Network Study on SecA – A Component of Sec Secretion System in Bacteria *Pseudomonas Aeruginosa*

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Abstract. *Pseudomonas aeruginosa* is a Gram-negative bacterium and infects plants, animals and humans. Secretion systems in *P. aeruginosa* play an important role in infections. Sec secretion system has eight components, of which SecA is an ATPase. However, gene network study on how SecA functions under different experimental conditions has yet to be done. In this study, network is used to analyze *P. aeruginosa* genes under four types of experimental conditions, i.e. stress, habitat, nutrition and mutation. Special attention is given to (i) how many clusters form under control and experimental conditions, (ii) how many genes in SecA cluster, (iii) how many genes change their membership together with SecA, and (iv) which gene connects with SecA under control and experimental conditions, and their functions. The results demonstrate how genes reorganize under experimental conditions, and discussion is given to the reasons for such reorganizations.

Keywords: Network · *Pseudomonas aeruginosa* · Secretion system

1 Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium living in soil and water. It can infect plants [1], animals [2], and humans including eye [3], burn wound [4], acute and chronic pulmonary infections, especially cystic fibrosis [5], which is associated with substantial morbidity and mortality [6]. Besides, *P. aeruginosa* is the major bacterium developing drug resistance in clinic [7].

Gram-negative bacteria have seven secretion systems [8], which secrete toxins, degradative enzymes, and others leading to damages and death of host cells [9]. Because Gram-negative bacteria have outer and inner membranes, the secretion process is carried out in two steps: (i) type II secretion system operates across outer membrane [10], and (ii) Sec system operates across inner membrane [11].

Sec system is composed of SecA, which is an ATP-dependent motor protein [12]; SecB, which is chaperone brings protein precursors to Sec system [13]; SecYEG, which is a complex of SecY, SecE and SecG forming a gated pore in the inner

T. Huang et al. (Eds.): ISNN 2018, LNCS 10878, pp. 807–814, 2018.
https://doi.org/10.1007/978-3-319-92537-0_91

membrane [14]; SecDF, which is composed of SecD and SecF facilitating protein secretion [15]; YajC and YidC, whose function is related to protein insertion [16].

SecA is important because it converts ATP into a mechanical force to drive proteins to go through Sec secretion system across the inner membrane [17]. However, it is not clear how SecA interacts with other genes in *P. aeruginosa*, especially under different circumstances. A way to address this question is to combine all available transcriptomic data and look at how SecA gene network functions under various experimental conditions. Currently, the most transcriptomic studies on *P. aeruginosa* are done using platform GPL84, and their results are documented in public domain, Gene Expression Omnibus (GEO) [18]. To our knowledge, network has not been used to investigate SecA, therefore this study was designed to analyze the gene network of SecA from *P. aeruginosa* under different experimental conditions.

2 Materials and Methods

2.1 Data

Platform GPL84 contains 5549 *P. aeruginosa* PAO1 genes [19], of which SecA, SecB, SecD, SecE, SecF, SecG, SecY and YajC are PA4403, PA5128, PA3821, PA4276, PA3820, PA4747, PA4243, and PA3822, respectively. Of the transcriptomic data in GEO [18], the experimental conditions can be classified into four types: mutation in *P. aeruginosa*, changing habitat, environmental stress and starvation.

2.2 Gene Network

In this study, each gene corresponds to a node, and the edge between two nodes is determined according to correlation between transcriptomic data for these two genes. When two genes work together under the same condition, their transcriptomic profiles can be correlated. In this way, network can reveal how genes organize under the control condition and how genes reorganize under experiment conditions [20]. Network analysis was conducted using iGraph R package [21] and Pajek [22].

3 Results and Discussion

Of various stresses on *P. aeruginosa* such as azithromycin [23], ciprofloxacin [24], hydrogen peroxide (H_2O_2) is important because H_2O_2 plays crucial roles in release of extracellular DNA [25], DNA repair proteins, catalases, intracellular iron transport, bacterial adaption to oxidative stress, pyocins, glycolysis [26, 27]. Figure 1 shows the gene network of *P. aeruginosa* under control (left panel) and stress (right panel) experimental conditions, i.e. *P. aeruginosa* PAO1 exposed to H_2O_2 [26]. In this type of figures, each symbol represents a gene, the line between two symbols indicates a good correlation in transcriptomic data between two genes, and the same colored symbols construct a cluster. Actually, each panel includes all 5549 *P. aeruginosa* PAO1 genes.

