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RESEARCH ARTICLE

FUTURISM OF SORTSASE ENZYME FAMILY

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ABSTRACT

Sortases are Gram-positive bacteria extracellular transpeptidase enzymes responsible for covalently attaching secreted proteins to the peptidoglycan cell wall. The name of the enzymes derived from the role the enzymes play in the protein sorting pathway, 'sorting' proteins into the cell wall compartment of Gram-positive bacteria. The peptidoglycan is composed of numerous glycan polymers made up of repeating chains of N-acetyl glucosamine and N-acetylmuramic acid which are cross linked to one another by peptide stems. The peptide stems are generally composed of five amino acids with an inter-peptide branch at position 3. Staphylococcus aureus comprises L-Ala-D-iGlu-L-Lys-DAla- D-Ala with a pentaglycine inter-peptide branching off the L-Lys. These peptide cross-links between the glycan strands vary between Gram-positive and Gram negative bacteria in the inter peptide branching region. Based on the primary sequences around 60% of sortase homologues in Gram-positive bacteria can be clustered into six families but class A and class B sortase enzyme studied in details. Sortase have important role in displaying virulence factors of bacterium which makes them promising drug targets. Class -specific structural features important for molecular basis of substrate recognition. Inhibition of sortase enzyme activity can decrease the virulence of some pathogenic bacteria so sortases are considered as targets for antibacterial drugs and development of new generation of antibiotics against very virulent bacterial species. Sortase enzymes utilized for development of biofilm vaccine, capsular and flagellar vaccine and mutagenic strains have wide industrial application.

Key words: Sortase, Sorting Signal, Bacterial Virulence, Antibiotic Resistance, Drug Targeting.

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INTRODUCTION

Antibiotics are substances used to destroy the bacteria. They are widely used for the prevention, control and treatment of diseases and infections. Improper use of antibiotics in livestock industries leads to accumulation of antibiotics in food products including meat, milk and chicken eggs (Mitchell *et al.,* 1998). Antibiotic resistance can occur through natural mutation and horizontal' transfer of resistance genes between bacteria. Horizontal transmission of resistance genes is a major cause of antibiotic resistance through which bacteria can develop resistance to several different types of antibiotics (Mitchell *et al.,* 1998). The resistance gene transferred between bacteria through transposons and plasmids. Plasmids can carry several resistance genes giving resistance to several different antibiotics. The particular genes that enable some bacteria to resist attack by antibiotics can be transferred to other bacteria of the same or of a different species. Plasmids carrying antibiotics resistance genes have been found in the *Salmonella* and *E coli* isolated from people in Europe, US, Asia and Africa.

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Handling pigs and poultry and working in a farm environment puts people at risk of picking up resistant bacteria from the animal's body or their faeces. Contamination of meat generally results from faecal material getting into the meat during the slaughter and evisceration process (Mitchell *et al.,* 1998). The resistant bacteria can be transferred in water, soil and air. Animals can excrete a significant amount of the antibiotics they are treated with antibiotic making their manure a potential source of both antibiotic residue and antibiotic-resistant bacteria which can enter soil and groundwater leads to persistence of antibiotic resistance for long periods. The highly antibiotic -resistant 'superbug' strain is termed Methicillin Resistant *Staphylococcus aureus* (MRSA) will developed. Sortase enzymes are extracellular transpeptidase with potent N terminal enzymes of Gram-positive bacteria which are responsible for covalently attaching secreted proteins to the peptidoglycan cell wall. The activity of the sortase enzyme was discovered in the early 1990s by Schneewind and co-workers. The first sortase enzyme isolated from *Staphylococcus aureus* in 1999 was named as sortase A (SrtA). The name is derived from the role the enzymes play in the protein sorting pathway and constructing 'sorting' proteins into the cell wall compartment of Gram-positive bacteria. The classification of different types of sortase enzymes are based on sequence

