ELUCIDATING PATHOGENIC DETERMINANTS IN STENOTROPHOMONAS MALTOPHILIA PATHOGENESIS

RENJAN THOMAS

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STENOTROPHOMONAS MALTOPHILIA PATHOGENESIS

By

RENJAN THOMAS

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in fulfilment of the requirements for the Degree of Doctoral of Philosophy

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In the name of

St. Gregorious Thirumeni of Parumala.
ELUCIDATING PATHOGENIC DETERMINANTS IN 
STENOTROPHOMONAS MALTOPHILIA PATHOGENESIS

By

RENJAN THOMAS

2014

Chairperson: Associate Professor Vasanthakumari Neela, Ph.D

Faculty: Medicine and Health Sciences

Stenotrophomonas maltophilia, Gram negative bacteria has been known to be an 
environmental microbe with numerous biotechnological applications. They are 
ubiquitously found in nature. In recent times, this bacterium has been documented to 
be one of the leading nosocomial pathogen next to Pseudomonas aeruginosa. Owing 
to the high incident rate in hospital setup, they have been ranked as an opportunistic 
pathogen and have been associated with bacteremic infection and pneumonia, both 
with high rate of mortality in immunocompromised patients. Mortality rate has been 
found to be high with patients who have a history of prolonged hospitalization, 
malignancy, neutropenia, immune suppression, breakdown of muco-cutaneous 
defence barriers (e.g., following catheterization, artificial implantation, tracheotomy, 
or peritoneal dialysis), exposure to broad spectrum antibiotics and those requiring 
mechanical ventilation.

Their intrinsic/acquired resistance to most antibiotics and their ability to colonize 
surfaces of medical devices makes S. maltophilia a potentially dangerous pathogen. 
Treatment of S. maltophilia infections is also complicated by the fact that isolates are 
inherently resistant to many of the currently available broad-spectrum agent 
including carbapenems. Whether S. maltophilia clinical isolates are colonizers or true 
pathogens is still controversial.

Despite the increase in the spectrum of clinical syndromes associated with 
S. maltophilia, very little is known about the extracellular enzymes profiles which 
may have potential roles in pathogenesis especially among clinical isolates 
associated with infections. In this study, we screened and compared an array of 
extracellular enzymes in S. maltophilia collected from invasive and non-invasive 
clinical specimens by substrate plate assays. We also grouped the isolates as device 
related and non-device related and compared the enzyme profile. Our study showed 
all clinical isolates produced substantial levels of biochemical enzyme assayed. 
However, lecithinase and heparinase were significantly associated with isolates of 
invasive origin. In contrast, device related and non-device related did not show any
major significant difference. These data suggest that clinical isolates of *S. maltophilia* are a reservoir for pathogenic potential enzymes.

The pathogenic potential of *S. maltophilia* strains isolated from clinical samples were screened for a panel of putative virulent genes such as putative lipase, putative iron complex outer membrane [ICOM], putative siderophore, *lux R*, *toxA*, *piliZ* and *tatD* which were fished out from closely related *P. aeruginosa* genome. The results showed that among the 108 isolates, 57.4%, 10.1%, 0.92%, 57.4% and 74% of the isolates harboured ICOM (n = 62), siderophore (n = 11), *luxR* (n = 1), Lipase (n = 62) and *tatD* (n = 80) harboured these genes. *ToxA* and *piliz* were not found in these clinical isolates. Relative quantification of these putative virulent genes showed ICOM, *tatD* and lipase genes to be overexpressed compared to others. Environmental strain *S. maltophilia* LMG 959 lacked these putative virulent genes.

The role of *S. maltophilia* on macrophages was studied to determine the inflammatory response and to study the phagocytic ability of this bacterium on RAW 264.7 macrophages. Both invasive and non-invasive isolates of *S. maltophilia* were able to enter the macrophage cells. Greater internalization ability was observed by clinical isolates of *S. maltophilia* in comparison to that of the environmental strain. *S. maltophilia* LMG959 (p < 0.05). Although all isolates of *S. maltophilia* gained entry, only the clinical isolates were able to replicate within the macrophages. Environmental strain was unable to replicate within the macrophage. The ability of clinical isolates of *S. maltophilia* to enter and survive the macrophages indicates its resistance to host defence system. Clinical isolates of *S. maltophilia* induced an amplified level of activation within macrophages which triggered immune response compared to environmental strains, as revealed by increased nitric oxide production and CD40 expression. Intracellular survivability of *S. maltophilia* was also ascertained by the presence of several bacteria which were observed as membrane bound. This intracellular phase during infection could play a prominent role in immune evasion and its pathogenicity.

