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The associated protein screening and identification in *Proteus mirabilis* swarm colony development

Yanxia Liu, Jing Xie, Junkang Liu*, Yuanxin Deng, Weiwei Xu, Linming Guo and Yu Deng

Bio-wave Research Center, Department of Pharmacology, College of Pharmacy, Third Military Medical University, Chongqing 400038, China.

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Proteus mirabilis **colonies exhibit striking geometric regularity, and swarm colony terraces correspond to one swarming-plus-consolidation cycle. Basic microbiological methods and imaging techniques were used to measure periodic macroscopic events in swarm colony morphogenesis. Here, adding a respiratory enzyme indicator, 2,3,5-triphenyltetra-zolium chloride (TTC) into the culture medium, the bacteria on the rings gradually changed into red and the change was not synchronous with the formation of ring. According to this phenomenon, we distinguished five phases as *P. mirabilis* colonize agar surfaces during swarming to investigate the bacteria swarm colony development mechanism by the methods of matrix assisted laser desorption/ionization (MALDI) mass spectrometry and real time fluorescence quantitative-polymerase chain reaction (RTFQ-PCR). Through these observations, we found that *P. mirabilis* swarm colony development was closely related to the gene *fusA*, *fliC1*, *tufB*, *ahpC*, *icd* and *flaB*. The research indicates that the functions of the genes *fusA*, *filC1* and *tufB* in swarming phase significantly influence the *P. mirabilis* swarming behavior. In consolidation phase, expression of *ahpC* connected to oxidative stress decreased as compared to other phases. The gene *icd* which affected the metabolism had high expression in consolidation phase I and there were more expression from the bacteria on the ring than that from the bacteria between two rings. The increase of *flaB* was concomitant with the development of cell growth from swarming phase to turn red phase II. The morphology of *P. mirabilis* was obviously distinct in different growth phase, and periodically changed. The movement and metabolize way of *P. mirabilis* were diverse in different growth phase. In swarming phase, the swarm colony and antioxidant ability was enhanced, but the functions of respiratory and metabolism was down-regulated. In conclusion, the bacteria fertility ability and respiratory function were enhanced in consolidation phase. These genes related with motion and metabolism played a key role in regulating *P. mirabilis* swarm colony development.**

Key words: *Proteus mirabilis*, real time fluorescence quantitative-polymerase chain reaction (RTFQ-PCR), matrix assisted laser desorption/ionization (MALDI)-TOF, swarm.

INTRODUCTION

Proteus mirabilis is known for its ability to differentiate between short swimmer cells and swarmer cells, a process crucial for the pathogenesis of these bacteria during urinary tract infections (Alilison and Hughes, 1991; Belas, 1996). It exhibits a striking form of multicellular behavior, called swarming migration, in which motile vegetative rods growing on solid media differentiate into extremely elongated hyperflagellated swarm cells that undergo rapid and coordinated population migration away from the initial colony (Rahman et al., 2009). In the

spectacular colonies of *P. mirabilis*, a series of concentric rings are developed as the bacteria multiply and swim following a scenario that periodically repeats itself (Czirok et al., 2001). This bacterium can undergo dramatic morphological and physiological changes in response to growth on surfaces or in viscous environments (Allison et al., 1993). These changes are ultimately required to produce the multicellular motile behavior that is characterized by flagellum-assisted swarming motility over nutrient agar media.

P. mirabilis swarm colony terrace corresponds to one swarming-plus-consolidation cycle, which distinguished three initial phases (lag phase, first swarming phase and first consolidation phase) followed by repeating cycles of subsequent swarming plus consolidation phases (Darnton et al., 2010). The lag phase is the differentiation of the first swarmer cell perimeter in preparation for swarming, ends when the first swarmer rafts emerge from the colony perimeter. The first swarming phase is characterized by relatively independent movement of swarmer cell rafts which tend to coalesce into radially oriented tubes, ends when migration which ceases and the leading edge remains stationary. As the first swarming phase comes to an end, a wave of cell multiplication and thickening of the newly colonized areas begins adjacent to the perimeter of the inoculation spot. The second and following swarming phases are more coherent than the first swarming phase, ends when the swarming front ceases to advance rather synchronously along its entire perimeter. The second and following consolidation phases form a uniform thickening of the newly formed terrace instead of spreading wave of multiplication (Rauprich et al., 1996).

