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ORIGINAL ARTICLE

Secretome of *Paenibacillus* sp. S-12 provides an insight about its survival and possible pathogenicity

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Abstract

Our aim in this study was to characterize and investigate the secretome of *Paenibacillus* sp. S-12 by nanoLC-MS/MS tool-based analysis of trypsin digested culture supernatant proteins. Using a bioinformatics and combined approach of mass spectrometry, we identified 657 proteins in the secretome. Bioinformatic tools such as PREDLIPO, SecretomeP 2.0, SignalP 4.1, and PSORTb were used for the subcellular localization and categorization of secretome on basis of signal peptides. Among the identified proteins, more than 25% of the secretome proteins were associated with virulence proteins including flagellar, adherence, and immune modulators. Gene ontology analysis using Blast2GO tools categorized 60 proteins of the secretome into biological processes, cellular components, and molecular functions. KEGG pathway analysis identified the enzymes or proteins involved in various biosynthesis and degradation pathways. Functional analysis of secretomes reveals a large number of proteins involved in the uptake and exchange of nutrients, colonization, and chemotaxis. A good number of proteins were involved in survival and defense mechanism against oxidative stress, the production of toxins and antimicrobial compounds. The present study is the first report of the in-depth protein profiling of *Paenibacillus* bacterium. In summary, the current findings of *Paenibacillus* sp. S-12 secretome provide basic information to understand its survival and the possible pathogenic mechanism.

Keywords Proteins · Secretome · Virulence · *Paenibacillus* · Pathogenicity

Introduction

In bacteria, the secretome contains several secreted proteins that are involved in cell-to-cell communication, adhesion, stress response, and other important processes related to the outer environment and the organism life style (Lee and Schneewind 2001). The secretome of a microbe represents several proteins targeted to the envelope/surface of bacterial cells or secreted into the extracellular environment. The surface proteins of bacteria facilitate their attachment to other bacteria or to the environmental surfaces, thereby assisting in the colonization of an environment. The various polymeric molecules get converted into mono- or oligomeric compounds by the secreted enzymes of the secretome that further can be absorbed by the microbes to use as carbon and nitrogen sources for growth (Gagic et al. 2013). Secretome keeps the dominant target of the host immune response and that are interested in the development of vaccines (Gagic et al. 2016). Secretome includes various surface-associated pili and flagella proteins, which play an important role in horizontal gene transfer, motility, and a bacterial surface attachment (Van Gerven et al. 2011).

Gram-positive (+ve) bacteria secrete proteins mainly via the general secretory system or sec-dependent pathway (Schneewind and Missiakas 2012) or they can also secrete by the Tat (twin-arginine transport) pathway (Goosens et al. 2014). Secretome proteins are exported to the bacterial periplasmic space with the assistance of signal peptides and are synthesized by N-terminal extensions. The major group of periplasmic proteins is transported by the general secretory or sec pathway (Pugsley 1993; Danese and Silhavy 1998). In Gram-negative (−ve) bacteria, the secretome proteins are secreted by specific pathways that are involved in detoxification of the external space and virulence,

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cell-to-cell communication, and killing of competitive bacteria (Tjalsma et al. 2004).

Paenibacillus species is a Gram-positive, endospore-forming, facultative anaerobic bacteria (Xie et al. 2012). It is included within the genus of *Bacillus* and then further in 1993; it was reclassified into separate groups (Elo et al. 2001). The *Paenibacillus* species have been isolated from different environments, many of which belong to humans, plants, and the environment (Sáez-Nieto et al. 2017). The major group of them is rhizobacteria that promote plant growth. Antimicrobial compounds are produced by many species of *Paenibacillus* that are useful for medicines or as pesticides, and it secretes many enzymes that are utilized in bioremediation or for the production of valuable chemicals (Grady et al. 2016). One of the *Paenibacillus* species, *P. polymyxa*, can fix nitrogen, inhibit the growth of plant pathogens, release phosphorus, and increase the porosity of the soil; thus, it can be considered a major part to enhance plant growth (Rivas et al. 2005).

The continuous use of antibiotics increases the global resistance to commercially used antibiotics that lead to the development of new infections, so it necessitates discovering new proteins through the mining of secreted novel proteins (Monciardini et al. 2014; Butler et al. 2006). Microorganisms found in extreme habitats show higher production of secondary metabolites under abnormal conditions; therefore, they represent unique sources of new antimicrobial compounds with target activity (Hemala et al. 2014; Sanchez et al. 2009). In this paper, we showed interest in *Paenibacillus* bacterium because previous studies suggested their importance for industrial (*P. amylolyticus*, *P. algorifonticola*, *P. chitinolyticus*, *P. dendritiformis*, *P. xylanilyticus*), agriculture (*P. alvei*, *P. ehimensis*, *P. riograndensis*, *P. polymyxa*), and in medical applications (*P. peoriate*). In a previous study (Ghio et al. 2018), secretome analysis of xylan cultures of *Paenibacillus* sp. A59 identified ten glycoside hydrolases and three endoxylanases, efficiently releasing soluble sugars from the hemicellulose fraction and can therefore be used to study xylo-oligosaccharides (XOS) production for prebiotics development. A recent study (Zainal Baharin et al. 2022) demonstrated that secretome proteins of *P. polymyxa* Kp10 (Kp10) showed strong inhibition against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE).

