Original Study Article
https://doi.org/10.36233/0372-9311-417

Molecular-genetic portrait of virulence of *Stenotrophomonas maltophilia*

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Abstract

Introduction. *Stenotrophomonas maltophilia* is an opportunistic pathogen that is intrinsically resistant to a wide range of antibiotics. The bacterium is associated with a number of serious diseases and makes a significant contribution to the pathogenesis of polymicrobial infections. *S. maltophilia* has a wide range of virulence factors, information about which is currently presented in the form of scattered and unconsolidated data.

Purposes and objectives: critically analyze and summarize current data regarding the molecular-genetic aspects of *S. maltophilia* virulence for better understanding of the pathogenesis of infections associated with this pathogen.

Materials and methods. An analysis of information from 80 modern literary sources devoted to the study of the virulent properties of *S. maltophilia* at the molecular-genetic level has been carried out. The analysis focuses on the mechanisms of production of virulence factors and their genetic determinants.

Results. The molecular mechanisms of virulence that determine the infectious process caused by *S. maltophilia* have been analyzed and summarized, including the adhesive function of the surface structures of the bacterial cell (lipopolysaccharides, pili/fimbriae, flagella), the production of extracellular enzymes, the ability to form biofilms on abiotic surfaces and on the tissues of the macroorganism, the functioning of efflux pumps, secretion of small molecules into the external environment by the intercellular information exchange system Quorum Sensing, as well as the influence of iron metabolism on the virulence properties of *S. maltophilia*.

Conclusion. The adaptation mechanisms that allow *S. maltophilia* to adapt to new habitat niches and survive in the human body and unfavorable environmental conditions have been poorly studied. An analytical review summarizing current information on the molecular-genetic aspects of *S. maltophilia* virulence will be of interest to clinicians and researchers studying the fundamental mechanisms of virulence.

Keywords: *Stenotrophomonas maltophilia, virulence factors, adhesins, biofilms, Quorum Sensing*

Funding source. The work has been carried out with financial support from the Ministry of Health of the Russian Federation under the State Assignment “Molecular-genetic mechanisms of the emergence and loss of antibiotic bacterial resistance in current opportunistic pathogens” (Number in the Integrated state information system for recording research, development and technological work for civil purposes (EGISU NIOKTR No.) 121060200152-8).

Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

For citation: Mikhailovich V.M., Heydarov R.N., Bocharova J.A., Chebotar I.V. Molecular-genetic portrait of virulence of *Stenotrophomonas maltophilia. Journal of microbiology, epidemiology and immunobiology.* 2023;100(5):380–390.
DOI: https://doi.org/10.36233/0372-9311-417
EDN: https://www.elibrary.ru/uvszan

Научный обзор
https://doi.org/10.36233/0372-9311-417

Молекулярно-генетический портрет вирулентности *Stenotrophomonas maltophilia*

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Аннотация

Введение. *Stenotrophomonas maltophilia* является условно-патогенным микроорганизмом, обладающим природной устойчивостью к широкому спектру антибиотиков. Бактерия ассоциирована с рядом серьёзных заболеваний и вносит значимый вклад в патогенез полимикробных инфекций. *S. maltophilia* обладает широким набором факторов вирулентности, информация о которых к настоящему времени представлена в виде разрозненных и необобщённых данных.

Цели и задачи: критически проанализировать и обобщить актуальные данные, затрагивающие молекулярно-генетические аспекты вирулентности *S. maltophilia*, для более глубокого понимания патогенеза инфекций, связанных с этим возбудителем.

Материалы и методы. Выполнен анализ информации из 80 современных литературных источников, посвящённых изучению вирулентных свойств *S. maltophilia* на молекулярно-генетическом уровне. Анализ сфокусирован на механизмах продукции факторов вирулентности и определяющих их генетических детерминантах.

Результаты. Проанализированы и обобщены молекулярные механизмы вирулентности, детерминирующие вызванный *S. maltophilia* инфекционный процесс, включая адгезивную функцию поверхностных структур бактериальной клетки (липополисахариды, пили/фимбрии, флагеллы), продукцию внеклеточных энзимов, способность формировать биоплёнки на абиотических поверхностях и на тканях макроорганизма, фукционирование эффлюкс-помп, секрецию во внешнюю среду малых молекул системой межклеточного обмена информацией Quorum Sensing, а также влияние метаболизма железа на вирулентные свойства *S. maltophilia*.