In conclusion, *S. maltophilia* has all the essential qualities to be termed as a serious nosocomial pathogen with the presence of these virulence factors such as the extracellular enzymes and the gene products which could have a deleterious effect owing to the fact that the virulent determinants act in combination. Evading host defences and having intracellular survival ability makes this bacterium a potent and serious nosocomial pathogen.
PENGENALPASTIAN PENENTU KEPATOGENAN YANG TERLIBAT DALAM PATOGENESIS *STENOTROPHOMONAS MALTOPHILIA*

Oleh

RENJAN THOMAS

2014

Pengerusi: Profesor Madya Vasantha Kumari Neela, PhD

Fakulti: Perubatan dan Sains Kesihatan


Meskipun terdapat peningkatan di dalam spektrum yang melibatkan sindrom klinikal dengan *S. maltophilia*, amat sedikit pengetahuan tentang profil – profil enzim luar sel yang berkemungkinan mempunyai potensi di dalam penglibatannya dalam patogenesis terutama sekali pemencilan yang diperolehi daripada jangkitan-jangkitan yang berhubung kait dengan kes klinikal. Di dalam kajian ini, satu siri pelbagai enzim-luar sel yang terdapat pada *S. maltophilia* yang dikumpulkan daripada spesimen klinikal invasif dan bukan invasif disaringkan dengan asai ‘substrate plate’. Semua pemencilan klinikal menghasilkan tahap – tahap asai biokimia enzim yang mempunyai kesan negatif. Walau bagaimanapun, enzim lesiitinase dan heparinase memainkan peranan yang penting di dalam pemencilan secara invasif. Apabila pemencilan – pemencilan ini diklasifikasikan sebagai berkaitan dengan peralatan dan bukan berkaitan dengan peralatan, ia tidak menunjukkan

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sebarang perbezaan signifikasi yang major di dalam profil-profil enzim. Kesemua data ini mencadangkan bahawa pemencilan secara klinik akan *S. maltophilia* adalah lombong untuk mendapatkan enzim – enzim yang mempunyai potensi untuk menunjukkan ciri-ciri patogenik.


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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been
accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.
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AM  Alveolar macrophages
AMP Adenosine monophosphate
APC Antigen presenting cells
ATCC American type culture collection
BCCM Belgian Co-ordinated Collections of Microorganisms
BEC Bladder epithelial cells
BSA Bovine serum albumin
CA Community acquired
CD40 Cluster of differentiation 40
CF Cystic fibrosis
CRP Cyclic AMP receptor protein
CSF Cerebrospinal fluid
DMEM Dulbecco’s modified eagle medium
DNA Deoxyribonucleic acid
DSF Diffusible signal factor
EDTA Ethylene diaminetetra acetic acid
EPS Exopolysaccharide
FACS Fluorescence activated cell sorting
HA Hospital acquired
HCA Healthcare associated
HIV Human immunodeficiency virus
ICOM Iron complex outer membrane protein
IFA Immunofluorescence assay
IL10  Interleukin 10
IL-1β  Interleukin -1beta
IL8  Interleukin 8
iNOS  inducible nitric oxide synthase
LB  Luria bertani
LPS  Lipopolysaccharide
MAMP  Microbe associated molecular patterns
MDR  Multiple drug resistant
MHA  Muller hinton agar
MOI  Multiplicity of infection
NED  N-1-naphthyl ethylenediamine Dihydrochloride
NET  Neutrophil extracellular traps
NGM  Nematode growth hormone
NO  Nitric oxide
OD  Optical density
ORF  Open reading frame
PBS  Phosphate buffer saline
PCR  Polymerase chain reaction
PL  Polysaccharide lyase
PRR  Pattern recognition receptors
QS  Quorum sensing
rRNA  Ribosomal ribonucleic acid
RTI  Respiratory tract infection
SMF-1  S. maltophilia fimbriae 1
SS-PCR  Species specific polymerase
chain reaction
T2S Type II secretion system
TEM Transmission electron microscopy
TLR Toll like receptor
TNF-α Tumor necrosis factor alpha
tRNA Transfer ribonucleic acid
UV Ultraviolet
CHAPTER 1

INTRODUCTION

*Stenotrophomonas maltophilia* is an emerging nosocomial, Gram-negative and multiple-drug-resistant (MDR) pathogen. This non-fermenting, bacilli has undergone numerous taxonomic changes over the time (Palleroni and Bradbury 1993). This bacterium has been associated with serious infections in humans (Sader, Jones et al. 2005; Crossman, Gould et al. 2008) and widely known to be an important nosocomial pathogen in immunosuppressed patients (Almeida, Rubio et al. 2007).