Many strains of *Paenibacillus* produce antimicrobial compounds, which showed their activity against a multidrug-resistant group of Gram-negative bacteria (Qian et al. 2012; Zengguo et al. 2007). Members of this genus have been isolated from various areas, including agricultural soil (Kim et al. 2016), rhizosphere (Son et al. 2010), desert (Lim et al. 2006), human feces (Clermont et al. 2015), and eutrophic lake and glacier (Montes et al. 2004; Kishore et al. 2010). A more expedient reason to study the secretome of *Paenibacillus* bacterium was to identify the most abundant proteins released which may be the suitable candidate to develop new diagnostic kits and possible new treatments. The MS-based protein identification has certain limitations like low-abundance, low molecular weight, and unstable proteins are less likely to be detected. Additionally, glycosylated proteins and proteins responsible for adhering to the cell wall may also escape detection. The secreted proteins like proteases, lipases, and cytotoxins may cause life-threatening infections in immunocompromised patients suffering from cystic fibrosis and chronic granulomatous (Carvalho et al. 2007). Little knowledge is available on the protein secretion of *Paenibacillus* species. Therefore, we investigated the secretome of *Paenibacillus* sp. S-12 by nanoLC-MS/MS tool-based analysis. Secretome profiling may be helpful in the identification of secreted proteins involved in virulence, metabolic pathways, and transporters and also the proteins involved in the survival of test organism in different environments.

Materials and methods

Bacterial culture and preparation of secretome

The bacterial strain *Paenibacillus* sp. S-12 was isolated from the rhizospheric soil of *Rauwolfia serpentina* following the serial dilution method. Its whole genome sequence was submitted with BioProject accession no. PRJNA861075. After the culture growth, a loopful of the bacterial colony was inculcated into LB-broth (HiMedia, India) medium and kept for incubation at 37 ℃ with shaking at 180 rpm. The culture was allowed to grow up to late exponential growth phase and centrifuged at 8,000 g at 4 ℃ for 30 min. The obtained supernatant was filtered to remove the presence of any bacterial cells using filters of 0.2 µm (Merck Millipore, USA) pore size. The test isolate was independently cultured three times and pooled the culture filtrate proteins together.

Protein concentration and digestion

The proteins in the supernatant were precipitated by employing the standard trichloroacetic acid (TCA)/acetone method (Lakshman et al. 2008) with minor modification. The TCA/acetone (10% *v*/*v*) solution containing 2-mercaptoethanol (0.07% *v*/*v*) was added to the culture in a 1:3 ratio and kept at – 20 ℃ overnight. The precipitated proteins were further dried using a speed-vac system for 30 min. The obtained dried secretome was resuspended in 1 mL of 100 mM NH_4HCO_3 (pH 8.0) and gently sonicated in an ice bucket till solubilization. The reduction was performed with dithiothreitol (DTT; 10 mM) at 30

℃ for 1 h, and further, alkylation was performed with iodoacetamide (40 mM) at room temperature for 30 min. Following reduction and alkylation, the protein sample was added to urea/thiourea (1 M/0.3 M) solution and trypsin at a concentration of 16 ng/µL.

nanoLC-MS/MS analysis

The digested secretome proteins (1 µg/µL) were analyzed using nanoLC-MS/MS. Desalting was performed on the enrichment C18 column (0.3 × 0.5 mm, Agilent) using 1% acetonitrile/0.5% formic acid as eluent followed by separation on Zorbax C18 column (75 µm × 150 mm, Agilent) using gradually increased concentration (5 to 40%) acetonitrile within 2 h, at a flow rate of 200 nL/min. The eluted proteins were applied to a mass spectrometer using the nanospray (Thermo Fischer Scientific, USA) with a fixed spray voltage of 1.8 kV. The regeneration step was comprised of 90% eluent B and an equilibrium step at 10% eluent B with one process taking 20 min. The fragmented mass spectra were acquired in AutoMS mode with a scan range of 300–1500 m/z. MS1 spectra were acquired in the Orbitrap (Max IT = 60 ms, AGQ target = 300%, RF lens = 70%, R = 60 K, mass range = 375–1500). Dynamic exclusion was employed for 30 s excluding all charge states for a given precursor. MS2 spectra were collected for top peptides (Max IT = 60 ms, R = 15 K, AGQ target = 100%). LC–MS/MS data were analyzed using Mascot software (Perkins et al. 1999). The peptide mass fingerprint data were searched against the Mascot database search (http://www.matrixscience.com) of the National Center for Biotechnology Information (NCBI) database. Protein identification was validated by Proteome Software (V.3.3.3) using the Protein Prophet algorithm. For accuracy, the identification of at least two peptides with a probability of > 95% and a total protein probability of > 95% was employed.