alignment and its biological function. Bacteria have complex cell envelopes which perform a wide range of functions. The composition of the bacterial envelopes varies between species. The Gram-positive cell wall structure differs from Gram-negative bacteria by their peptidoglycan content (Peterson *et al.,* 2004). The peptidoglycan is composed of numerous glycan polymers made up of repeating chains of N-acetyl glucosamine and N-acetylmuramic acid which are cross linked to one another by peptide stems. The peptide stems are generally composed of five amino acids with an inter-peptide branch at position 3. *Staphylococcus aureus* comprises L-Ala-D-iGlu-L-Lys-DAla- D-Ala with a pentaglycine inter-peptide branching off the L-Lysine. These peptide cross-links between the glycan strands vary between Gram-positive and Gram negative bacteria in the inter peptide branching region. Gram-positive stem peptides frequently have a pentaglycine branch and the Gram-negative bacteria have a diaminopimelic acid branch. The bacterial cell envelope is the display of various surface proteins anchored into the thick peptidoglycan layer and plays an important role in bacterial adhesion and development of virulence. Sortase enzymes are 206-amino acid protein with a potential N-terminal signal peptide which act as a membrane anchor and also has a unique cysteine at position 184 The cell wall sorting reaction is sensitive to chemical reagents that modify sulfhydryl groups. Sortase A homologs are present in several Gram-positive pathogenic and some of the beneficial bacterial cell wall. The LPXTG penta protein has been characterized in gram positive organism and all sortase enzymes homolog display absolute conservation of the cysteine codon at position 184 (Spring*et.al* 2011). Most of the sortase enzymes have both protease and transpeptidase activities and sortase enzymes are found to be an membrane associated protein in western blotting. The several LPXTG proteins of Staphylococci contribute to the virulence of *S. aureus* and inactivation of sortase enzyme genes leads to loss of virulence of the bacterium which is very important to control antibiotic resistance in bacterial organism (Danne *et al.,*2012). Proteins targeted for processing by sortases have a cell wall sorting signal (CWSS) at the C-terminus that comprises a pentapeptide recognition motif such as LPXTG and hydrophobic rich region of approximately 20 residues and a lysine/arginine rich positively charged (Paterson *et.al,.* 2004). The proteins are secreted via the Sec apparatus of the general secretary pathway (Schneewind *et al*., 1993).

The role of sortase in infections produced by *Streptococcus pneumoniae* and other Gram-positive bacteria were studied in laboratory animal model. Higher rate of the sortase mutation may be observed in the case of bacteria that have fewer toxins and secreted factors compared with virulent strains. Sortase is an important target to identify inhibitors that could be used for development of an effective antimicrobial therapy.

