, S. & Allen, B. L. (1985). Molecular Cloning and Expression of An Extracellular Nuclease
of Serratia marcescens In Escherchia Coli. FEMS Microbiol Lett 27, 257–262.

Coulthurst, S. J., Williamson, N. R., Harris, A. K., Spring, D. R., and Salmond, G. P. (2006).
Metabolic and Regulatory Engineering of Serratia marcescens: Mimicking Phage-
Mediated Horizontal Acquisition of Antibiotic Biosynthesis and Quorum-Sensing
Capacities. Microbiology 152, 1899-1911.

Grimont, P. A. & Grimont, F. (1978). The Genus Serratia. Annu Rev Microbiol 32, 21–248.

Liu, L., Nakano, M., Lee, O.H. and Zuber, P. (1996) Plasmid-amplified comS enhances genetic
competence and suppresses sinR in Bacillus subtilis. Journal of Bacteriology 178, 5144-
5152

Henke, J.M., Bassler, B.L., (2004). Bacterial Social Engagements. Trends Cell Biol. 14, 648–
656.

Hejazi, A. & Falkiner, F. R. (1997). Serratia marcescens. J Med Microbiol 46, 903–912.

Hines, D. A., Saurugger, P. N., Ihler, G. M. & Benedik, M. J. (1988). Genetic Analysis of
Extracellular Proteins of Serratia marcescens. J Bacteriol 170, 4141–4146.

Lerat, E., and Moran, N. A. (2004). The Evolutionary History of Quorum-Sensing Systems in Bacteria. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson. Mol. Biol. Evol. 21(5):903-913.

Li H., Tanikawa T., Sato Y., Nakagawa Y., Matsuyama T. (2005). *Serratia marcescens* gene required for Surfactant Serrawettin W1 production encodes Putatuve Aminolypid Synthetase belonging to Nonribosomal Peptide Synthetase Family. Microbiol. Imunol. 49(4), 303-310.

Maniatis, T., Sambrook, J., and E. F. Fritsch. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Matsuyama, T., M. Fujita, and I. Yano. (1985). Wetting agent produced by Serratia marcescens. FEMS Microbiol. Lett. 28:125-129.

Matsuyama, T., K. Kaneda, I. Ishizuka, T. Toida, and I. Yano. (1990). Surface-active novel glycolipid and linked 3-hydroxy fatty acids produced by Serratia rubidaea. J. Bacteriol. 172:3015- 3022.

Matsuyama, T., K. Kaneda, and I. Yano. (1986). Two kinds of bacterial wetting agents: aminolipid and glycolipid. Proc. Jpn. Soc. Mass Spectrom. 11:125-128. (In Japanese.)

Matsuyama, T., T. Murakami, M. Fujita, S. Fujita, and I. Yano. (1986). Extracellular vesicle formation and bio-surfactant production by Serratia marcescens. J. Gen. Microbiol. 132:865-875.

Matsuyama, T., M. Sogawa, and Y. Nakagawa. (1989). Fractal spreading growth of Serratia marcescens which produces surface active exolipids. FEMS Microbiol. Lett. 61:243-246.

Matsuyama, T., M. Sogawa, and I. Yano. (1987). Direct colony thin-layer chromatography and rapid characterization of Serratia marcescens mutants defective in production of wetting agents. Appl. Environ. Microbiol. 53:1186-1188.

Matsuyama, T., and H. Uetake. (1972). Chromosomal locations of Salmonella conversion phages: mapping of prophages g341, -15F3 in Salmonella anatum. Virology 49:359-367.

Nakano, M. N., M. A. Marahiel, and P. Zuber. (1988). Identification of a genetic locus required for biosynthesis of the lipopeptide antibiotic surfactin in Bacillus subtilis. J. Bacteriol. 170:5662-5668.

Nakano, M. N., and P. Zuber. (1989). Cloning and characterization of srJB, a regulatory gene involved in surfactin production and competence in Bacillus subtilis. J. Bacteriol. 171:5347-5353.