Bioinformatics analysis

The functional annotation was performed using the Gene Ontology using Blast2GO (Conesa et al. 2005), and annotation was performed using default parameters (Myhre et al. 2006). The prediction of non-classical secreted proteins and classical secreted proteins with a signal sequence was performed by Secretome 2.0 server (http://www.cbs.dtu.dk/service/SecretomeP) and SignalP 4.1 (Petersen et al. 2011) (http://www.cbs.dtu.dk/service/SignalP), respectively. The subcellular localization and trans-membrane helices were identified by PSORTb v4.0 and TMHMM 2.0, respectively. The secretome proteins associated with metabolic pathways were identified by using the KEGG (Kyoto Encyclopedia of Genes and Genomes) (http://www.kegg.jp/show_organism?menu_type=pathway_map). Prediction of virulence factors for the secreted proteins was performed by the VFDB database (http://www.mgc.ac.cn/VF) analysis. The secreted proteins were aligned against the VFDB dataset by the BLAST algorithm, and the matrix was created by VFDB output consisting of a BLAT score > 80.

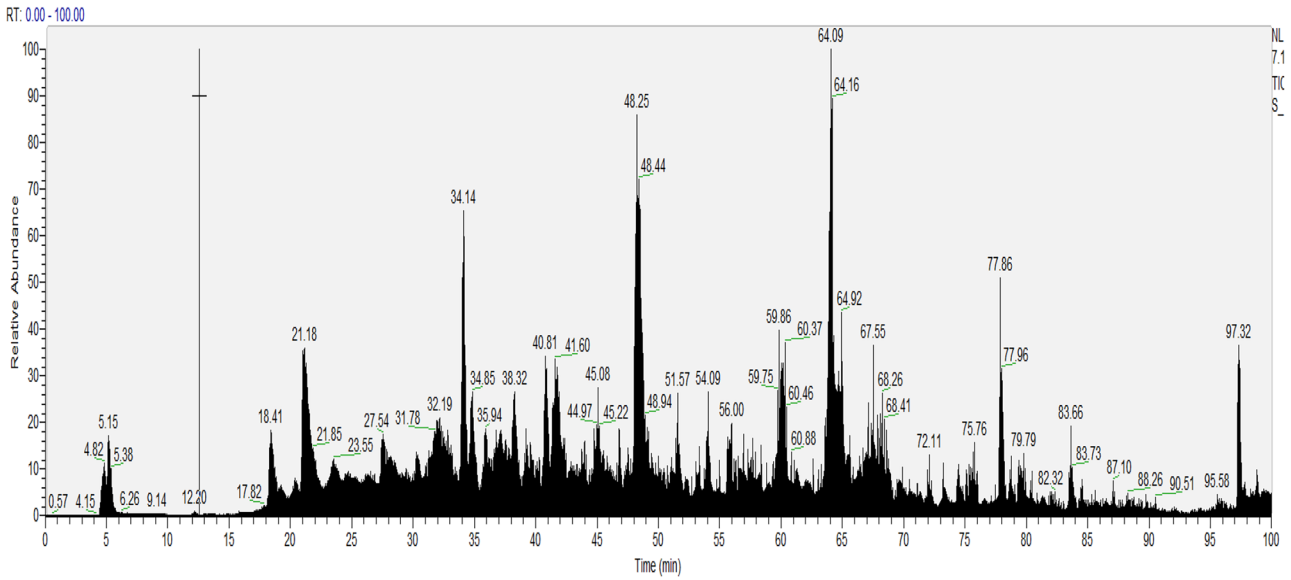

Fig. 1 LC–MS chromatogram of *Paenibacillus* sp. S-12 secretome

Results

Gene Ontology (GO) annotations

The amino acid FASTA files of the secretome of *Paenibacillus* sp. S-12 were analyzed using the Blast2GO or WEGO tools. The LC–MS/MS spectra have been provided in Fig. 1. A total of 657 proteins (Suppl File S1) were identified in the secretome of *Paenibacillus* sp. by LC–MS/MS-based analysis, in which 60 proteins were assigned by GO annotations. The identified proteins were divided into 3 groups based on their GO terms which are "biological process," "cellular component," and "molecular functions," each of them containing 20 proteins. In the biological process, most of the proteins were dominantly involved in the metabolic process (74.52%), and the rest of the proteins associated with the biosynthetic process (8.67%), cellular response (2.2%), regulation process (3.87%), the establishment of localization (3%), transmembrane transport (2%), biogenesis (1.87%), catabolic process (3.2%), cell division (0.33%), and cell wall organization or biogenesis (0.33%) (Fig. 2a). In molecular functions, 11 proteins were associated with a different kind