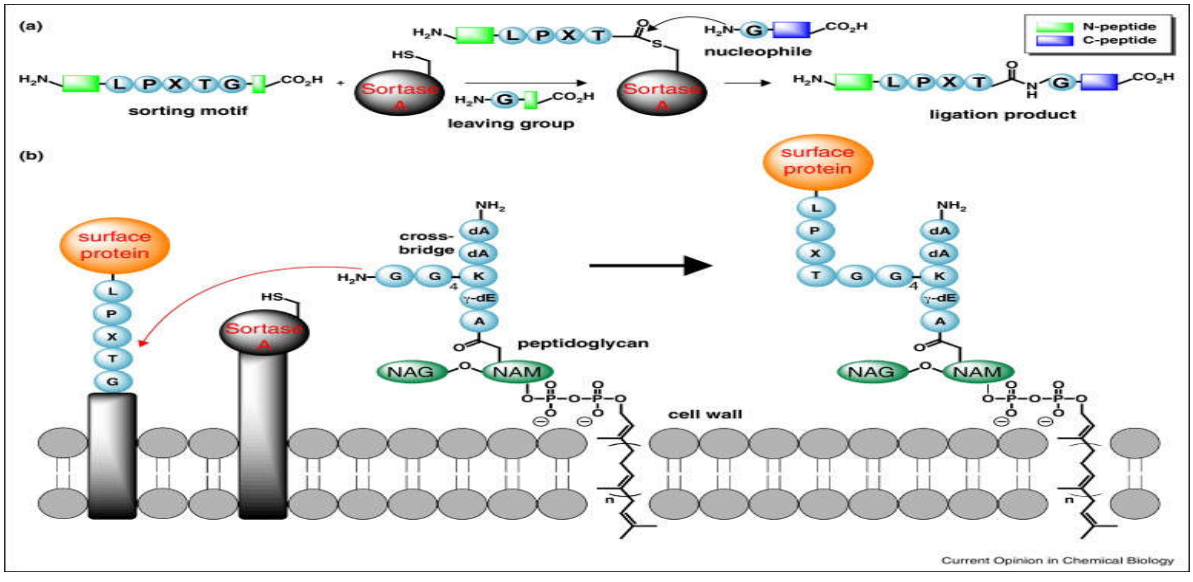
Functions of sortase enzymes

Sortase enzyme families play an important role in cell wall formation of Gram positive bacteria. Sortaseenzymes are ubiquitous in Gram-positive bacteria where they attach proteins to the cell wall and construct pili (Hendrick *et al.,* 2011). Sortase enzymes responsible for virulence, infection and colonization of bacteria (Mandlik *et al.,* 2008). Sortase have important role in displaying virulence factors which makes them promising drug targets. Considerable effort has been put forth to elucidate the molecular mechanism of catalysis, class-specific structural features that dictate function and the molecular basis of substrate recognition. Many of the sortase enzymes serves as the bacterial antigen. Some of the sortase helps for the development of pili and flagella structure of bacteria. Sortase play a major role in bacterial conjugation and promote bacterial adhesion. These enzymes also play a role in polymerization of pilin subunits and promote bacterial adhesion (Mandlik *et al.,* 2008). Some of the sortases function as polymerases, while others attach proteins to the cell wall will require the development of robust biochemical assays to monitor pilus assembly and novel substrate analogs to visualize nucleophile recognition (Mazmanian *et al.,* 2001). Sortase plays intermediate role for development of virulence and transmission of virulence and antibiotic resistance to other bacteria by transposon. Some of the sortase act as a intermediate agent for Immune evasion mechanism of bacteria.

Applications of sortase enzymes

Inhibition of sortase activity may decrease the virulence of some pathogenic bacteria, hence sortases are regarded as targets for antibacterial drugs in case of multi drug resistance bacterial organism. Sortase are important of development of new generation of antibiotics against very virulent bacterial species and of biofilm vaccine, capsular and flagellar vaccine

Sortase enzyme's structure



and bacterial spore vaccine (Kline *et al*., 2009). Sortase mutagenic strains or recombinant strains of bacteria have wide industrial applications. Enzymes used to directly couple the drug–linker to antibodies. Sortase plays a vital role in intestinal mucosal adhesion of Lactobacillus spp which is utilized for mucosal delivery of oral vaccine. Sortase inhibitor compounds identified for controlling multi drug resistance bacterial species (Maresso *et al*., 2008)

Classification of sortase enzymes

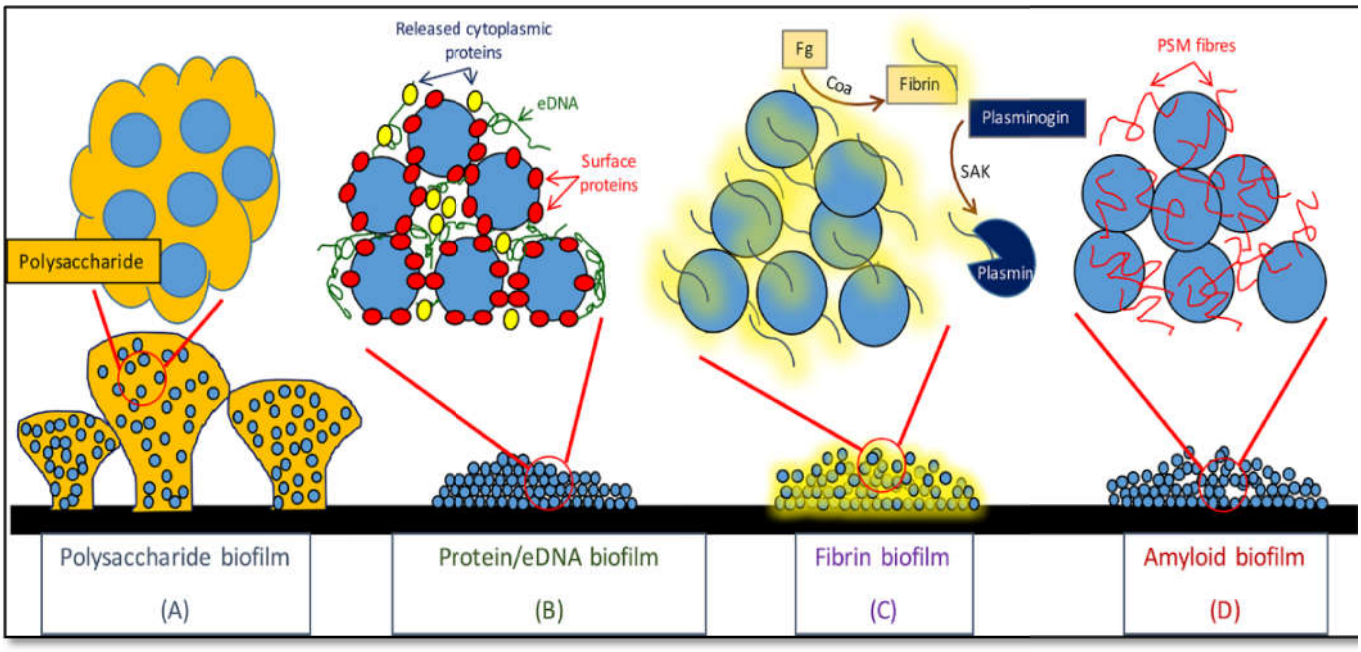
Sortase A: Sortase A enzymes are present in almost all Gram-positive bacteria called as 'housekeeping' sortases which are responsible for sorting the largest number and different types of cell surface proteins. Most of the Firmicutes subfamily contain a single class sortase A and its primary sequence is most closely related to the prototypical Sa-Sortase A enzyme. Detailed research work on Class A sortase enzymes from different bacteria has revealed that each enzyme anchors a large number of functionally distinct proteins to the cell wall (Mazmanian *et al*., 1999). Sortase A performs a housekeeping function in the bacterial cell. The most important enzyme in this group appears to be the sortase A from *Listeria monocytogenes* will display 43 types of distinct proteins. Most surface protein contain a LP*X*TG motif within their cell wall single. Class sortase A which is promotes the bacterial adhesion, nutrient acquisition, host cell invasion and immuno evasion. Sortase A enzymes have significant role in potential drug targets because a number of clinically important pathogenic organism like *S. aureus, L. monocytogenes, Streptococcus pyogenes* and *Streptococcus pneumoniae* uses these sortases to display virulence factors and they are lost their virulence if their sortase A gene is eliminated or inactivated (Schneewind *et al.,* 1993)