Cell density, surface contact, inhibition of flagellar rotation and cell-to-cell signalling all provide critical stimuli, and close cell alignment and the production of secreted migration factors facilitate mass translocation (Armbruster and Mobley, 2012). The first pivotal stimulus of swarm cell differentiation is surface contact. Differentiation is induced when viscosity of the growth medium is increased, or when flagella are tethered with antibodies. Alavi and Belas (2001) reported that hyperflagellation is also the most prominent feature of swarm cells, and differentiation requires efficient flagella assembly. Cell density is critical to swarming as the duration of the lag phase that precedes *P. mirabilis* migration is strongly influenced by inoculum density. Swarming cells also require extracellular components (e.g. polysaccharide and surfactants) that allow mass migration of differentiated cells over difficult terrain. AL-2, putrescine and cyclic dipeptide have also been proposed as extracellular signals that are capable of mediating cell-cell communication (Schneider et al., 2002; Sturgill et al., 2002; Sturgil and Rather, 2004).

In the past 20 years, there has been a significant acceleration in our understanding of *P. mirabilis* as it pertains to the process of migration and differentiation, which refer to regulation of the gene, lipopolysaccharide and peptidoglycan synthesis, bacterial division, ATP production, putrescine biosynthesis, proteolysis and morphological changes (Allison and Hughes, 1991a,b; Williams and Schwarzhoff, 1998). The process of elongation takes place with only a slight increase in cell width and is due to an inhibition in the normal septation mechanism, although the molecular mechanism of inhibition is not known. Elongation of the swarmer cell can give rise to cells 60 to 80 µm in length. During this process, DNA replication proceeds without significant change in rate from that in the swimmer cell (Gmeiner et al., 1985). Not surprisingly, the rate of synthesis of certain proteins, e.g, flagellin, the protein subunit of the flagellar filament, is altered markedly in the swarmer cell (Armitage et al. 1979; Armitage, 1981; Falkinham and Hoffman, 1984). The differentiation process leading to the development of a swarmer cell involves between 25 and 50 genes that are coordinately expressed (Belas, 1994; Mobley and Belas, 1995). Thus, since *P. mirabilis* is frequently associated with urinary tract infections, particularly in patients with chronic urinary catheterization, knowledge of the regulation of swarmer cell gene expression is crucial to understanding the pathogenesis of this organism.

While several signals are believed to induce differentiation (for example, surface contact, cell density and amino acids) the pathways of signal integration are still poorly understood, in particular the apparent surface contact-sensing by flagellar filaments and the basis of the cell-cell communication assumed to underlie coordinated migration. Our previous studies on some preliminary work shows the influence of strain, pH, the concentration of agar, and the inoculate way on growth. In our study, we observed the changes of modulative gene in the process of swarm colony development. Although many attempts have been made to explain the mechanism of the swarming in *P. mirabilis*, including slime production and chemotaxis, the process of swarmer cell differentiation and multicellular swarming motility remains elusive. In our study, the experiment aimed to investigate the proteins in five phases of *P. mirabilis* by the methods of RTFQ-PCR, which provides a new experimental evidence for clarifying the mechanisms of the swarm colony development.

MATERIALS AND METHODS

Strain, media, and culture conditions

Clinical isolates of *P. mirabilis* HI4320 were obtained from the

*Corresponding author. E-mail: liujunkang@163.com. Tel: +86 13657699681. Fax: +86 02368752191.

microbiology laboratory of the Third Military Medical University (Chongqing, China). We routinely grew *P. mirabilis* colonies on LB agar containing 0.1% glucose, 1% tryptone and 0.5% yeast powder. It was grown in LB medium at 37°C. To obtain homogeneous populations of differentiating bacteria, 0.2% 2,3,5-triphenyl four azole nitrogen chloride (TTC) was spread onto a LB (2%) agar plate, and the latter was incubated for various periods (from 0 to 24 h).

Bacterial sample preparation

An overnight culture of cell was spot inoculated in the center of each LB agar plates. Following incubation at 37°C for 12 h, cells were scraped off the agar surface with bacterial rings. We divided the growth cells into 5 phases: swarming phase, consolidation phase I (cells on the ring), consolidation phase II (cells between two rings), turn red phase I (cells on the ring) and turn red phase II (cells between two rings). The cells were collected by the inoculating loop and suspended in 1 mL phosphate-buffered saline (PBS).

Extraction of total protein

Total protein was extracted from bacteria by using the Bacterial Protein Extraction Kit (Sangong, Shanghai, China) which was designed for the extraction of biologically active soluble proteins and high purity inclusion bodies from bacterial cells. Briefly, cells were collected and immediately washed completely with iced PBS (pH=7.2) at 4°C (centrifuging at 5000 ×g for 10 min). After removing the media and washing the pellet with PBS, the cells were re-suspend pellet in 1 ml of 1×cell lysis buffer. Adding 40 µl phenylmethylsulfonyl fluoride (PMSF) and 80 µl lysozyme, the cell suspension were incubated at 37°C for 30 min and then on a rocking platform for 10 min. The mixture was added 20 µl DNasel/RNase and incubation was continued on a rocking for another 10 min at 37°C. Finally, the insoluble debris were removed by centrifugation at 3000 ×*g* for 30 min at 4°C and then collecting the supernatant in a fresh tube. The bicinchonininc acid (BCA) protein assay kit (Beyotime, Shanghai, China) was used according to the manufacturer's instructions using the microplate procedure (20 µl sample/200 µl BCA working reagent; 37°C/30 min; 562 nm).

SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by use of slab gels consisting of a 12% acrylamide gradient resolving gel and 5% concentrating gel. The samples were placed at -20°C. Prior to electrophoresis, samples were vortexed, and 1×loading buffer were added to adjust the supernatant to an equal volume of 50 µg. The samples were boiled at 100°C for 5 min, and centrifuged for 5 s. The samples were loaded on a 5% concentrating gel and run until the bromophenol blue was at the very bottom of the gel at 50 V for 30 min and 100 V for 90 min. The gels were stained with Coomassie brilliant blue and destain. The molecular weights of proteins were estimated from their mobilities relative to a set of commercially available proteins.

MALDI-TOF MS analysis

According to the results of SDS-PAGE, we cut off the corresponding protein bands and sent the samples to the Burn Department of Southwest Hospital. The matrix assisted laser desorption/ionization (MALDI) mass spectrometry was employed. The differentially expressed proteins were digested with trypsin. We selected eight bands and subjected them to matrix-assisted laser desorption/ionization time of flight mass spectrometry followed by searching ncbinr (NCBI) database to identify these proteins (Wu et al., 2009). All MALDI-TOF mass spectrometry results were compared with results from conventional methods, and discrepancies were resolved by 16S rRNA gene sequencing.

RTFQ-PCR analysis

According to the MALDI-TOF results, the corresponding protein mRNA levels were verified with real-time fluorescence quantitative PCR (RTFQ-PCR) analysis for *P. mirabilis* by using a R+eal-time Quantitative PCR Detecting System (biosystems, USA). PCR was performed in a 20 µl reaction volume that contained ultrapure water, PCR buffer, 50 mM $Mgcl_2$, 2.5 mM dNTPs, 0.5 µl Upstream primer, 0.5 µl Downstream primer, 0.3 µl SYBR, 0.2µl Taq DNA Polynerase and 1 µl cDNA. The PCR protocol was as follows: initial denaturation for 2 min at 95°C, 40 cycles at 95°C for 10 s, 60°C for 30 s, and 60°C for 45 s. Real-time PCR was performed on the corresponding cDNA synthesized from each sample. We choose 16SrRNA as the internal control gene to normalize the PCRs for the amount of RNA added to the reverse transcription reactions. The data were analyzed by $\triangle\triangle C_T$ method, where, $\triangle\triangle C_T$ = (C_T,Target - C_T,Actin)Time *x* - (C_T,Target - C_T,Actin)Time 0 (Kenneth and Thomas, 2001).

RESULTS

Culture results

P. mirabilis in LB medium was incubated at 37°C for 12 h, cells were scraped off the agar surface with bacterial rings, while adding 1% TTC aqueous into LB medium, the color of bacteria on the concentric rings was changed to red (Figure 1). However, the color of bacteria between two rings was not change.

SDS-PAGE analysis

Denatured polyacrylamide gel electrophoresis was employed to assess the differences of protein pattern in the experimental samples that belong to *P. mirabilis*. In SDS-PAGE analysis, a total of eight protein bands were determined (Figure 2). Five bands with molecular weights of 95 - 130 kDa (A1), 43 - 55 kDa (A2), 34 - 43 kDa (A3 and A4) and 26 kDa (A5) in swarming phase were all putative different proteins as compared to the other phases. Results show that the contents of protein (A1, A3 and A4) were decreased regularly in groups I, II, and III while the contents of protein in Group IV and Group V were increased. In addition, in Figure 2, the Group II~V samples corresponded to B1 band while the Group I sample did not have. The result of SDS-PAGE analysis revealed that the absence and/or presence of bands were observed in the five phases.

MALDI-TOF analysis

These eight proteins bands treated by tryptic in-gel

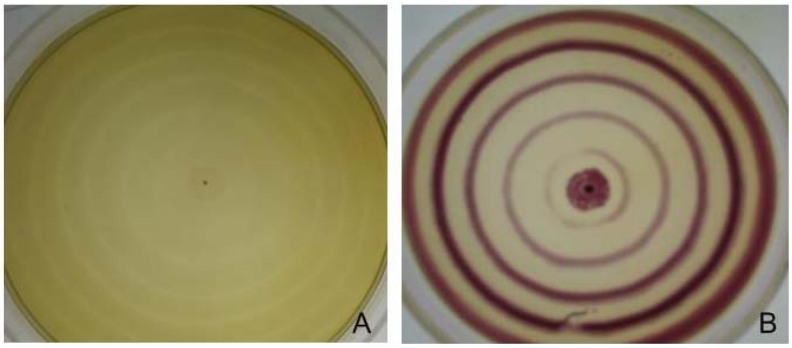

Figure 1. The periodic growth patterns of *P. mirabilis.* A: Without TTC in the LB culture medium. B: With TTC in the LB culture medium.