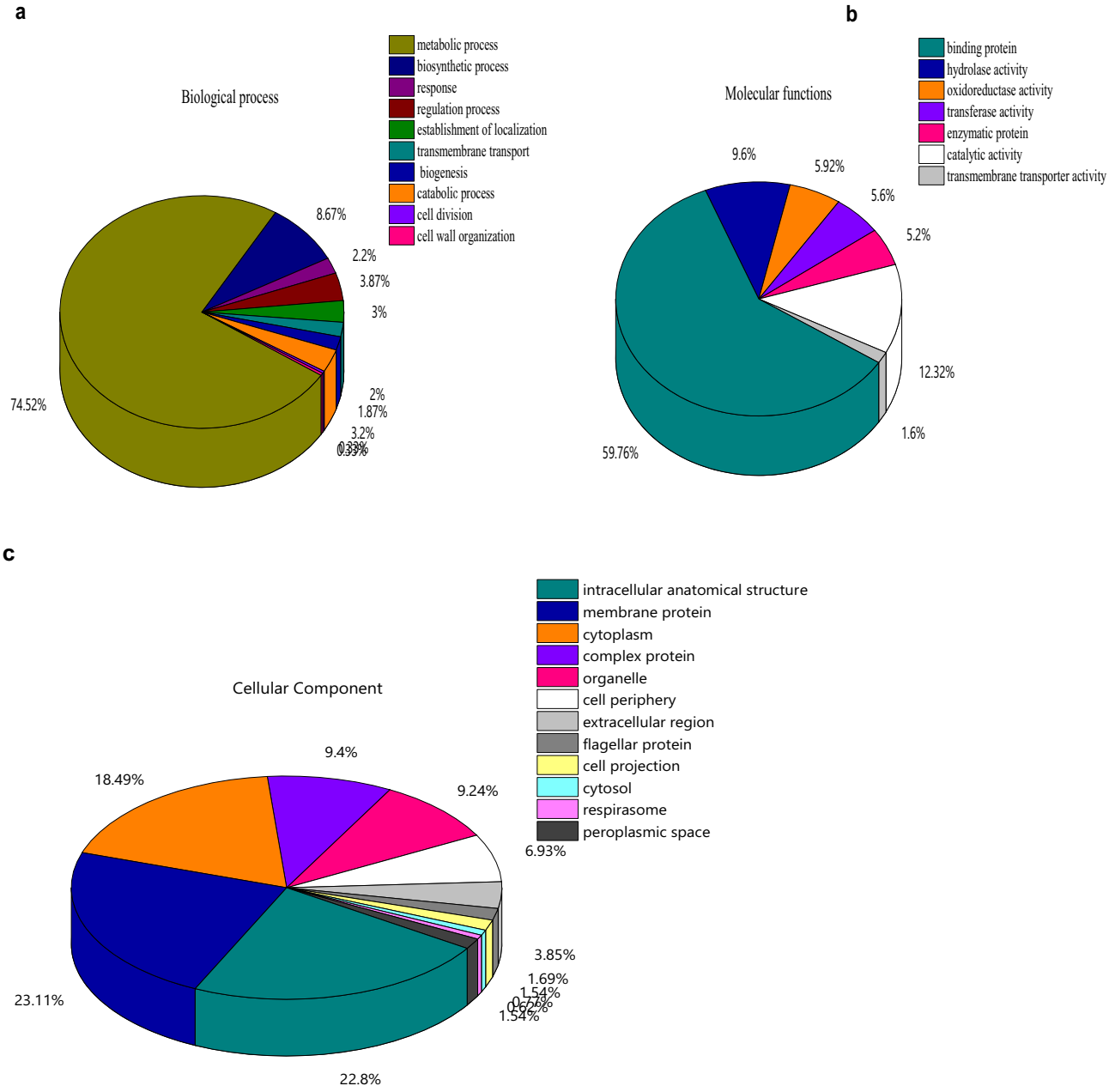

Fig. 2 Gene ontology analysis of *Paenibacillus* sp. S-12 secreted proteins using Blat2GO and online software WEGO. The secretome proteins were categorized into 3 groups: biological, molecular functions, and cellular component based on their properties and functions

of binding process (59.76%) (Fig. 2b), whereas in the cellular component, most of the proteins were involved in the membrane (23.11%), intracellular anatomical structure (22.8%), cytoplasm (18.49%) region, etc. (Fig. 2c).

Categorization of secretome

The secretome of *Paenibacillus* S-12 was categorized into three different groups based on signal sequences. Tools that are used for the categorization of secretome proteins are PREDLIPO, SignalP 4.1, and SecretomeP 2.0. Out of 657 proteins, 479 were categorized into three groups such as lipoproteins, classical, and non-classical proteins. Around 44 proteins (9.19%) were identified as lipoproteins mainly found in the bacterial cell membrane, 153 proteins (31.94%) were reported as classical proteins having signal sequences that suggest the general secretary "Sec" pathway for their secretion, and 282 proteins (58.87%) were non-classical proteins that lack signal sequences (Fig. 3).

Localization of secretome

For the determination of subcellular localization of the secreted proteins predicted with LC–MS/MS, the Psortb tool was used. We predicted that 317 proteins are localized in the cytoplasm (48.32%), 50 in the cytoplasmic membrane (7.62%), 22 are extracellular (3.35%), 18 in outer membranes (2.74%), and 40 in periplasmic space (6.1%), whereas localization of 209 proteins (31.86%) could not be predicted (Fig. 4).