Заключение. Адаптационные механизмы, позволяющие *S. maltophilia* приспосабливаться к новым нишам обитания, выживать в организме человека и неблагоприятных условиях окружающей среды, изучены недостаточно. Аналитический обзор, обобщающий актуальные сведения о молекулярно-генетических аспектах вирулентности *S. maltophilia*, будет интересен клиническим специалистам и исследователям, изучающим фундаментальные механизмы вирулентности.

Ключевые слова: *Stenotrophomonas maltophilia, факторы вирулентности, адгезины, биоплёнки, Quorum Sensing*

Источник финансирования. Работа выполнена при финансовой поддержке Министерства здравоохранения Российской Федерации по Государственному заданию «Молекулярно-генетические механизмы возникновения и утраты антибиотикорезистентности у актуальных оппортунистических патогенов» (ЕГИСУ НИОКТР № 121060200152-8).

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Для цитирования: Михайлович В.М., Гейдаров Р.Н., Бочарова Ю.А., Чеботарь И.В. Молекулярно-генетический портрет вирулентности *Stenotrophomonas maltophilia*. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2023;100(5):380–390. DOI: https://doi.org/10.36233/0372-9311-417 EDN: https://www.elibrary.ru/uvszan

Introduction

Stenotrophomonas maltophilia is a gram-negative microorganism that is widespread in nature and is often isolated from water sources, soil, and plant and animal samples [1]. According to the Bergey's Manual[1], the genus *Stenotrophomonas* includes three species. Modern alternative taxonomic resources attribute to this genus at least 19 species, which demonstrate a wide variety of metabolic pathways, as well as genetic and phenotypic heterogeneity both within the genus and between strains of each individual species [2–4].

S. maltophilia is well adapted to exist in a variety of habitats, including environments with a low content of nutrient substrates, is capable of utilizing a wide

[1] Palleroni N.J. Stenotrophomonas // Bergey's Manual of Systematic of Archaea and Bacteria. URL: https://onlinelibrary.wiley.com/doi/10.1002/9781118960608.gbm01237

DOI: https://doi.org/10.36233/0372-9311-417

range of carbon sources (including trichlorethylene, gasoline, chloroform), and is naturally resistant to salts of heavy metals [5, 6].

S. maltophilia is an opportunistic agent that is intrinsically multidrug resistant to a wide range of antibiotics. The microorganism is associated with a number of serious diseases and is isolated in respiratory and urological infections, bacteremia, endocarditis, etc. [7]. The bacterium is also of interest as an active member of polymicrobial bacterial communities, which affects the metabolism of surrounding microorganisms, including through antagonistic suppression of representatives of other species (interspecific antagonism). A notable example of such a community is observed in cystic fibrosis, where *S. maltophilia* colonizes the respiratory tract of patients and often coexists with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae, Burkholderia cenocepacia*, nontuberculous mycobacteria, *etc.* [8, 9].

The pathogenetic basis of the bacterium is determined by virulence factors — molecular structures that ensure the development of the infectious process. *S. maltophilia* has a fairly wide range of virulence factors (or factors potentially associated with virulence), which include the surface structures of the bacterial cell (lipopolysaccharides (LPS), pili/fimbriae and flagella, the production of extracellular enzymes (in particular, proteases, elastases, lipases, DNases and RNases, fibrinolysin), the ability to form biofilms on abiotic surfaces and on tissues of the macroorganism and secrete small molecules into the external environment through QS (quorum sensing) systems called "diffusible signal factor" — DSF) [6, 11].

Adhesins as a virulence factor

The key stage of the initial microorganism-host interaction is adhesion, i.e. the attachment of a bacterium to the cells of the macroorganism's tissue. Already at the adhesion phase, bacteria initiate their own biochemical processes aimed at proliferation, invasion, secretion of toxins and activation of response signaling cascades of host cells.

Bacterial adhesion factors (adhesins) are represented by proteins and LPS. Protein adhesins are divided into fimbrial and afimbrial. Lipopolysaccharide and polysaccharide adhesins are associated with the cell membrane (cell wall, outer membrane and capsule). It should be noted that the functions of LPS in pathogenesis are not limited to the primary "bacterium-macroorganism" interaction: their significant role remains at subsequent stages of the infectious process.