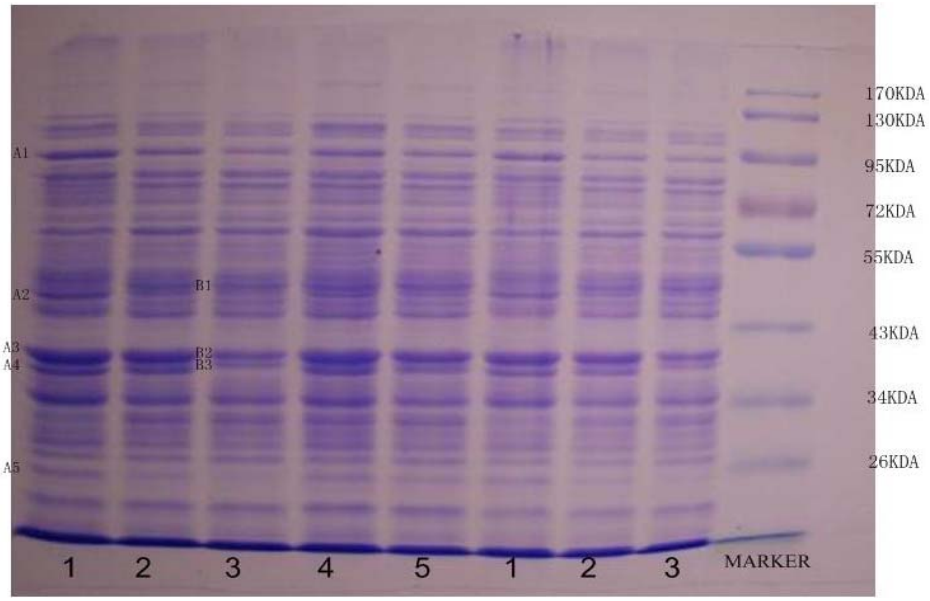

Figure 2. The SDS-PAGE of bacteria complete protein. Lane 1, Swarming phase; Lane 2, bacteria on the ring in consolidation phase; Lane 3, Bacteria between the rings in consolidation phase; Lane 4, bacteria turn red and in the ring; Lane 5, bacteria between the rings turned red.

digestion were characterized by MALDI-TOF MS and peptide mass finger printings of all were obtained. A novel protein or polypeptide was identified by database retrieval. NCBI was used to identify nine proteins of *P. mirabilis* HI4320 based on the MS Search Score greater than 70. The result is shown in Table 1.

RTFQ-PCR analysis

As shown in Table 2, we chose six target proteins including alkyl hydroperoxide redutase (*ahp*C), fructose bisphosphate aldolase (*fba*B), isocitrate dehydrogenase (*icd*), EF-G (*fus*A), EF-Tu (*tuf*B), and flagellin 1 (*fli*C1) to do the RTFQ-PCR analysis. Primers were designed for these six genes (Table 2 for protein name, gene name, primer sequences). It was generally accepted that gene-expression levels should be normalized by a carefully selected stable internal control gene. The expression level of 16sRNA is shown in Table 3. Three genes expression levels (*fus*A, *fli*C1 and *tuf*B) which involved bacterial growth were obtained. The expression levels of *fus*A (Figure 3) and *fliC1* (Figure 4) in swarming phases were higher than other tested phases, and the level of *fus*A gradually reduced from Group I to Group V, while the level of *fli*C1 reduced no regularity. An opposite trend was observed in the expression of *tuf*B (Figure 5), the *tuf*B levels in swarming phase were lower than the

Table 1. Summary of differentially expressed proteins of *P. mirabilis* by MALDI-TOF analysis.

Run name	Protein name	Mean peptide spectral intensity	AA coverage (%)	MS search score	Distinct peptides
A1	Elongation factor G	9.85e+007	48	430.50	24
A1	Formate acetyltansferase	3.62e+007	10	89.65	5
A2	Elongation factor TU	2.61e+008	50	246.80	15
A2	Phosphopentomutase	1.19e+008	21	114.67	6
A3	Flagellin 1	1.99e+008	61	322.32	19
A4	Flagellin 1	2.89e+007	26	106.45	6
A4	Outer membrane porin	3.02e+007	20	85.03	6
A5	Alkyl hydroperoxide reductase	5.89e+006	34	81.46	5
B1	Isocitrate dehydrogenase	6.18e+007	41	203.95	13
B1	Phosphopentomutase	2.12e+007	14	81.32	4
B2	Flagellin 1	8.21e+007	57	282.80	17
B3	Fructose-blsphosphate aldolase	1.63e+007	26	94.84	5
B3	Flagellin 1	2.44e+007	14	72.16	4

Table 2. The primer information of RTFQ-PCR analysis for *P. mirabilis* mRNA levels.