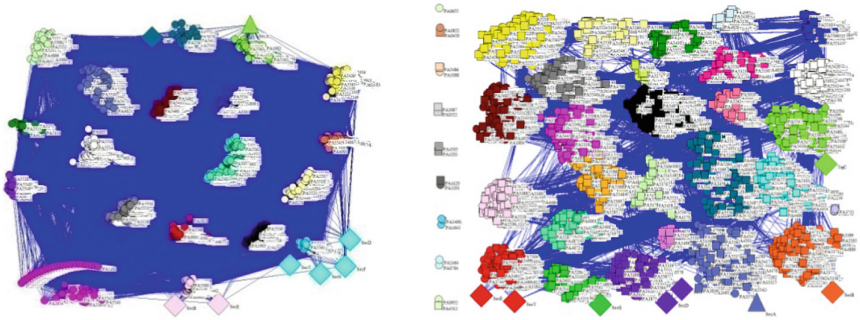

Fig. 1. Gene network of *P. aeruginosa* under control (left panel) and H_2O_2 (right panel) conditions. Triangle symbols represent SecA, diamond symbols represent the rest of Sec genes, circle symbols represent the rest 5541 genes under control condition, and square symbols represent the rest 5541 genes under experimental condition. The data are GSE3090 in GEO [18, 26]. (Color figure online)

Of various habitats for *P. aeruginosa* such as plant [28], biofilms [29], animal gut [30], burn wounds of humans [31], *P. aeruginosa* in cystic fibrosis is most important [32] because most cystic fibrosis patients have infection of *P. aeruginosa* [33] although cystic fibrosis is an inherited disease [34]. Figure 2 displays the gene network of *P. aeruginosa* from planktonic culture (left panel) and from clonal isolate of cystic fibrosis (right panel).

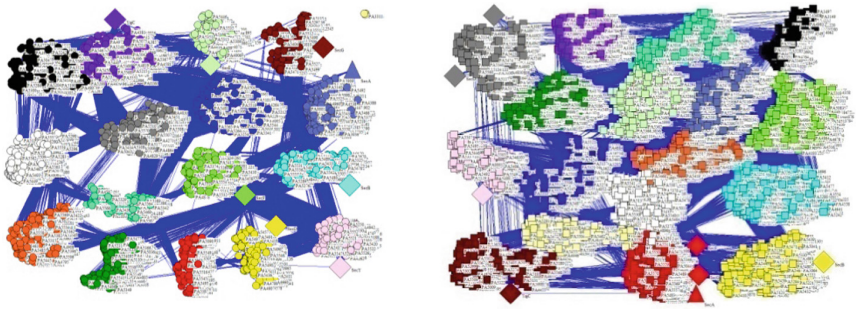

Fig. 2. Gene network of *P. aeruginosa* from planktonic culture (left panel) and from clonal isolate of cystic fibrosis (right panel). The data are GSE10304 in GEO [18, 32]. (Color figure online)

Of variety of nutrition in *P. aeruginosa* such as iron starvation [35], sulfate starvation [36], low oxygen tension [37], phosphate abundance [38], iron is very important for the growth of *P. aeruginosa*. Figure 3 demonstrates the gene network of *P. aeruginosa* without supplement of PQS (left panel) and with PQS (right panel).

Of various mutations in *P. aeruginosa* such as mutations in quorum sensing [40], in regulation of fatty acid [41], in biofilm formation [42], in cell-surface signalling

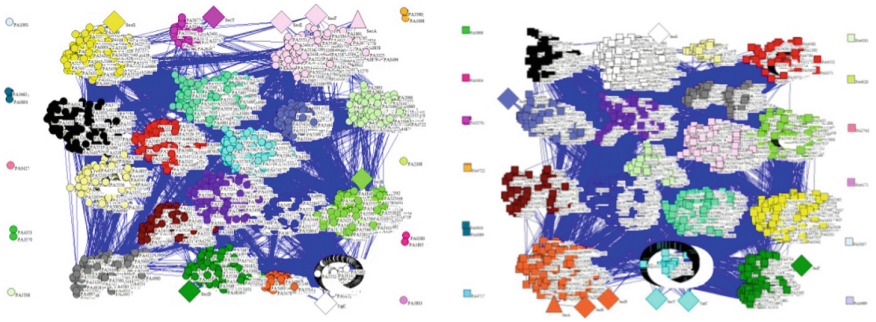

Fig. 3. Gene network of *P. aeruginosa* without supplement of PQS (left panel) and with PQS (right panel). The data are GSE3836 in GEO [18, 39]. (Color figure online)

systems [43], in agmatine and putrescine catabolism [44], the mutation in cystic fibrosis is clinically most important [45]. Figure 4 illustrates the gene network of *P. aeruginosa* from non-clonal isolate (left panel) and clonal isolate with mutation (right panel).

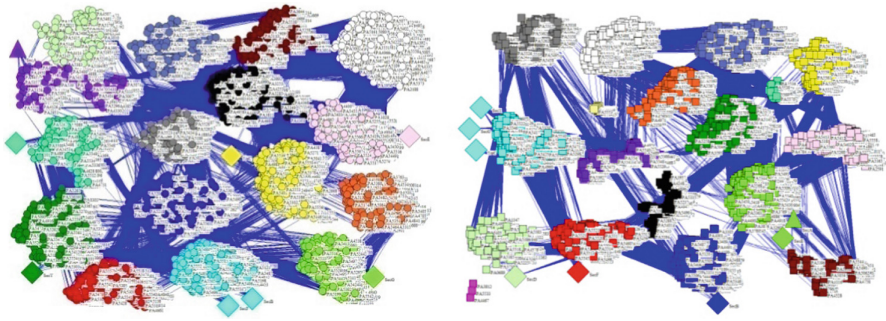

Fig. 4. Gene network of *P. aeruginosa* from non-clonal isolate (left panel) and clonal isolate with mutation (right panel). The data are GSE6122 in GEO [18, 45]. (Color figure online)

Interestingly, Sec components, i.e. SecA, SecB, SecD, SecE, SecF, SecG, SecY, and YajC, belong to different clusters, and not many connections exist between them in these figures, although they all work for Sec secretion system. These suggest that the regulation of Sec secretion system could work differently for each component.