LPS (endotoxin) of *S. maltophilia* consists of lipid A, core oligosaccharide, and O-antigen (O-polysaccharide) [12, 13]. Lipid A in LPS is a potential inducer of tumor necrosis factor alpha (TNF-α) production by macrophages, as demonstrated by V.J. Waters et al. in a mouse model [14]. Despite the relatively low invasiveness of *S. maltophilia*, the level of TNF-α after stimulation of the RAW macrophage cell line with purified lipid A of *S. maltophilia* was significantly higher than the level obtained when stimulated with lipid A isolated from the reference strain *P. aeruginosa* PAO1 [14]. Core oligosaccharides play an important role in the formation of LPS structure and, consequently, virulence. It has been established for many microorganisms that defective forms of core oligosaccharides lead to a significant decrease in virulence or the emergence of avirulent strains, for example, *P. aeruginosa* [15] and *Bordetella bronchiseptica* [16]. An equally important contribution to the formation of virulence is made by O-antigens, the complete loss of which or the presence of defects in their structure due to impaired biosynthesis can reduce virulence of the microorganism, which has been demonstrated, in particular, in species *Burkholderia pseudomallei* [17], *P. aeruginosa* [15], *Brucella abortus* [18]. LPS from different *S. maltophilia* strains are highly heterogeneous: at least 31 O-antigen variants are known [19].

A number of genes are involved in the processes of sugar metabolism and their incorporation into LPS in *S. maltophilia*. The *spgM* gene, encoding the bifunctional enzyme phosphoglucomutase/phosphomannomutase, is similar to the *algC* gene responsible for the synthesis of alginate in *P. aeruginosa* [13, 20]. Two operons play an important role in the biosynthesis of O-antigen: *rmlBACD* and *xanAB*. T.P. Huang et al., having performed SDS-PAGE analysis of purified LPS from *S. maltophilia* strains with mutations in the *rmlA*, *rmlC* and *xanB* genes, found that these genes are directly involved in the control of O-antigen biosynthesis, and the *xanB* gene is also involved in the synthesis of the core component of LPS [21]. The authors showed that both operons also influence the synthesis of exopolysaccharides produced by *S. maltophilia*, which are key components of biofilms.

In addition to surface LPS, flagella are involved in the adhesion stage. *S. maltophilia* has from one to several flagella located at the pole(s) of the bacterial cell, which, in particular, contribute to the primary attachment to the cells of the tracheal mucosa of mice and induce a specific immune response of the macroorganism [22, 23]. When BALB/c mice were infected with purified flagellin *S. maltophilia*, an increased level of cytokines was recorded in the animals after 4 hours: interleukins (IL)-1β, -10 and TNF-α. The number of neutrophils, leukocytes and monocytes also increased, which increased nonspecific protection of mice against both *S. maltophilia* and *Staphylococcus aureus* [24].

A. Pompilio et al. compared the severity of the disease in mice after aerosol infection with a wild strain of *S. maltophilia* SM111 and a mutant variant (Δ*fliI*) lacking flagella. The authors found no statistically significant differences in animal weight loss, lung tissue damage, or mortality rates, although TNF-α values

were higher in animals infected with the wild strain. Finally, the authors made a peculiar assumption that the presence of flagella (and therefore motility) may not be associated with the virulent properties of *S. maltophilia* in the pathogenesis of lung diseases [25]. It is hypothetically possible that during a chronic infection, a microorganism lacking such a significant immunogenic factor as flagellin will have advantages by reducing the host's immune response from patients with cystic fibrosis, which, in particular, was observed in non-flagella-producing strains of *P. aeruginosa* [26]. However, most studies indicate a positive correlation between motility and primary adhesion, *e.g.* [22, 23, 27]. Apparently, the above-mentioned assumption about the absence of a relation between the presence of flagella and the virulent properties of *S. maltophilia* can only apply to later, chronic stages of infection, when the stages of adhesion of the microorganism preceding the disease have already been completed.

The motility of *S. maltophilia* and the level of flagellin expression depend on environmental factors and are controlled by a complex and incompletely understood genetic system. It has long been established that cyclic diguanosine monophosphate (c-di-GMP), an important signaling molecule (second messenger) that controls the physiology of the microorganism, its motility and the process of biofilm formation, is involved in the regulation of expression [28]. High c-di-GMP concentration in the cell is associated with decreased motility [29].