KEGG pathway analysis

All protein sequences of the secretome of *Paenibacillus* sp. S-12 were further analyzed by KEGG pathway annotations,

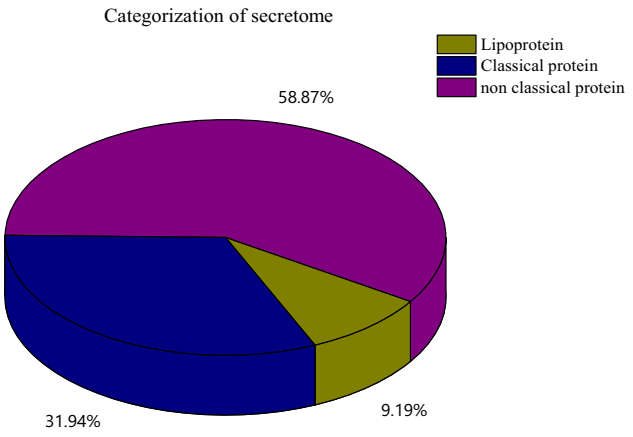

Fig. 3 The prediction of non-classical and classical secreted proteins was performed by Secretome 2.0 server (http://www.cbs.dtu.dk/service/SecretomeP) and SignalP 4.1 server

and analysis of 88 different pathways was identified in which a number of proteins play different functions in the pathway. The top five pathways were related to glycolysis/gluconeogenesis (9.41%), pyruvate metabolism (8.42%), aminoacyl-tRNA biosynthesis (8.42%), amino sugar and nucleotide metabolism (6.44%), and carbon fixation pathways in prokaryotes (5.94%) in which carboxykinase, dehydrogenase, isomerase, kinase, carboxylase, synthase, hydrolase, reductase, ligase, and transaminase play an important role in above-mentioned pathways (Fig. 5). A 5.45% protein related to pathways like purine metabolism, pentose–phosphate pathway, and propanoate metabolism was observed. Similarly, 4.46% proteins were related to cysteine/methionine metabolism; pyrimidine metabolism and methane metabolism were observed (Fig. 5).

Identification of virulence proteins

VFDB and VirulentPred online database were used for the identification of virulence proteins in the secretome of *Paenibacillus* sp. S-12. Around 179 virulence proteins were identified which included the major classes like adherence, immune modulators, flagellar-hook associated protein, exotoxins, and chaperones. The adherence proteins belonging to alpha-keto acid dehydrogenase subunit (PdhB), ABC transporter aspartate/glutamate-binding protein (PebA), fibrinogen binding protein (Fss2), fibronectin domain-containing lipoprotein (FlpA), endocarditis and biofilm-associated pilus tip protein (EbpA), *Listeria* adhesion protein (Lap), lipoprotein adhesin (LigB), surface adhesin (VpadF), etc. were identified (Table 1). Various immune modulating proteins such as asparagine synthase belonging to glutamine-hydrolyzing (WbmC), asparagine synthase related to glutamine-hydrolyzing (EndoS), NADP-dependent phosphogluconate dehydrogenase (GndA), D-alanyl-D-alanine carboxypeptidase (PbpG), acyl-CoA transferase (WcbT), and polyketide synthase (Pks) were identified (Table 2). Flagellar proteins FliC, FliL, FliD, CheW, and FliK (Table 3) and exotoxins ClbD, ClbL, CylF, and CylG were identified (Table 4). Various chaperone proteins such as DnaK, ClpB, and PrsA2 were also identified (Table 4).

Discussion

Several reports of secretome reveal the secretion of virulence factors or immunogens extracellular that could be used in the development of some important diagnostic biomarkers as well as vaccines (Zhang et al. 2017; Zubair et al. 2020). *Paenibacillus* genus comprises more than 250 species, and many of them are uncharacterized. Researchers have always wanted to isolate novel species of the genus *Paenibacillus* from different environmental areas due to its strong antagonistic activity against phytopathogens and promotion of plant growth (Grady et al.

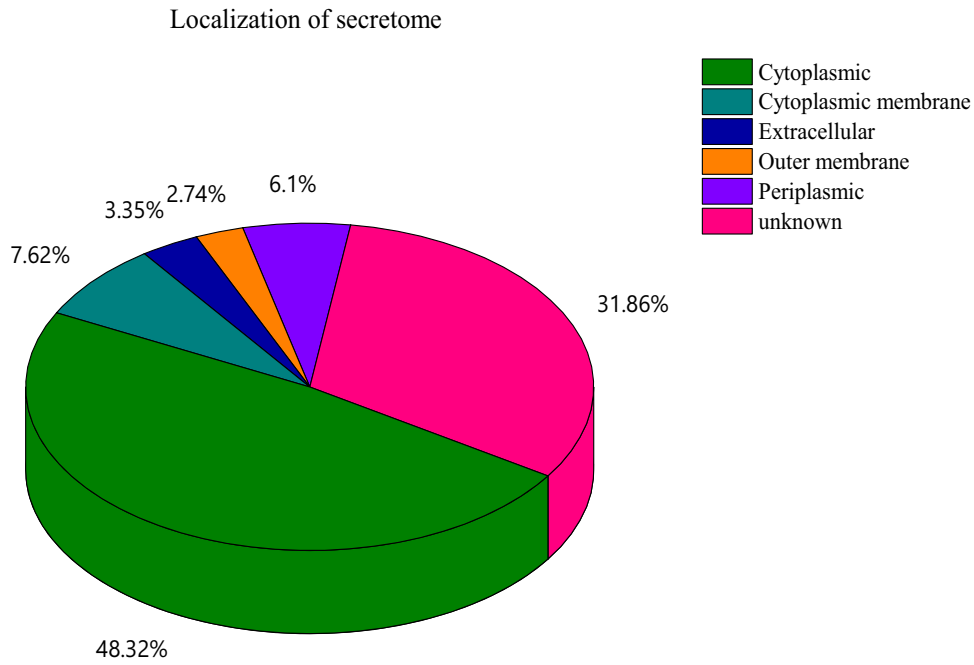

Fig. 4 The FASTA protein sequences were analyzed by PSORTb for their subcellular localization like extracellular, cytoplasmic, outer membrane, periplasmic, and cytoplasmic membrane