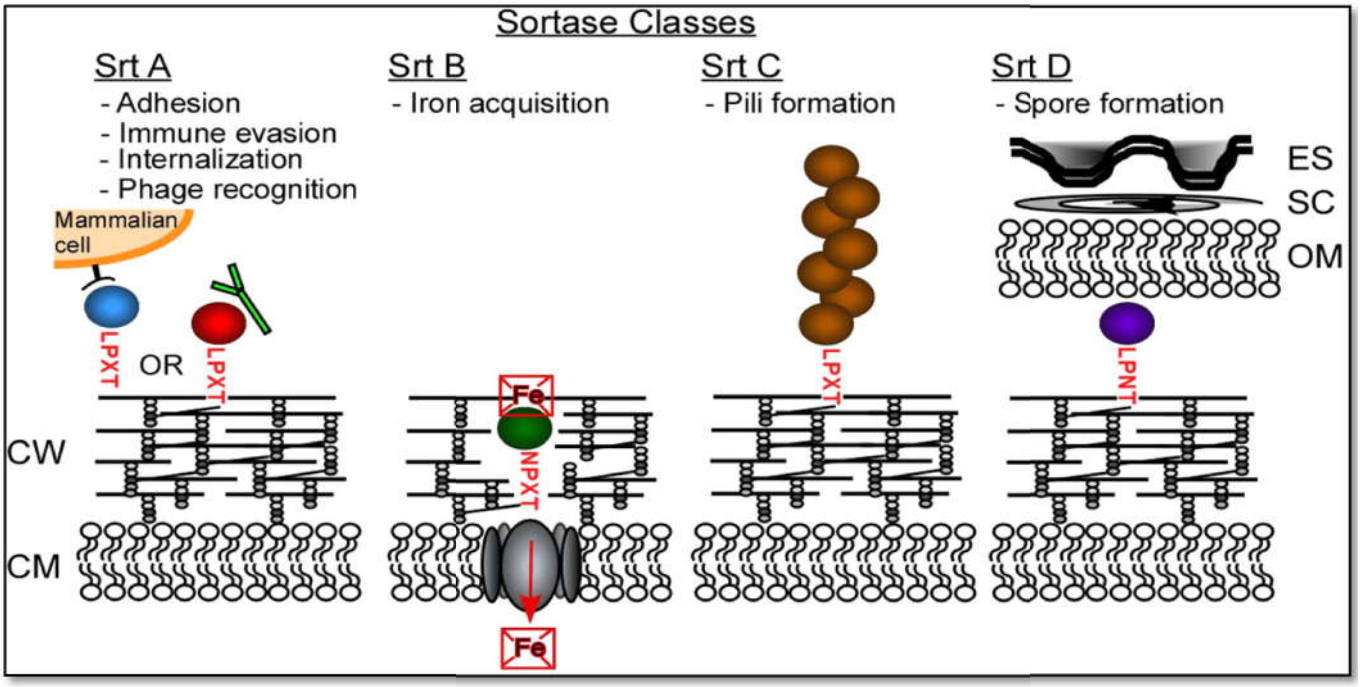
Sortase B enzyme: Sortase B enzymes are responsible for sorting a single substrate (IsdC), sortase B only found in few bacterial species grown under iron deprived condition. Sortase B enzymes are widely distributed in Firmicutes and have primary sequences are most closely related to the sortase B enzyme from *S. aureus* (Mazmanian *et al*., 2001). In some pathogenic microbes class B sortases attach haemoproteins to the cell wall (Maresso *et al*., 2008). Recent studies shows that the Class B sortase enzymes in *S. pyogenes* assemble pili (Kline *et al*., 2009). The function of sortase B mainly based on primary sequence homology. In case of *S. aureus* and *B. anthracis* a single class sortase B enzyme anchors the Isd Chaemoprotein to the cell wall (Mazmanian *et al*., 2001; Maresso *et al*., 2008). IsdC is facilitate haem capture from human haemoglobin by relaying haem from upstream haem-receptors to a membrane transporter complex that imports haem into the cell (Maresso *et al*., 2008). Sa-SrtA and Sa-SrtB both substrate IsdC are expressed only in iron depleted conditions (Mazmanian *et al*., 2008). The class A and B enzymes in *S. aureus* may attach their substrates to different sites within the cell wall. Sa-SrtA attaches its substrates to surface exposed sites that are heavily cross-linked. Protease sensitivity and immunoblot experiments reveals Sa-SrtB attaches IsdC to the peptidoglycan layer which is not heavily cross-linked (Mazmanian *et al*., 2001). Gene knockout studies in *S. aureus* and *L. monocytogenes* indicate that their class B enzymes are needed to establish persistent infections but less important for virulence than class A enzymes (Peterson *et al*., 2004). Recent studies shows the Sa-SrtB enzyme could play a more prominent role in pathogenesis as *S. aureus* has been shown to have enhanced specificity for human haemoglobin such that murine models of infection may underestimate the pathogenic importance of the Sa-SrtB anchored IsdC protein and other iron-regulated surface determinant proteins that participate in the capture of haem-iron from human haemoglobin during infections. Sortases B enzymes are also present in *Clostridium perfringens*, *Clostridium difficile* and *Enterococcus faecalis*.