The intracellular concentration of c-di-GMP is regulated by changes in the activity of two classes of enzymes: diguanylate cyclases and phosphodiesterases. The former synthesize c-di-GMP from 2 molecules of guanosine triphosphate, and phosphodiesterases hydrolyze c-di-GMP to linear diguanosine monophosphate or guanosine monophosphate (GMP) [30–32].

For a number of microorganisms, some genetic determinants are known, the so-called master regulators and their homologues, which initiate and regulate the expression of flagellin genes, for example, *FlrA* (*FleQ*) in *P. aeruginosa* and *Vibrio cholera*, *flaK* and *flaM* in *Vibrio parahaemolyticus* [33–36]. For *S. maltophilia*, the mechanisms by which c-di-GMP controls the synthesis and number of flagella remain poorly understood, and only a few studies are devoted to the fundamental aspects of their functioning.

In 2014, J. Yang et al. found that the regulation of the expression of flagellar genes in *S. maltophilia* is carried out by the master regulator FleQ (Smlt2295) homologous with *P. aeruginosa* [37]. This transcription factor (enhancer-binding protein), acting in complex with the putative ATPase FleN, is inhibited by binding to c-di-GMP, which in turn leads to a decrease in flagellar gene expression and promotes the initiation of biofilm formation. In the absence of c-di-GMP, i.e. in the unbound state, the situation is reversed: FleQ promotes increased expression of flagellins and, accordingly, reduces the ability to "sedentary" way of vital activity in biofilms.

W. Liu et al. have demonstrated a correlation between increased expression of the *BsmR* gene (regulatory protein, phosphodiesterase with an EAL-binding domain) and increased motility, as well as decreased aggregation ability in the *S. maltophilia* strain CGMCC 1.1788 [38]. Thus, *BsmR* acts as a negative regulator of biofilm formation. The *BsmR* operon controls the expression of at least 349 genes, 34 of which are involved in flagellin synthesis under the positive regulation of the transcription factor FsnR, which initiates transcription by binding to the promoter regions of two operons: *Smlt2303* and *Smlt2318* [39, 40].

In 2022, X. Zhang et al. analyzed genes potentially affecting c-di-GMP levels in *S. maltophilia*, namely those encoding proteins containing the GGDEF, EAL and HD-GYP domains [41]. The authors found 33 genes with the desired sequences in the genome of the microorganism and constructed mutant strains with inactivated genes. 13 out of the 33 mutant strains had reduced motility, indicating the potential role of the corresponding genes in its regulation. In addition, as a result of the analysis of diguanylate cyclases and phosphodiesterases, the authors identified a new Fe^{2+}-dependent phosphodiesterase SisP, which, with an increase in the concentration of iron cations, increased its enzymatic activity in direct proportion, i.e. hydrolyzed c-di-GMP in a dose-dependent manner.

Type 1 fimbriae (SMF-1) play the role of adhesins, ensuring the attachment of *S. maltophilia* to epithelial cells. In particular, adhesion to biotic and abiotic surfaces has been shown to be inhibited in the presence of anti-SMF-1 antibodies [42]. Fimbriae are also involved in haemagglutination and biofilm formation and [42] the administration of fimbrin to BALB/c mice stimulated the production of IL-1β, TNF-α and an increase in phagocyte activity in them [43]. It should be noted that, in contrast to clinical isolates, *S. maltophilia* strains isolated from the environment lacked such fimbriae [44], which suggests their significant role in adhesion/colonization of the respiratory tract in patients with cystic fibrosis. The production of fimbriae in *S. maltophilia* is controlled by the operon *Smlt0706–Smlt0709* [45]. Although the amino acid sequences of fimbrin in *S. maltophilia* are similar to those of pathogenic *E. coli* strains, the N-terminal region of the SMF-1 protein in *S. maltophilia* differs significantly from other bacterial families (50–61% agreement), which suggests a fairly strong phylogenetic distance of this species [42].

Type IV pili also play an important role in the adhesion of *S. maltophilia* to biotic and abiotic surfaces, including the biofilm formation [46]. Despite the fact that type IV pili are considered by many authors as an important virulence factor, no significant correlations between virulence and the presence of the *pil* gene

DOI: https://doi.org/10.36233/0372-9311-417

family associated with pili formation were found in *S. maltophilia*. The phenomenon of the formation of more massive biofilms in strains with increased motility was described by A. Pompilio et al., but this phenomenon was observed in a small sample of strains isolated from sputum samples of patients with cystic fibrosis [5]. Of the 9 strains studied that were not motile, only 2 did not form biofilms. The authors concluded that motility was not a necessary factor influencing the ability to form biofilms. At the same time, *S. maltophilia* strains isolated from other diseases had an increased ability to form biofilms in comparison with isolates from patients with cystic fibrosis. Obviously, it should be clarified here that many authors mean by motility both "swimming activity" and "twitching" of the bacterial cell.