2016; Rybakova et al. 2016). Protein secretion shows the virulence mechanism in bacteria; however, the analysis of *Paenibacillus* secretome was unknown; therefore, we employed a mass spectrometry approach (Zijnge et al. 2012; Bao et al. 2015, 2017) to perform the protein profiling of *Paenibacillus* sp. S-12. We found that the secretome of *Paenibacillus* was enriched with a diverse set of proteins that were analyzed by various bioinformatic tools.

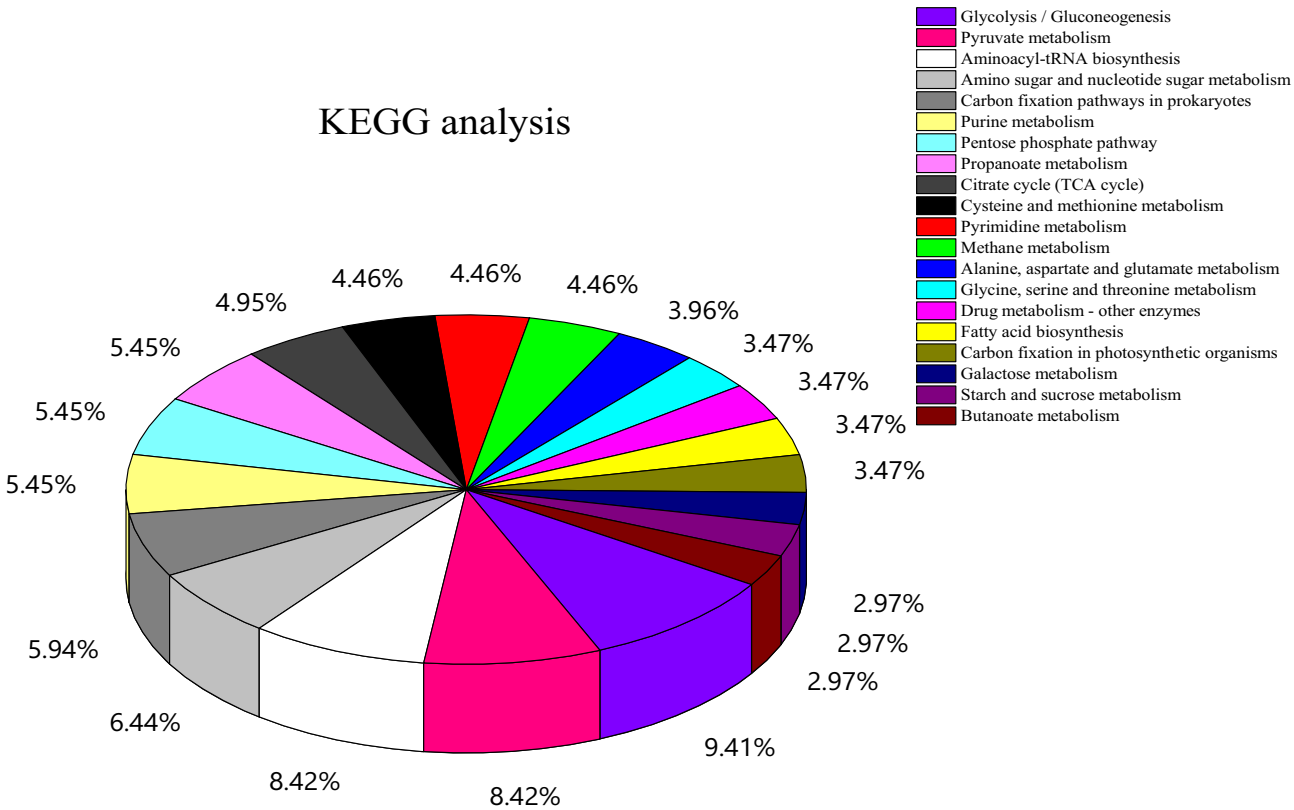

Fig. 5 The secretome proteins of *Paenibacillus* sp. S-12 were annotated for metabolic classification by KEGG following Blast2GO analysis