Ubiquitous in nature, *S. maltophilia* are found in a variety of microenvironments and geographical regions and occupies distinct ecological niches such as water, vegetables and soil. Owing to the fact that *S. maltophilia* are environmental bacteria and they inherit the multiple-drug-resistant (MDR) property, these microbes have also been isolated from aqueous sources within and outside the clinical setup. In the environment, it has been isolated from soil (Minkwitz and Berg 2001), plant roots (Berg 2009), animals (Hejnár, Kolář et al. 2010), and invertebrates (Petridou, Filioussis et al. 2010). In hospitals, isolation of *S. maltophilia* has been reported from hospital instruments such as ventilators (Kollef, Silver et al. 1995), central venous catheters (Muder, Harris et al. 1996), arterial pressure monitors, dialysis equipment (Flaherty, Garcia-Houchins et al. 1993), endoscopes (Kovaleva, Degener et al. 2010), hospital suction tubing (Yorioka, Oie et al. 2010), ice machines (Denton and Kerr 1998), tap water (Cervia, Farber et al. 2010), sinks (Brooke, Vo et al. 2008) and disinfectants (Mukhopadhyay, Bhargava et al. 2003). They have also been isolated from surfaces of materials used in intravenous cannulae, prosthetic devices and nebulizers (Denton, Rajgopal et al. 2003).

Although not a primary pathogen, *S. maltophilia* has emerged as an opportunistic nosocomial (hospital-acquired) microorganism. Most commonly *S. maltophilia* infections are associated with respiratory tract infections like pneumonia (Sefcick, Tait et al. 1999) and acute exacerbations of chronic obstructive pulmonary disease [COPD] (Nseir, Di Pompeo et al. 2006), bactereemia (Lai, Chi et al. 2004), biliary sepsis (Papadakis, Vartivarian et al. 1995), infections of the bones and joints, urinary tract, soft tissues (Sakhnini, Weissmann et al. 2002; Landrum, Conger et al. 2005; Bin Abdulhak, Zimmerman et al. 2009), endophthalmitis (Akçakaya, Sargın et al. 2011), eye infections, keratitis, scleritis, dacryocystitis (Mauger, Kuennen et al. 2010; Lin, Ma et al. 2011; Wladis 2011), endocarditis (Takigawa, Noda et al. 2007) and meningitis (Rojas, Garcia et al. 2009). Hospital-acquired infection of *S. maltophilia* has been increasing among immunocompromised population with high rates of mortality ranging from 20 to 70% (Farrell, Sader et al. 2010). Prolonged hospitalization, chemoprophylaxis, heart surgery (Del Toro, Rodriguez-Bano et al. 2006; Paez and Costa 2008) and burns (Tsai, Chen et al. 2006) are major risk factors involved in *S. maltophilia* colonization/infection. High rates of isolation in immunocompromised patients, increasing multidrug resistant strains, and lack of controlled clinical treatment trials makes this bacterium a cause of serious concern (Rolston, Kontoyiannis et al. 2005; Nicodemo and Paez 2007; Safdar and Rolston 2007).
Whether *S. maltophilia* is a colonizer or true pathogen is still unanswered and critical as isolation of pure *S. maltophilia* from lungs of pneumonic patients is difficult (Pathmanathan and Waterer 2005). Production of several extracellular enzymes that includes DNase, RNase, fibrinolysin, lipases, lecithinases, hyaluronidases, proteases and elastases associated with virulence has been reported in *S. maltophilia* infection and pathogenesis (Denton and Kerr 1998; Crossman, Gould et al. 2008). Adherence to biotic and abiotic surfaces, biofilm forming ability, antibiotic resistance to a whole wide group of antibiotics, presence of outer- membrane lipopolysaccharide and resistance to complement mediated killing are other properties that qualifies *S. maltophilia* as a pathogen to initiate and establish infection (Looney, Narita et al. 2009). Besides, studies have shown that *S. maltophilia* has immunostimulatory property to induce tumor necrosis factor alpha (TNF-α) which contributes significantly to airway inflammation (Waters, Gomez et al. 2007), the actual information pertaining to the immune response between host and this bacteria is still lacking. Regardless the association of *S. maltophilia* with several serious illnesses and presence of virulence factors that aids in damaging the host tissues making the host permissive for infection the pathogenicity potential or virulence property is not clearly understood.

In conclusion there is an immediate need to study and characterize the importance of virulence factors involved in the pathogenesis of *S. maltophilia* which is emerging as a significant nosocomial pathogen. In this context, the present study employing clinical isolates of *S. maltophilia* was undertaken to identify and screen isolates for their ability to produce different virulence factors or gene products. Their capability to elicit an immune response was studied.

The specific objectives pertaining to this study are as follows:

1. To determine the extracellular enzyme profiling of *S. maltophilia* isolated from clinical samples.
2. To investigate the prevalence of putative virulent genes in *S. maltophilia* infection.
3. To study the intracellular ability of *S. maltophilia* on RAW 264.7 macrophages and immune response *in vitro*.

**Thesis Organization**

This study was organized into 6 chapters. The chapters are formatted according to the style 2 of the Guide to Thesis Preparation April 2009, School Of Graduate Studies, Universiti Putra Malaysia. Chapters 1 and 2 are identified as introduction and literature review, respectively. Chapters 3 to 5 are identified as research chapters and study stands on its own. Chapter 6 is identified as summary, conclusions and recommendations.
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