Sortase C enzyme: Sortase C enzymes act as polymerase enzymes and are co-expressed with pilin proteins to form pili in some Gram-positive bacteria. Gram-positive bacteria uses class C enzymes to build pili that promote microbial adhesion and biofilm formation. The pili extend 0.2–3.0 μm from the cell surface and are assembled in a two-stage process. First, one or more class C enzymes form the long thin shaft of the pilus by linking together pilin subunits through isopeptide bonds. The base of the pilus is anchored to the cell wall by a housekeeping sortase or sortase C enzyme itself and there is great species-specific variation in pilus structure and the mechanism of biogenesis. Pili in Gram-positive bacteria are constructed by two or three types of pilin subunits. In two-component pili the shaft of the pilus is formed by multiple copies of a major pilin subunit and the tip of the pilus contains a single copy of a minor 'tip' pilin that functions as an adhesion molecule. The three-component pili are similar but they also contain a minor 'basal' pilin subunit that is covalently attached to the cell wall. Some pilin subunits within the pilus contain intra-protein isopeptide bonds and this bonds presumably stabilize the structure of the pilus.

Sortase D enzyme: Sortase D enzymes are responsible for sorting and proteins involved in sporulation in sporulating Gram-positive bacteria. Most Gram-positive bacteria have at least SrtA and many have one or more additional accessory sortase enzyme which are more specialized for certain environments. Class D enzymes are present in *B. anthracis* (Schneewind *et al.,* 1993). This microbe encodes a single class D called enzyme (Ba-SrtC) that attaches *BasH* and *BasI*proteins to the cell wall. Deletion of this enzyme sequence reduces the efficiency of spore formation under oxygen limiting conditions**.** The sortase D enzyme attaches *BasI* to the diaminopimelic acid moiety of the peptidoglycan of pre divisional cells and it attaches *BasH t*o the forespore. The *srt C* and *basI* genes are co expressed from the same operon before formation of the septum that divides the mother cell from the fore spore enabling conventional anchoring of the *BasI* protein to the cell wall. In contrast, the *BasH* gene is located elsewhere in the genome and is primarily expressed in the fore spore after septum formation. *BasH* is then presumably attached to the fore-spore by class D enzymes that are transferred to the forespore from the mother cell membrane. *B. anthracis* contains class A and D sortase enzymes that recognize closely related sorting signals. The enzymes function non-redundantly suggesting that they may have evolved a high degree of specificity for their respective sorting signals. Class D enzymes in other bacilli may perform similar functions as genes encoding these enzymes are frequently clustered with genes encoding proteins with a LPNTA sorting signal motif.