Secretion systems and extracellular enzymes

Clinical strains of *S. maltophilia* produce siderophores, proteases (StmPr1-4), lipases (including phospholipases C and D), nucleases, gelatinase, elastase, fibrolysin/streptokinase, esterases, hyaluronidases, hemolysin and cytotoxins, which act as virulence factors and promote colonization and persistence of the microorganism, participating in adhesion, damage and destruction of host cells, capture of iron ions necessary for bacterial reproduction [47, 48].

Based on genome sequencing data, of the 9 known bacterial secretion systems, systems of types I, II, IV, V and VI were found in *S. maltophilia* [45, 49, 50]. While the role of secretion systems in the formation of virulence is well known for many microorganisms, it was described in sufficient detail for *S. maltophilia* only for systems of types II (Xps type II) and IV.

The clinical strain of *S. maltophilia* K279a has a T2SS system (*gsp* and *xps* genes), through which at least 7 proteins are secreted, including three serine proteases StmPr1-3, which cause a cytotoxic effect in lung epithelial cells, degradation of fibronectin, fibrinogen and IL-8 [51, 52]. The StmPr4 protease produced by *S. maltophilia* is also known, but its functional role has not been clarified yet [53, 54].

S. maltophilia produces 13 potential antibacterial effector proteins. At the same time, the nucleotide sequences encoding them are highly conserved for different strains of *S. maltophilia* [55]. These effectors are produced by the type IV secretion system (T4SS), which is found in both strains isolated from natural sources and clinical isolates of *S. maltophilia* [56]. This system, called VirB/D4 T4SS, is similar to T4SS in bacteria of the closest genus, Xanthomonas, and is encoded by the chromosomal genes virB1-virB11 and virD4. Effector proteins are secreted by the T4SS system into the environment or by direct contact immediately into a competing bacterial cell, thereby realizing interspecific antagonism.

The interspecific antagonism inherent in *S. maltophilia* was elegantly described by M.Y. Nas et al. [56]. The authors showed that *S. maltophilia* strains, using T4SS, caused the death of the natural isolate of *P. aeruginosa* 7700, *P. aeruginosa* PAO1 and *P. aeruginosa* PAK strains. Interestingly, *S. maltophilia* also affects some other species of the genus *Pseudomonas*, but very selectively. For example, *S. maltophilia* kills *P. mendocina*, but not *P. fluorescens*, *P. putida* or *P. stutzeri*.

It is noteworthy that from the above-mentioned 13 antibacterial effectors of *S. maltophilia*, two potential proteins were isolated — RS14245 and RS14255, which have bactericidal properties against representatives of the genera *Pseudomonas* and *Escherichia*. The effectors RS14245 and RS14255, neutralized using blocking proteins, when added to the medium, did not lead to the death of laboratory and clinical strains of *P. aeruginosa* and *E. coli*. Mutant strains lacking these proteins or the T4SS system had significantly reduced bactericidal properties [55].

The data obtained are interesting from various points of view. On the one hand, the secretion of effectors that suppress other bacterial species is a significant factor that increases the virulence of *S. maltophilia*; on the other hand, the isolation and study of such effectors can potentially be used to create new antimicrobial therapeutics for targeted therapy of infections caused by *Pseudomonas* and *Escherichia*.

It should be noted that the T4SS system is used by *S. maltophilia* not only for competitive interspecific inhibition. The products of this system also perform other important functions — they inhibit the apoptosis in host epithelial cells and initiate it in macrophages [56].

Biofilms

During the biofilm formation, the primary adhesion (weak reversible attachment) of planktonic counterparts of *S. maltophilia* occurs within 30–60 minutes. The second stage develops after 4 hours, at which the microorganism is firmly attached to the surface with the participation of semi-flexible fimbriae, flagella filaments and surface LPS. The attached cells begin to produce exopolysaccharides, forming the extracellular matrix, and after approximately 10 hours they form the first surface microcolonies. After 18–24 hours, the process enters the third stage, at which cell differentiation occurs in the maturing biofilms, microchannels are formed for the transport of water, salts, nutrients and the exchange of communication signaling molecules (QS), which will be discussed below. At the last stage, mature biofilms gradually "bud off" planktonic counterparts of bacteria, which, due to their inherent motility, begin to spread and colonize new niches [20].