How 5549 genes from *P. aeruginosa* organize and reorganize in terms of correlation network can be further elaborated in Table 1. At first, the number of cluster (row 1) and number of genes in SecA cluster (row 3) are different in control groups, which is plausible because the samples of *P. aeruginosa* come from different sources.

In the first row in Table 1, H_2O_2 has strong influence on the number of clusters. This is reasonable since bacteria often face various reactive oxygen species during their lifetime and *P. aeruginosa* has a defense system against reactive oxidants [26]. Along the third row in Table 1, the number of genes in SecA cluster is quite different one from another. As a cluster indicates that the genes in the cluster have similar transcriptomic

Table 1. Network statistics (No. A: Number of cluster, No. B: Number of genes in SecA cluster, No. C: Number of genes changing their numbership with SecA, Color: color of SecA cluster)

Group	Figure 1		Figure 2		Figure 3		Figure 4	
	Control	Stress	Control	Habitat	Control	Nutrition	Control	Mutation
No. A	19	35	17	17	27	29	16	18
Color	Lime green	Cadet blue	Cadet blue	Red	Pink	Orange	Purple	Lime green
No. B	398	258	348	437	360	391	314	545
No. C	22		28		26		58	

profiles, so it is more likely that these genes could function simultaneously with SecA. It is interesting to note that the number of genes in SecA cluster decreases in Fig. 1 whereas the number of genes in SecA cluster increases in Figs. 2, 3 and 4. This suggests that *P. aeruginosa* mobilizes more genes for secretion together with SecA under habitat, nutrition and mutation conditions. Furthermore, the fourth row in Table 1 indicates how many genes change their membership together with SecA. Evidently, mutation leads more genes to change their membership together with SecA because a single mutation could change the metabolic pathway completely [45].

It is intriguing to look at which gene connects with SecA in these figures. For Fig. 1, ten genes (PA4626, PA4718, PA5026, PA5163, PA5320, PA5358, PA5387, PA5459, PA5539, PA5563) and two genes (PA4791, PA4993) connect with SecA in control and experimental groups. For Fig. 2, twelve genes (PA4410, PA4605, PA4703, PA4729, PA4766, PA4810, PA4968, PA5007, PA5069, PA5189, PA5473, PA5565) and six genes (PA4627, PA4749, PA4852, PA4937, PA5010, PA5301) connect with SecA in control and experimental groups. For Fig. 3, two genes (PA4512, PA4619) and three genes (PA4729, PA5268, PA5500) connect with SecA in control and experimental groups. For Fig. 4, six genes (PA4782, PA5099, PA5241, PA5268, PA5391, PA5485) and sixteen genes (PA4574, PA4680, PA4723, PA4776, PA4835, PA4865, PA4872, PA4917, PA5019, PA5065, PA5067, PA5091, PA5214, PA5244, PA5281, PA5316) connect with SecA in control and experimental groups.

When look at these genes according to *Pseudomonas* proteins classification [46], a general patterns can be observed. For control groups, the connected genes are mainly related to (i) coenzyme transport and metabolism, (ii) cell cycle control, cell division, chromosome partitioning, (iii) cell wall/membrane/envelope biogenesis, (iv) energy production and conversion, and (v) inorganic ion transport and metabolism. For experimental groups, the connected genes are mainly related to (i) amino acid transport and metabolism, (ii) carbohydrate transport and metabolism, (iii) translation, ribosomal structure and biogenesis, and (iv) inorganic ion transport and metabolism.

It is reasonable that SecA connects with the genes related to transport and metabolism under both control and experimental conditions because Sec secretion system secretes proteins; related to cell division/wall/membrane/envelope biogenesis under control condition because SecA is located in inner membrane; related to energy production and conversion because SecA itself is an ATPase under control condition. However, it is not clear why the genes related to inorganic ion transport appear in both

control and experimental groups, because SecA is not involved in ion transport. This should be a point for pursuit in future. Interestingly enough, a recent study shows that SecA interacts ribosomes [47], which could explain why SecA connects with genes related to translation, ribosomal structure and biogenesis under experimental condition.

In conclusion, we conduct a network study on transcriptomic data from *P. aeruginosa* under four types of experimental conditions, demonstrate how genes reorganize under experimental conditions, and discuss the reasons for such reorganizations.

Acknowledgements. This study was partly supported by National Natural Science Foundation of China (31460296 and 31560315), and Special Funds for Building of Guangxi Talent Highland.

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