Sortase E and F enzymes: Sortase E and F sortases are not commonly present in but some high G + C bacterial species may use a class E enzyme as their housekeeping sortase (Schneewind, 1993). The genes encoding class A and E enzymes are never found in the same organism and the genes





encoding class E enzymes are not positioned adjacent to genes encoding potential protein substrates. Comparative genome analyses suggest that class E enzymes recognize an LA*X*TG sorting signal instead of the canonical LP*X*TG motif processed by class A enzymes. This unique specificity has been demonstrated for the class E enzyme in *C. diphtheria*, which is the only class E enzyme that has been characterized experimentally. Cd-SrtF also attaches assembled pili to the cell wall, In *Streptomyces coelicolor*, class E enzymes display chaplin surface proteins containing an LA*X*TG sorting signal, which presumably mediate aerial hyphae formation. Class F enzymes are present in *S. coelicolor* and other Actino bacteria but the function still unknown. Class D and E sortases were collectively grouped into a single family called class D sortases. The class D and E sortase enzymes share only limited primary sequence homology with one another and are predicted to process distinct sorting signals and genes encoding class E enzymes are not genomically clustered near their substrates.

Sortase as targets of novel therapeutics

Targeting virulence factor of the pathogen is very difficult and complex procedure. *S. aureus* is possessing a vast array of virulence factors ranging from proteins involved in adhesion and immune evasion to ciderophores and toxins formation. Sortase at the centre of a pathway that processes multiple virulence factors, so targeting sortase effectively target a large number of virulence factors simultaneously. Sortases offer a potentially promising target for the treatment of bacterial infections by inhibition of virulence. Sortases are an exclusively bacterial family of enzymes and very specific sortase inhibitors are exhibit particularly deleterious side effects. Cysteine proteases such as calpains, caspases and cathepsin are the functional homologue not structural homologue with sorase enzymes. Antisortase therapy is an important approach for Multi Drug Resistance Species of microbes. The organism must possess a sortase, the sortase must anchor proteins that are essential for virulence or viability

and inhibition of the sortase must abolish the functional presentation of the essential proteins. Different structures of sortases have been determined with a wide range of inhibitors or substrate analogues bound to the active site from which interactions with the protein can be identified and upon which structure-based drug design can be done. **(**Z)-3-(2,5-dimethoxyphenyl)- 2-(4-methoxyphenyl) acrylonitrile are sortase inhibitor and reduce mortality from *S. aureus* in mouse models of kidney and joint infection 3-(4-pyridinyl)-6- (2-sodiumsulfonatephenyl) triazolothiadiazole reduces virulence of *S. aureus*. Sortase inhibitors using bacteriophages has also been explored with *in silico* screening of inhibitor binding. Compounds identified using *in silico* screens and their inhibitory activity demonstrated *in vitro*. *S. aureus* sortase inhibitors are identified and characterized both *in silico* and *in vitro*, more experimental data using *in vivo* model systems plays important role that sortases take in virulence, infection and colonization by a range of pathogens.