In 2020, L. Ramos-Hegazy et al., having analyzed the library of mutant transposons they had created, identified the *GpmA* gene encoding phosphoglycerate mutase, a glycolytic enzyme potentially involved in the initial stages of biofilm formation both on polystyrene and on human bronchial epithelial cell lines [57].

S. maltophilia strains with the knocked out *GpmA* gene had a significantly reduced rate of biofilm formation in the first hours compared to the wild-type strain. Interestingly, after 6 hours, the difference in the rate of biofilm formation in the wild and mutant strains was completely leveled, which suggests the participation of the *GpmA* gene as a mediator at the initial stages of adhesion and biofilm formation [57, 58].

A. Pompilio et al. analyzed 85 strains isolated from both cystic fibrosis patients and other infections. The vast majority of all strains (88.2%) formed biofilms in the plate test. At the same time, strains associated with cystic fibrosis demonstrated lower optical density of biofilms, but had greater multiple resistance in comparison with "non-cystic fibrosis" strains [59]. It is likely that enhanced biofilm formation may serve as a protective survival mechanism specifically for susceptible microorganisms.

The analysis of transcriptomic profiles of cells from biofilms (in comparison with planktonic counterparts) showed that only a relatively small proportion of genes are involved in the transition to existence in biofilms: the expression level decreases in 1–3% of genes and increases in 6–9% of genes [60]. Nevertheless, the analysis of the available information on the role of numerous virulence factors leads to the conclusion that the transition of cells from the planktonic lifestyle to the "sedentary" one in biofilms is initiated by many mechanisms that require further study.

Efflux pumps as virulence factors

As a rule, the efflux pumps are considered to be among the mechanisms that provide microorganisms with resistance to antimicrobial therapeutics. In the meantime, the functions of some types of efflux pumps are broader — they go beyond the scope defined by the term "antibiotic bacterial resistance" and are involved in the molecular mechanisms of the formation of virulence properties.

The efflux pumps contribute significantly to the natural resistance of *S. maltophilia* to antimicrobial therapeutics. Pumps of various types found in bacteria remove a wide range of therapeutics: fluoroquinolones, tetracycline and doxorubicin under the control of the SmrA pump; aminoglycosides, macrolides and polymyxins — through the MacABCsm pump belonging to the same family of ABC (from ATP-binding cassette) pumps [61].

The EmrCABsm pump from the MFS (major facilitator superfamily) transporter superfamily is responsible for the removal of nalidixic acid, erythromycin, carbonyl cyanide-3-chlorophenylhydrazone and tetrachlorosalicylanilide) [62]. FusA (ABC type) removes fusaric acid [63]. In addition, *S. maltophilia* has 8 types of RND-type pumps (Sme*), for 7 of which (except SmeMN) their role in the formation of antibiotic bacterial resistance has already been established. In addition to the antimicrobial therapeutics listed above, they are also involved in the transport of sulfamethoxazole, chloramphenicol, trimethoprim and trimethoprim-sulfamethoxazole [64].

It is interesting to note that, besides the known (and currently considered the main) function of removing xenobiotics from the bacterial cell, the efflux pumps SmeYZ and MacABCsm also influence the formation of flagella, the motility of *S. maltophilia* and the formation of biofilms. At the same time, MacABCsm differs from homologous pumps of other microorganisms. In particular, the expression of its operon is constitutive, of an "innate" nature, and the pump has its own original outer membrane protein MacCsm. In addition, in comparison with the homologous MacAB-TolC pump from *E. coli*, it has, as already noted, an expanded spectrum of excreted anitibiotics, including macrolides, aminoglycosides and polymyxins [61].

Another noteworthy function of the efflux pumps was described by C.J. Wu et al., who showed that the SmeYZ, SmeDEF and SbiAB pumps influenced the secretion of the siderophore stenobactin and the utilization of iron ions [65].

For some microorganisms, the role of the efflux pumps, in particular, their outer membrane structures — porins, in increasing the invasive properties of bacteria [66] and protecting the latter from phagocytosis [67], has been demonstrated, but such information has not been published for *S. maltophilia*.