Target protein	Target gene name	Primer
Alkyl hydroperoxide redutase	*ahpc*	CTTCTGGCCAATGGACTTCA ACGCCATGCGTTATGTACGA
Fructose bisphosphate aldolase	*fbaB*	GCCGGATGTAACTGTGTTGC GTCGCACCTACAGCAACAGC
Isocitrate dehydrogenase	*icd*	AGGTGGTGGTATCCGTTCAT CCACTCAATACCGGCATAGA
EF-G	*fusA*	TCACATCCGCTGCAACTACT GAGGCTGAACACCACCAACT
EF-Tu	*tufB*	CCATTGAAGCCGGATGTAAC GTCGCACCTACAGCAACAGC
Flagellin 1	*fliC1*	AGTGCAATCGAGCGTCTGTC TCGTTCGCATTACGTGAAGC

Table 3. The expression of 16S rRNA gene.

Sample name	Target name	Cт	Cт Mean
	16srRNA	13.64741	
1	16srRNA	13.78528	13.74631
	16srRNA	13.80623	
	16srRNA	13.51925	
2	16srRNA	13.46205	13.48805
	16srRNA	13.48284	
	16srRNA	13.66578	
3	16srRNA	13.57320	13.61869
	16srRNA	13.61710	
	16srRNA	13.35785	
4	16srRNA	13.45795	13.44047
	16srRNA	13.50561	
	16srRNA	13.65908	
5	16srRNA	13.86087	13.6018
	16srRNA	13.28546	

other phases. The expressed product of *ahp*C (Figure 6) gene is alkyl peroxide reductase which acts on the oxidative stress. In the swarming phase, the level of *ahp*C had the highest expression amount, the other four phases showed tendency to increase. These genes (*icd* and *fba*B) were associated with bacterial metabolism, in which isocitrate dehydrogenase encoded by *icd* gene was the rate-limiting enzyme of tricarboxylic acid cycle (TAC), and diphosphofructose enzyme encoded by *fba*B gene was the rate-limiting enzyme of glycolysis. In this paper, the expression levels of *icd* (Figure 7) and *fba*B (Figure 8) in swarming phase were both significantly lower than the other phases.

DISCUSSION

The mechanism of the swarm colony development on protein expression of *P. mirabilis* was investigated using a

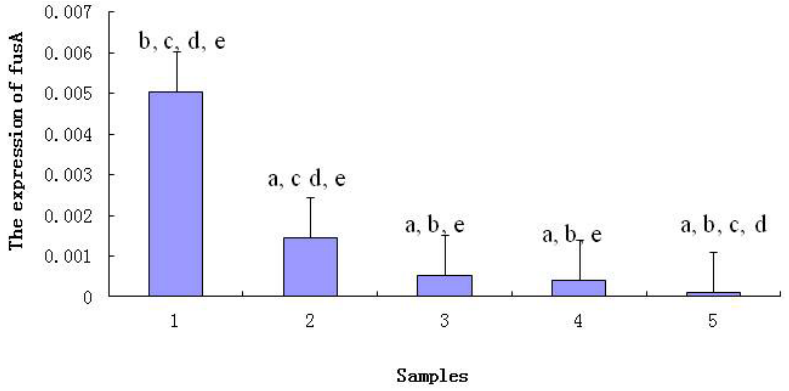

Figure 3. The expression of *fusA.* a: Compared with group 2 (p<0.05 or p<0.01); b: compared with group 3 (p<0.05 or p<0.01); c: compared with group 4 (p<0.05 or p<0.01); d: compared with Group 5 (p<0.05 or p<0.01); e: compared with group 5 (p<0.05 or p<0.01).

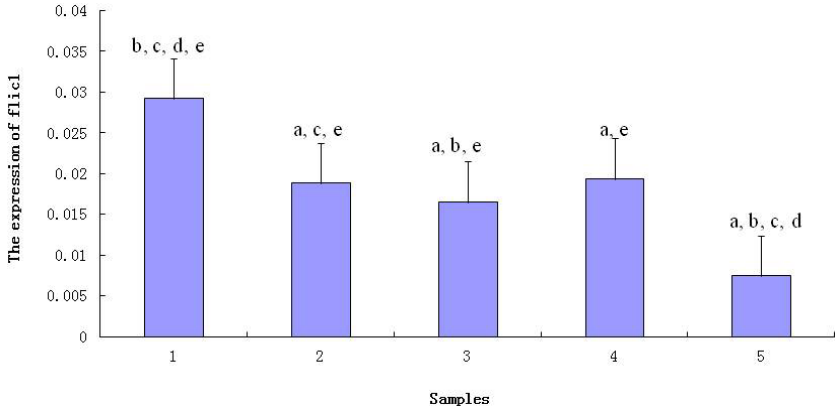

Figure 4. The expression of *fliC1.* a: Compared with group 2 (p<0.05 or p<0.01); b: compared with group 3 (p<0.05 or p<0.01); c: compared with group 4 (p<0.05 or p<0.01); d: compared with group 5 (p<0.05 or p<0.01); e: compared with group 5 (p<0.05 or p<0.01).