Role of sortase in lactobacillus intestinal mucosal adhesion of Lactobacillus

Sortase enzymes are found in all Gram-positive microbes including lactobacillus and other beneficial microbes. Lactobacillus spp. used safely in dairy food industry and consumption of the beneficial bacteria associated with health benefits including competitive exclusion of pathogens and maintenance of epithelial barrier function and maintenance of good gut environment (Vollmer *et al.,* 2008). The sortase enzyme and SDPs in LAB are used to understand mechanisms of host-bacterial interaction. Sortase A enzymes have been identified in a handful of LAB members and sortase C enzyme has only been functionally characterized in *Lactobacillusrhamnosus* GG (Kankainen *et al*., 2009). The sortase cell wall anchoring machinery in *Lactobacillus* used for the development of vaccines which could administered orally as strains generally recognized as safe (GRAS). Surface display is a twofold process composed of both protein targeting and protein attachment to the cell exterior. Protein targeting to the cell exterior is typically achieved through either the secretary (Sec) pathway or the twin-arginine translocation (TAT) pathway (Dieye *et al.,* 2010). The Secretary pathway recognizes unfolded protein targets containing N-terminal leader peptide hydrophobic core and C-terminal sequence that promotes binding of Secretary machinery which depending on the peptide sequence in the C-terminal region. The Proteins that are N-terminally anchored in the membrane and processed by the Sec pathway represent a large proportion of membrane-anchored proteins in lactobacilli. The TAT pathway helps to transport folded protein to the cell's exterior. This pathway appears to be much more uncommon in species of lactic acid bacteria (LAB) and this pathway has only been identified in *Streptococcus thermophilus* and not in lactococci or lactobacilli. Association of these proteins targeted to the membrane and cell exterior can either be achieved through covalent linkages or non-covalent interactions. The N-terminal region of sortasedependant protein contains a signal peptide. This signal peptide enables secretion of the sortase substrate by the Sec pathway and the C-terminal charged tail anchors the substrate once it reaches the cell membrane. The anchoring in the cell membrane by the C-terminal tail brings the SDP and the sortase enzyme also embedded in the cell membrane and it may carry out the transpeptidation reaction required for cell wall anchoring. The first step in the transpeptidase reaction is the cleavage of the sortase substrate between the glycine and the threonine residue forming a sortase enzyme and SDP complex. The resulting thioester acyl bond between these two proteins is subjected to nucleophilic attack and subsequent linkage to lipid II. The lipid II is composed of both the peptidoglycan precursors, *N*-acetylglucosamine and *N*-acetylmuramic acid as well as the pentapeptide peptidoglycan cross bridge. The SDPs have been shown to link specifically to the pentapeptide (Spirig *et al.,* 2011). Once linked to the cross bridge, SDPs are incorporated into the cell wall with lipid II as it is translocated to the outer surface of the cell.

The conserved C-terminal anchor motif recognized by sortase in Gram-positive microbes used for antigen display in vaccine development. The use of the LPXTG motif has been investigated for oral vaccine delivery using food grade probiotic lactobacilli as the presentation vector for the antigen. Food grade LAB and notably probiotic lactobacilli present an alternative delivery vehicle as they have a safe to use in foods and dietary supplements are able to survive passage through the GIT for vaccine delivery to the mucosal immune system. Lactobacillus have Sec and C-terminal cell wall anchoring machinery which can be exploited for antigen immobilization. LAB and sortase-mediated cell wall anchoring have been studied in the display of potential vaccine antigens including tetanus toxin fragment C and human papillomavirus type 16 E7 antigen and *Salmonella enterica* serovar *typhimurium* flagellin (Dieye *et al.,* 2010). The functionality of sortase-mediated cell wall localization in Lactobacillus was demonstrated through display of the M6 protein in LPXTG-anchored virulence factor of *S. pyogenes,* in *L. lactis*. The M6 protein was also successfully displayed on the cell wall of other LAB including *L. fermentum* LEM83, *L. sakei* 23K, and *S. thermophilus* CNRZ302 (Roos *et al.,* 2002). The antigenic display was achieved through cloning and expression of the gene encoding the M6 protein into the LAB and then examining the distribution of the M 6 protein using Western blot analysis. Recent research showed inefficient cell wall localization of their reporter protein, staphylococcal nuclease A, in *L. lactis* when it was coupled to the M6 protein cell wall anchor and signal peptide by switching the signal peptide to one of lactococcal origin (Usp45). The M6 protein was efficiently displayed in *L. lactis* as well as in other LAB including *L. casei*, *L. sakei*, and *L. plantarum*. The sortase machinery is functionally different across LAB and have the capacity to recognize substrates from an unrelated microbe. Localization of antigen to the cell wall had been shown to not only be effective and increases immune responses compared to the intracellular or secreted form of the antigen (Roos *et al.,* 2002). Sortase enzymes have mechanism for display of cell surface proteins, it is very important for commensal and probiotic microbes associated with the intestinal mucosa. The sortase machinery present in *Lactobacillus spp.* will be utilized for oral vaccine delivery and the antigen display in intestinal mucosa. Sortase enzymes plays an crucial roles in bacterial physiology as well as mediating bacterial-host interactions in different species of Lactobacillus (Dieye *et al.,* 2010). The ability to access and examine sortase enzymes and their targets using genomic analysis tools has been crucial. The mechanism of sortase action and covalent linkage of SDPs to the cell wall is a successful method of surface display in Gram-positive bacteria which has enable some pathogenic organisms to gain advantage of their host. The genetic engineered recombinant strain of *L. acidophilus* expressing the *Salmonella* flagellin (FliC) which was covalently linked to the cell wall using an LPXTG motif and potential of FliC to serve as a vaccine