Newly obtained data indicate that our current understanding of the main function of the efflux pumps, which is limited only to the removal of xenobiotics from the cell, is not an absolute dogma and requires critical revision and further study.

Relationship between virulence factors and iron availability

Iron is a vital element for the normal metabolism of non-glucose-fermenting gram-negative bacteria, including *S. maltophilia*. Competition for iron between bacteria and the host cells in chronic infections can negatively affect the host cells. The capture of iron by bacteria can be accompanied by both local tissue damage and systemic damage, for example, severe anemia. Therefore, systems that ensure the capture and transport of iron into the bacterial cell are considered to be significant virulence factors [68].

Siderophore- and heme-mediated transport systems of iron ions into the bacterial cell were found in *S. maltophilia*. The *entAFDBEC* operon encodes the synthesis of the siderophore enterobactin, which belongs to the class of catecholamines, binding and transporting Fe^{3+} into the bacterial cell. The heme-mediated system is under the control of the *hgbBC* and probably *hmuRSTUV* operons [69].

It is very interesting that the iron capture system is not only a virulence factor itself, but can also in-

DOI: https://doi.org/10.36233/0372-9311-417

duce the activity of other mechanisms responsible for virulence properties. This occurs when there is a deficiency of iron in the environment. For example, the microorganism becomes more virulent in lung tissue in the presence of human iron-binding proteins (transferrin, lactoferrin) in the microenvironment, which reduce the level of free iron in the environment [70, 71]. In particular, under iron deficiency, the reference strain *S. maltophilia* K279a produced an increased amount of exopolysaccharides, DSF signaling molecules and formed thicker and more massive biofilms [69, 71]. It has been established that the iron supply system Fur and the transcriptional regulator σ-factor are involved in the regulation of this metabolic change, and the microorganism's response to oxidative stress and the secretion of extracellular enzymes potentially depend on the bioavailability of iron [69, 71].

System Quorum Sensing

Like most gram-negative bacteria, *S. maltophilia* (in particular, reference strain K279a) has a QS system — unique signaling mechanism for intercellular bacterial information exchange [72]. The system is in charge of the production of extracellular signaling molecules called autoinducers, their detection and response (changes in the expression of certain genes) to the emergence of signaling molecules in the environment. Autoinducers accumulate in the environment, and when a certain threshold concentration is reached, the surrounding bacterial cells are able to detect them. Through this exchange of signaling molecules, cells regulate their metabolic mechanisms responsible for colonization and virulence, including changes in motility, biofilm formation, production of extracellular effectors and resistance properties [73, 74].

T.P. Huang et al. found that the main molecule in the QS system of *S. maltophilia* is the autoinducer DSF, which is cis-Δ2-11-methyl-lauric acid, a monobasic saturated fatty acid, the synthesis of which is regulated by the *rpfF* and *rpfB* (from regulation of pathogenicity factors) genes [72]. The *rpf* gene cluster, a regulator of virulence factors, is responsible for the synthesis of own DSF and recognition of "foreign" signaling molecules, for which two variants are known: *rpf1* and *rpf2*, dividing the entire population of *S. maltophilia* into pheno- and genotypically different subpopulations [74]. The *rpf* cluster encodes the synthesis of RpfF synthase and the two-component RpfC/RpfG system, which are responsible for the detection and transduction of DSF. In its active form, RpfG phosphodiesterase hydrolyzes c-di-GMP to linear GMP, thereby regulating the expression of a number of virulence genes [75]. It should be noted here that only strains with the *rpf*-1 gene variant are initially able to produce DSF in detectable quantities without an external stimulus and, therefore, control the biofilm formation, as well as the motility and virulence of surrounding bacteria [74, 76]. In strains with *rpf-2*, the N-terminal (sensory) end of RpfF synthase is shortened. It is assumed that such strains with a reduced sensor domain require preliminary activation from the outside (for example, under the influence of DSF from other bacteria or from *S. maltophilia* strains with the *rpf*-1 system) to produce their own signaling molecules (DSF) into the environment [76].

It is interesting to note that strains carrying the *rpf-2* gene variant (particularly genogroups C) exhibited greater levels of resistance to colistin and increased virulence against *Galleria mellonella* wax moth larvae, which are used as one of the models for assessing virulence. Apparently, this is due to the increased ability of *rpf-2* strains to form biofilms [77]. At the same time, another model for determining virulence, which uses the *Caenorhabditis elegans* nematode, did not reveal such an association [77]. Genotyping and identification of the *rpf* variant are useful and important tools for epidemiological monitoring, which should take into account that *S. maltophilia* strains can potentially exchange *rpf* clusters through recombination during horizontal transfer of genes [76].