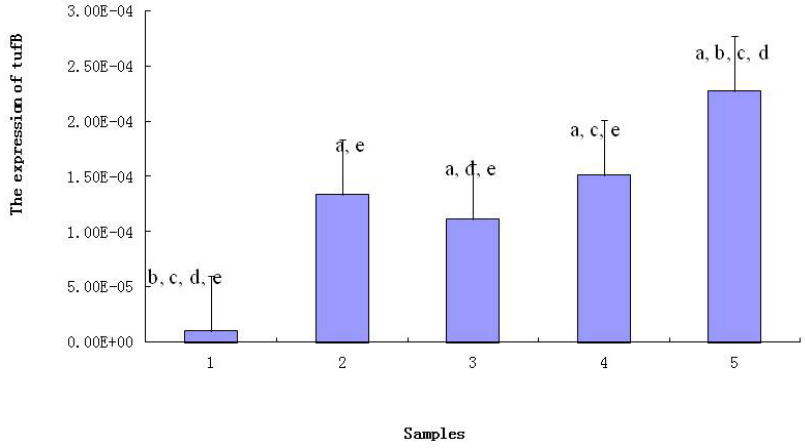

Figure 5. The expression of *tufB.* a: Compared with Group 2 (p<0.05 or p<0.01); b: compared with group 3 (p<0.05 or p<0.01); c: compared with group 4 (p<0.05 or p<0.01); d: compared with group 5 (p<0.05 or p<0.01); e: compared with group 5 (p<0.05 or p<0.01).

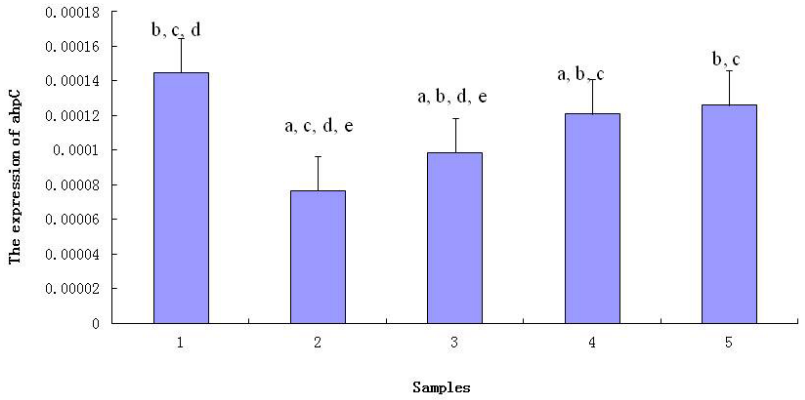

Figure 6. The expression of *ahpC.* a: Compared with group 2 (p<0.05 or p<0.01); b: compared with group 3 (p<0.05 or p<0.01); c: compared with group 4 (p<0.05 or p<0.01); d: compared with group 5 (p<0.05 or p<0.01); e: compared with group 5 (p<0.05 or p<0.01).

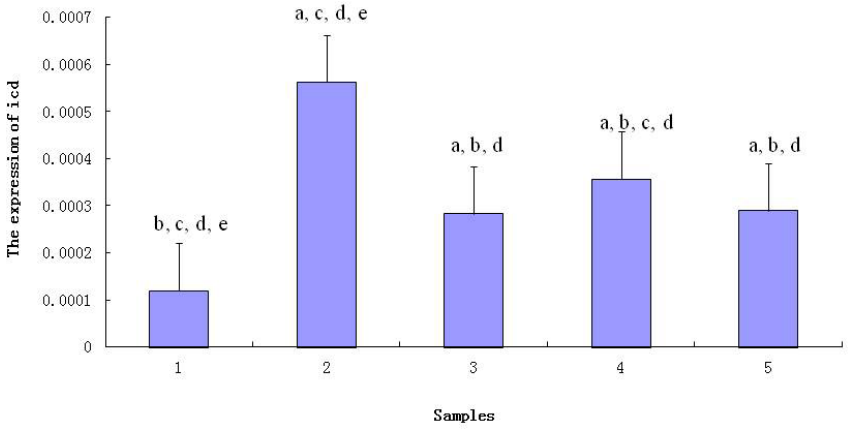

Figure 7. The expression of *icd.* a: Compared with group 2 (p<0.05 or p<0.01); b: compared with group 3 (p<0.05 or p<0.01); c: compared with group 4 (p<0.05 or p<0.01); d: compared with group 5 (p<0.05 or p<0.01); e: compared with group 5 (p<0.05 or p<0.01).