Pitt TL, Erdman YJ (1984). Serological typing of Serratia murcescens. Methods Microbiol; 15: 173-2 1 1.

Schauder, S., Bassler, B.L., (2001). The L...

Stock, I., Grueger, T. & Wiedemann, B. (2003). Nat... Serratia marcescens and The S. liquefaciens ... proteamaculans and S. grimesii. Int J Antimicrob ...

Sunaga, S., Li, H., Sato, Y., Nakagawa, Y., and Ma... 2004. Identification and characterization of the pswP gene required for the ...duction of prodigiosin and serrawettin W1 in *Serratia marcescens*. Microbiol. Jecular ...8: 723-728

Tanikawa T, Nakagawa Y Matsuyama T. (2006). T... ptional Downregulator HexS Controlling Prodigiosin and Serrawettin W1 Biosynthesis in *Serratia marcescens*. Microbiol. Immunol., 50 (8), 587-596.

Thomson, N.R., Cox, A., Bycroft, B.W., Stewart, G.S.A.B., Williams, P. and Salmond, G.P.C. (1997). The Rap and Hor Proteins of Erwinia, Serratia and Yersinia: A Novel Subgroup in Growing Superfamily of Proteins Regulating Diverse Physiological Processes in Bacterial Pathogens. Molecular Microbiology, Vol.26, pp.531544.

Traub, W. H. (2000). Antibiotic Susceptibility of *Serratia marcescens* and Serratia liquefaciens. Chemotherapy 46, 315–321.

Wasserman, H. H., J. J. Keggi, and J. E. McKeon. (1961). Serratamolide, a metabolic product of Serratia. J. Am. Chem. Soc. 83:4107-4108.

Wasserman, H. H., J. J. Keggi, and J. E. McKeon. (1961). The structure of serratamolide. J. Am. Chem. Soc. 84:2978-2982.

Wei, J. R., Lai, H. C. (2006). N-Acylhomoserine Lactone-Dependent Cell-To-Cell Communitcation and Social Behavior In The Genus Serrtia. International Journal of Medical Microbiology 296 117–124

Williams, R. P., Gott, C. L., Qadri, S. M. & Scott, R. H. (1971). Influence of Temperature of Incubation and Type of Growth Medium on Pigmentation In Serratia marcescens. J Bacteriol 106, 438–443.

Williams, P., Camara, M., Hardman, A., Swift, S., Milton, D., Hope, V.J., Winzer, K., Middleton, B., Pritchard, D.I., Bycroft, B.W., (2000). Quorum Sensing and The Population Dependent Control of Virulence. Queen's Medical Centre, University of Nottingham, UK Biol. Sci. 355, 667–680.

Williamson, N. R., Simonsen, H. T., Ahmed, R. A., Goldet, G., Slater, H., Woodley, L., Leeper, F. J. & Salmond, G. P. (2005). Biosynthesis of the Red Antibiotic, Prodigiosin, In Serratia: Identification of a Novel 2-Methyl-3-N-Amyl-Pyrrole (MAP) Assembly

Pathway, Definition of the Terminal Condensing Enzyme, And Implications for Undecylprodigiosin Biosynthesis In Streptomyces. Mol Microbiol 56, 971–989.

Winzer, K., Hardie, K.R., Williams, P., (2003). Luxs And Autoinducer-2: Their Contribution To Quorum Sensing and Metabolism In Bacteria. Adv. Appl. Microbiol. 53, 291–396.

Yanagida, N., Uozumi, T. & Beppu, T. (1986). Specific Excretion of Serratia Protease Through The Outer Membrane of Escherichia Coli. J Bacteriol 166, 937–944.

Zhu H, Sun SJ, Dang HY. (2008). PCR Detection of Serratia Spp. Using Primers Targeting Pfs And Luxs Genes Involved In Al-2-Dependent Quorum Sensing. Center for Bioengineering and Biotechnology, China University of Petroleum (East China), Qingdao, 266555, People's Republic of China. Curr Microbiol, 57(4): 326-30.