It has been fond that *S. maltophilia* has a two-component signal transduction system called BfmA–BfmK (Smlt4209–Smlt4208). The BfmA transcription factor included in the system binds to the *bfmA–bfmK* promoter region and *Smlt0800 (acoT)*, a gene encoding acyl-coenzyme A thioesterase, which is associated with biofilm formation [40].

Unlike *P. aeruginosa*, no full-fledged canonical LuxI/LuxR QS system based on signaling molecules of acyl-homoserine lactones has been found in *S. maltophilia*. However, R. Martínez et al., having performed a comparative analysis of genomes, showed that *S. maltophilia* has a gene *Smlt1839*, similar to the LuxR regulator, encoding the SmoR regulator (*Stenotrophomonas maltophilia* orphan regulator), which *in vitro* bound the synthetic lactone oxo-C8- HSL, a natural analogue of which is synthesized by *P. aeruginosa*. The addition of pooled supernatant of the medium on which *P. aeruginosa*, which produces lactones, was cultivated, gave an impetus to increased motility of *S. maltophilia* on Petri dishes [78]. In other words, despite the absence of the canonical LuxI/LuxR system, its own homologous intercellular exchange systems allow *S. maltophilia* to recognize QS-signaling molecules of other species with the LuxI/LuxR system. It is hypothetically possible that these systems are associated with the T4SS and, under certain conditions, can initiate the secretion of effectors aimed at inhibiting the growth of competitors (see above).

When considering the DSF system in *S. maltophilia*, it is worth mentioning the phenomenon of secretion through outer membrane vesicles [79]. Vesicles are small nanostructures secreted by bacteria that are capable of transporting nucleic acids, proteins and other small molecules, such as β-lactamases. S. Devos et

al. found that the secretion of these vesicles was dramatically increased by *S. maltophilia* in the presence of imipenem [80]. The composition of the detected molecules carried in them is also interesting: these were two types of beta-lactamases encoded by chromosomes, outer membrane proteins and flagellins Smlt0387 and Smlt0184. These flagellins are homologues of Ax21, a protein that influences motility and biofilm formation in *Xanthomonas oryzae*. The functional role of this protein for *S. maltophilia* has not been established yet, but it is assumed that its secretion is initiated by DSF. The system of secretion through vesicles is classified as a potential virulence factor based on data that it affects the motility and biofilm formation of *X. oryzae* [81].

Conclusion

In the last decade, close attention has been paid to the study of the mechanisms of virulence of *S. maltophilia*. The natural multidrug resistance of the microorganism, its rapid adaptation to unfavorable environmental conditions and to new habitat niches, the graceful switching of metabolic processes by the bacterium — all this is of considerable interest both to specialists studying the fundamental mechanisms of virulence and to clinical researchers.

When discussing the virulent properties of *S. maltophilia*, it is necessary to take into account that this bacterium is characterized by pronounced intraspecific variability: strains isolated in one hospital and even from one patient can belong to fairly distant phylogenetic groups and have different phenotypes [4]. The rapid accumulation of adaptive mutations arising under the influence of the selective pressure of hospital conditions or the host cells, and horizontal transfer of genes are considered as probable reasons for such heterogeneity. Understanding the molecular processes that ensure rapid adaptation and, accordingly, survival of the microorganism in unfavorable conditions, will allow detecting potential targets for the development of new antibacterial therapeutics, as well as better understanding interspecific interactions in polymicrobial infections and establishing mechanisms of switching metabolic pathways during the transition of opportunistic pathogens from “natural” lifestyle to infectious intervention.

In this review, we have briefly presented current data on the molecular aspects of *S. maltophilia* virulence factors, without addressing the mechanisms of antibiotic bacterial resistance for the sake of brevity. We hope that the review article will be of interest to molecular biologists, clinical microbiologists and biochemists.

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Участие авторов. Все авторы внесли существенный вклад в проведение поисково-аналитической работы и подготовку статьи, прочли и одобрили финальную версию до публикации.

Статья поступила в редакцию 04.07.2023;
принята к публикации 03.10.2023;
опубликована 28.10.2023

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Author contribution. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published.

The article was submitted 04.07.2023;
accepted for publication 03.10.2023;
published 28.10.2023