Table 1 List of adherence proteins identified in S-12 secretome

Description	Protein	AA length
Alpha-keto acid dehydrogenase subunit	PdhB	328
ABC transporter aspartate/glutamate-binding protein	PebA	261
Chaperonin	GroEL	540
Choline binding protein E	Pce/CbpE	148
Elongation factor Tu	Tuf	395
Fibrinogen binding protein	Fss2	1540
Endocarditis and biofilm-associated pilus tip protein	EbpA	652
Surface protein Fss1 (fibrinogen binding protein)	Fss1	1539
Fibronectin domain-containing lipoprotein	FlpA	140
HU family DNA-binding protein	UN	89
Internalin J	InlJ	856
Immunogenic lipoprotein A	IlpA	269
Listeria adhesion protein	Lap	865
Lipoprotein adhesin	LigB	832
M23 family metallopeptidase	UN	286
NDMA-dependent alcohol dehydrogenase	AdhD	368
N-Acetylglucosamine-binding protein	GbpA	209
Putative pilus tip protein	UN	413
sn-Glycerol-3-phosphate ABC transporter substrate-binding protein	UgpB	293
Streptococcal C5a peptidase	ScpA/ScpB	671
SDR family NAD(P)-dependent oxidoreductase	SadH	230
Surface adhesin	VpadF	354
Signal peptidase I	LepB	175
Sortase	SrtC-2/SrtC	223
Glycerol-3-phosphate ABC transporter	UgpB	186
Type I glyceraldehyde-3-phosphate dehydrogenase	Plr/GapA	332

The secreted proteins play an important role in microbial interaction with their environment, as these secreted proteins interact with receptor molecules on host cells or cell-surface proteins to modulate the signaling pathways that are involved in the immune response (Vargas-Romero et al. 2016). A total of 657 proteins were identified in the secretome of *Paenibacillus* S-12 by LC–MS/MS tools of which 179 proteins were virulence proteins that might be involved in the pathogenicity of *Paenibacillus* sp. S-12.

Paenibacillus secretome includes several proteins, including chaperone protein DnaK, post translocation chaperone PrsA2, ATP-dependent chaperone ClpB, and chaperonin GroEL. They can express their role in the cell surface to use as adhesins and might also release into the extracellular space to act as signaling virulence factors (Goulhen et al. 1998; Henderson et al. 2006). Molecular chaperones are involved in the pathogenesis of bacteria by helping them in coping with the host environment, phagosome fusion with lysosomes, and oxidative burst (Hosogi and Duncan 2005). Many Gram-positive bacteria such as *B. subtilis*, *S. aureus*, and *S. mutans* demonstrated that PrsA molecular chaperone proteins are involved in the synthesis of the cell walls (Guo et al. 2013; Hyyrylainen et al. 2010), resistance to antibiotics and other external stressors, and pathogenicity of bacteria (Lee et al. 2015). These observations suggest the significance of PrsA that contributes to the pathogenesis of several Gram-positive bacteria that may cause disease and infections (Lee et al. 2015).

About 60 proteins of *Paenibacillus* secretome were grouped into biological processes, cellular components, and molecular functions. The secretome proteins were mapped to 87 different KEGG pathways, with glycolysis/gluconeogenesis, pyruvate metabolism, and aminoacyl-tRNA biosynthesis being the dominant ones. The glycolysis pathway consisted of the enzymes carboxykinase, dehydrogenase, isomerase, epimerase, hexokinase, type IV glucokinase, hydratase, synthase, etc., several of which have been experimentally shown to be essential for glycolysis as well gluconeogenesis (Acosta et al. 2019). The other pathway proteins belonging to purine, propanoate, cysteine, and methionine metabolism suggest that S-12 secretome proteins are enriched with metabolic activities that help the bacterium to utilize nutrients from the extracellular milieu (Cezairliyan and Ausubel 2017).

Table 2 Immune modulating proteins in secretome of S-12

Description	Protein	AA length
Adenosine synthase A	AdsA	628
Asparagine synthase (glutamine-hydrolyzing)	WbmC	169
Acyl carrier protein	AcpXL	73
Acyl-CoA transferase	WcbT	437
ATP-binding cassette domain-containing protein	DdrA	313
BMP family ABC transporter substrate-binding protein	UN	354
CapA, required for poly-gamma-glutamate transport	CapA	335
D-Alanyl-D-alanine carboxypeptidase	PbpG	325
Asparagine synthase (glutamine-hydrolyzing)	EndoS	438
Glucose-6-phosphate isomerase	Pgi	520
LytR family transcriptional regulator	LytR	284
Mannose-6-phosphate isomerase, class I	ManA	313
NADP-dependent phosphogluconate dehydrogenase	GndA	467
Neutrophil activating protein	NapA	145
NDMA-dependent alcohol dehydrogenase	AdhD	368
Nucleoside diphosphate kinase	Ndk	133
Phosphoglucosamine mutase	MrsA/GlmM	444
Peptidoglycan N-deacetylase	PdgA	443
Phosphomannomutase	ManB/YhxB	530
Probable polyketide synthase	Pks	2085
6-Phosphogluconate dehydrogenase	UN	290
SDR family NAD(P)-dependent oxidoreductase	SadH	230
(pks15/1) type I polyketide synthase	UN	850
UDP-N-acetylglucosamine pyrophosphorylase/glucosamine-1-phosphate N-acetyltransferase	LpxA/GlmU	439