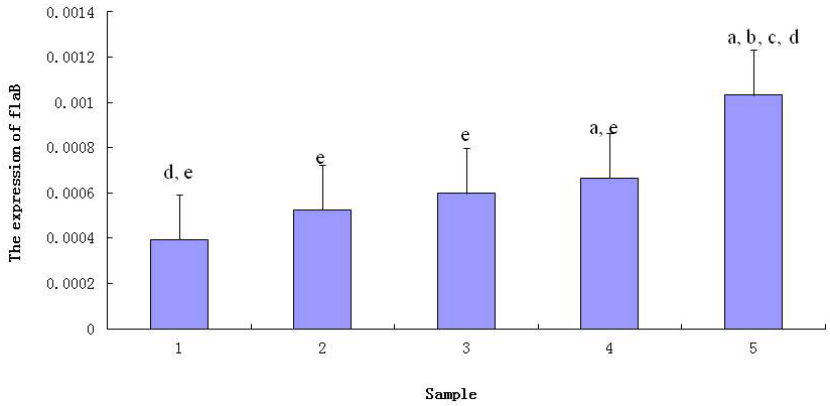

Figure 8. The expression of *flaB.* a: Compared with group 2 (p<0.05 or p<0.01); b: compared with group 3 (p<0.05 or p<0.01); c: compared with group 4 (p<0.05 or p<0.01); d: compared with group 5 (p<0.05 or p<0.01); e: compared with group 5 (p<0.05 or p<0.01).

proteomic method. The genetic studies were successful in identifying key events leading to the swarming behavior differentiation, such as the regulation of master operon *flh*DC and its products, which was central to the control of cell division, as well as the transcriptional regulation of flagellar biogenesis. However, genetics did not reveal the dynamics of interactions involved in the long-range correlations which occur during swarming (Claret and Hughes, 2000; Chilcott and Hughes, 2000). The eightfold increase in *fli*C1 expression during induction of swarmer cell differentiation is less than expected from observations of the increase in flagellar flaments when swimmer cells differentiate into swarmer cells (Robert, 1994). In our study, the expression of *fli*C1gene in swarming phase was increased when compared with the other phases. On the basis of the evidence presented, only *fliC*1 was actively expressed in cells, the transcription of *fliC*1 results in the synthesis of flagella composed solely of *fliC*1. It is possible related to the posttranslational control which was involved in controlling the amount of *flic*1 synthesized during swarmer cell differentiation. With the increase of *fli*C1 gene, the number of flagellin 1 encoding by *fliC*1 was increased, which was an important substance of flagella. Far away from the swarming phase, the expression of *fli*C1 was the lowest, the content of flagellin and synthesis of flagellar were relevantly reduced. The results thoroughly agree with our previous bacterial flagella staining (unpublished).

P. mirabilis is a facultative aerobe, with normal growth and under conditions of oxygen and no oxygen, the growth mode did not change. In recent years, scholars both at home and abroad have done a great deal of researches on the metabolism and energy sources, which showed that there were numerous differences in physiology of swarmer cells as compared to non-swarmer cells. For example, swarmer cells have reduced amino acid uptake and have increased sensitivity to hydrophobic antibacterial agents, suggesting that reorganization of the outer membrane might affect active transport processes and protective properties (Armitage et al., 1975; Falkinham and Hoffman, 1984). During swarming, the incorporation of precursors into DNA, RNA, proteins and the rate of oxygen uptake were also reduced (Armitage et al., 1979). Then, at the end of the swarm period, macromolecule synthesis and oxygen uptake were restored to levels equivalent to pre-swarming levels. Furthermore, a study showed that membrane vesicles from swarm cells have reduced rates of NADH, malate and succinate respiration (Falkinham and Hoffman, 1984). Interestingly, some researchers pointed out that the energy required during swarming was generated by fermentation (Himpsl et al., 2008). However, fermentation would be much less energetically favorable for fueling the flagellar motor as compared to membrane respiration. Christopher et al., (2012) found that swarming bacteria use a complete aerobic tricarboxylic acid (TCA) cycle but not employ oxygen as the terminal electron acceptor. They previously identified two *P. mirabilis* transposon mutants disrupting genes encoding proteins (pyruvate dehydrogenase and succinate dehydrogenase) in the oxidative TCA cycle that each displayed an aberrant swarming phenotype, which suggested a close link between the TCA cycle and swarming motility.