adjuvant for LAB vaccines. Protection of *Salmonella* flagellin fragment on the surface of *L. acidophilus*, the recombinant *L. acidophilus* cell suspensions were supplemented with either sodium bicarbonate or soybean trypsin inhibitor (SBTI). Both of these treatments were found to protect the antigen from degradation when incubated in simulated gastric juice. SBTI has a greater protective effect likely due to its sequestration of bile away from the bacterial cells thus contributing to increase viability and robust antigen production (Dieye *et al.,* 2010).

Industrial applications

Sortases enzyme family have been utilized for a wide range of industrial applications. 'Sortagging' is a process in which a protein is tagged with a cell wall synthesizing sortase and a sortase is used to ligate it to another protein. This allows the conjugation of two or more proteins in a very specific way with a potentially powerful technique (Ritzefeld, 2014). A wide range of uses of sortagging include immobilization of proteins on a surface and labelling of proteins for immune diagnostic. Protein cyclization and protein dimerization and engineering of sortases and substrates for specific functions is potentially important field. New method has been developed for the 'evolution' of sortases that generated a novel Sa- SrtA mutant with over 140 times higher activity of the wild-type and the protein can be entirely chemically synthesized. Heck et al (2014) developed a simple assay for monitoring enzyme activity on a solid support that was demonstrated with Sa-SrtA. In addition to show-some simple assay was developed for monitoring enzyme activity on a solid support that was demonstrated with Sa-SrtA. Sortase A important for attaching substrate in solid surface as well for assessing sortase activity which could be of use in a variety of industrial applications and assessing sortase inhibitor efficacy. Sortase mediated biotin labelling, sortase mediated fluorescent labelling, antibody sequencing, cell based ELISA, therapeutic Mabs and viral vaccine

Future research focus on sortase enzyme family

- Further molecular structural analysis of additional sortase proteins with and without substrate and activators has to be studied in future
- Determination of the substrate specificity of different sortase enzymes should be analysed
- Structural and functional basis for this specificity and the role of LPXTGase and its inhibitor in cell wall anchoring should be further analysed.
- High throughput screening for chemical and natural sortase inhibitors and their evaluation in animal models.
- The function of many LPXTG proteins is unknown so there is a need to characterize their activities.
- Presence of sortase enzyme in other than gram positive bacteria has to be explored
- Solid surface synthesis and Applications of sortagging activity of sortase enzymes to be an extremely useful and versatile molecular biology tool.

Conclusion

Sortase enzyme assembles the surfaces of Gram-positive bacteria with proteins that play key roles in microbial physiology, pathogenesis and virulence development. Genome sequencing efforts have identified nearly a thousand sortase homologues whose functions are only discovered. All sortases characterized based on cysteine transpeptidases that either covalently join proteins to the cell wall peptidoglycan or link proteins together to construct pili. Based on the primary sequences around 60% of sortase homologues in Gram-positive bacteria can be clustered into six families (class A to F enzymes) Class A and C sortases have been studied extensively. Many class Asortase enzymes appear to have a housekeeping role in the cell wall and attach a large number of functionally distinct proteins to the cell wall and class C enzymes useful for assemble pili. In structure-function studies of sortase will performed to identify enzyme determinants that control substrate specificity and to understand sortases selectively route different proteins to the cell surface by recognizing their unique sorting signals. It will also be important to learn the molecular basis through which sortases work with other enzymes and accessory factors on the cell surface and better understanding of the targeting and retention mechanisms of these proteins as well as the generation of robust *in vitro* systems to study pilus assembly.

Sortase enzymes are important for display of cell surface proteins which is a significant niche related trait of commensal and probiotic microbes associated with the intestinal mucosa. Sortase enzymes play crucial roles in bacterial physiology as well as mediating bacterial-host interactions has accelerated the study of this enzyme in different species of Lactobacillus. The mechanisms of sortase action and covalent linkage of sortase dependent protein to the cell wall is a successful method of surface display in Gram-positive bacteria which has enabled some pathogenic organisms to gain advantage of their host. Sortase based assay development have very wide industrial application. The sortagging activity of sortase enzymes to be an extremely useful and versatile molecular biology tool but very minimum research work carried out in related to sortase enzymes but in future sortase plays vital role for per oral vaccine delivery system and navel antimicrobial therapy for multi drug resistance species.

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