Motility is an important feature for plant-associated and endosymbiont bacteria, which assists the bacteria in colonization and also its spread within the plants (Hardoim et al. 2008). By KEGG enrichment analysis, we identified several proteins involved in the chemotaxis, motility, and formation of flagellar proteins. The bacterial chemotaxis signaling pathway consists of an adaptor protein or chemotaxis protein CheW (Capra et al. 2012). There is a specific secretion system to secrete proteins that are involved in the formation of flagellar hook, filament, and cap. Flagellar T3SS are found in both Gram-positive and Gram-negative bacteria, and it has been reported that the type III secretion system was involved in the

Table 3 List of flagellar proteins in secretome of S-12

Description	Protein	AA length
AlgW protein	AlgW	374
Cystine ABC transporter substrate-binding protein	TcyJ	265
Chemotaxis protein	CheW	175
Flagellin	FliC	289
Flagellar basal-body rod protein	FlgG	262
Flagellar protein export ATPase	FliI	392
Flagellar hook-length control protein	FliK	404
Flagellar filament capping protein	FliD	506
Flagellar hook-associated protein 1	FlgK	602
Flagellar hook-associated protein	FlgL	310
Flagellar basal-body rod modification protein	FlgD	121
Negative regulator of flagellin synthesis	FlgM	98
Short chain dehydrogenase/reductase family oxidoreductase	FlmH	182
MinD/ParA family protein	UN	174
Response regulator	UN	119

pathogenesis of bacteria (Erhardt et al. 2010; Hueck 1998). Flagella were an essential part of the motility of *Paenibacillus* bacterium, and the organization of *Paenibacillus* flagellar genes is quite similar to that of *B. subtilis*.

The bacterial flagellum consisted of more than 20 different proteins. The basal body of the flagellum is connected with the cell wall, whereas the hook connects the whip-like flagellar filament to the basal body that extended it throughout the bacterial cell. The major component of the flagellar subunit flagellin and/or FliC plays an important role in innate immunity and acts as the dominant antigen in the adaptive immune response. The flagella filament is composed of flagellin FliC, which is incorporated below the FliD a pentameric cap that acts as a plug for the assembly of monomeric flagellin protein FliC (Chevance and Hughes 2008). FliI is a flagellar ATPase protein that was not involved in export but serve to become more efficient for the secretion process. The various flagellar hook-associated proteins were identified in the *Paenibacillus* secretome. FlgK and FlgL are flagellar hook-associated protein that connects filament to hook. FlgD and FlgG are flagellar basal proteins and flagellar basal rod proteins, respectively (Haiko and Westerlund-Wikstrom 2013).

Secreted proteins of the secretome in bacteria play an important role in the physiological behavior of bacterial cells and their communication with the environment. *Paenibacillus* sp. S-12 is capable of producing several non-ribosomal peptides (NRPSs) that are previously reported (Selim et al. 2005; Aleti et al. 2015; van Belkum et al. 2015). Some of the predicted NRPSs are involved in the biosynthesis of known compounds, such as paenibacterin, polymyxin, fusaricidin, bogorol A, octapeptin C4, lichenysin, and pelgipeptin. Previous studies (Grady et al. 2016; Olishevska et al. 2019) have demonstrated the role of polymyxin against Gram-negative bacteria, whereas fusarcidins were effective against fungi and Gram-positive bacteria. Paenibacterin was identified in *Paenibacillus* sp. in the year 2012; bogorol A was reported in *Bacillus* sp. and Octapeptin C4 in *B. circulans.* These secondary metabolites were involved in the antibacterial activity (Liu et al. 2019). The pelgipeptins showed their activity against both Gram-positive and Gram-negative bacteria, as well as some anaerobic bacteria (Takeuchi et al. 1979). The activity of pelgipeptins C and D against methicillin-resistant was shown in *S. aureus* with a MIC of 12.5 µg/mL (Ding et al. 2011).

Table 4 List of exotoxins identified in the secretome of S-12

Description	Protein	AA length
Alveolysin	UN	501
Colibactin biosynthesis dehydrogenase	ClbD	284
Colibactin biosynthesis amidase	ClbL	241
Putative aminomethyltransferase CylF	CylF	307
35.8-kDa mosquitocidal toxin	UN	233
3-Ketoacyl-ACP-reductase	CylG	236
Chaperones		
Chaperone protein	DnaK	658
Post-translocation chaperone	PrsA2	260
ATP-dependent chaperone	ClpV	289

Conclusion

The current proteomics data on the secretome provides information about the putative virulence factors of *Paenibacillus* bacterium. The particular importance is the presence of a number of adherence and fimbriae proteins to enhance bacterial colonization and virulence through their multifunctional roles. The presence of various immune modulators suggests its ability to counteract the host immune response during the invasion processes. In the future, the predicted virulence factors in the secretome may be studied by over-expressing the respective genes in a suitable vector and by generating corresponding knockout mutants for further studies using in vitro or in vivo models.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12223-023-01032-4.

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Author contribution KK and PKS analyzed the secretome data. Y Ma edited the manuscript and helped in revision. RPS supervised the work and wrote the original draft.

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Data availability The data that support the findings of this study are in this published article and available from the corresponding author upon reasonable request.

Declarations

Ethics approval This article does not contain any studies with human participants or animal performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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