Proteomics analysis of proteins with different expressions reveals that most of the identified proteins were metabolism related. In this study, we screened isocitrate dehydrogenase and fructose bisphosphate aldolase which are metabolism-related proteins, as research object (Thorsness and Koshland, 1987). Isocitrate dehydrogenase was an important rate-limiting enzyme of the TCA which is encoded by *icd* and could convert isocitrate to alpha ketoglutarate. It was revealed by RT-PCR that *icd* gene expression had minimum quantity in the swarming phase. Results indicate that the current primary coverage of bacterial activity in this phase was forward movement, rather than the metabolism. In agreement with the TTC in the LB medium, bacterial color was pale yellow in swarming phase, indicating the activity of succinate dehydrogenase was also low. Succinate dehydrogenase and isocitrate dehydrogenase are the rate-limiting enzyme of TCA, a small amount of metabolism at this time was only for flagella to rotate to provide energy. In addition, no matter which bacteria is on the ring in consolidation phase (group 2) and bacteria turn red in the ring (group 4), expression of *icd* gene were higher than bacteria out of the ring (groups 1, 3 and 5). Bacteria on the rings were considered as in consolidation phases, which showed that *P. mirabilis* aerobic respiration in this stage was restored to normal, and the ability of metabolism was enhancement. Simultaneously, red rings appeared in the medium supplemented with TTC and this leaded to an increase in succinate dehydrogenase concentration.

Corresponding to the periodic motion, bacterial metabolism was a cyclical change. Fructose-bisphosphate aldolase was a rate-limiting enzyme of glycolysis which is encoded by *fbaB* and could catalyze the formation of glycerone phosphate and D-glyceraldehyde 3-phosphate from D-fructose 1, 6-bisphosphate. In our work, level of *fbaB* gene expression was minimum in swarming phase, and more away from swarmer cells, more *fbaB* gene were expressed, the result also confirmed this view that the bacteria metabolism ability was reduced and movement ability was enhanced in swarming phase.

Aerobic metabolism leads to oxidative stress from generation of reactive oxygen intermediates (ROI). *Ahp*C gene which encodes alkyl peroxide reductase protected both bacterial and human cells against reactive nitrogen intermediates (Chen et al., 1998). In the swarming phase, the level of *ahp*C had the highest expression amount, the other four phases from groups 2 to 5 showed a tendency

to increase. The results indicated that in swarming phase oxygen was a negative factor on bacteria. As mentioned before, the metabolism of this bacterial phase do not need oxygen, in such a bad environment; bacteria were moving but not for reproduction process. These properties of *P. mirabilis* and tumor cells were just different in approaches but equally with satisfactory result, and metabolism of anaerobic respiration was the most primitive in tumor cells. When the external environment was not conducive for the bacteria growth, bacteria would be reversion of anaerobic respiration. However, the intrinsic relation should be further explored.

Besides metabolism-related proteins, genetic information processing related proteins are discovered during acid adaptation. Elongation factor Tu (EF-Tu) and elongation factor G (EF-G) are translation-related proteins and are needed for protein synthesis, they are structural homologues and share near-identical binding sites on the ribosome, which encompass the GTPase-associated center (GAC) and the sarcin-ricin loop (SRL) (Yu et al., 2007; Zavialov and Ehrenberg, 2003). The SRL is fixed structure in the ribosome and contacts elongation factors in the vicinity of their GTP-binding site. In contrast, the GAC is mobile and we hypothesized that it interacts with the alpha helix D of the EF-Tu G-domain in the same way as with the alpha helix A of the G′-domain of EF-G. The mutual locations of these helices and GTP-binding sites in the structures of EF-Tu and EF-G are different. Thus, the orientation of the GAC relative to the SRL determines whether EF-G or EF-Tu will bind to the ribosome (Sergiev et al., 2005). EF-Tu and EF-G are GTP-dependent elongation factors, and they are responsible for binding of aminoacyl tRNA to rRNA and translocation, respectively. Another interesting discovery is EF-Tu (Sanderson and Uhlenbeck, 2005; Furness et al., 1997). This protein is encoded by *tuf*B and can promote the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis (Swiss-Prot) (Karring et al., 2003). In this study, the RT-PCR analysis showed that the expression of *fus*A in swarm phase was significantly increased and more than 3 to 14 times of other phases. Furthermore, the more close to the swarming phase in the process of bacterial growth, the higher the amount of *fus*A expressed. However, the expression of *tuf*B was just the opposite. This indicated that the swarming behavior was closely related to the *fusA* and *tufB* genes, even there exists competition relationship in the process of *P. mirabilis* swarming development (swarm cells to swimmer cell). Especially differentiation processes might necessitate large amount of protein synthesis; it referred to cell growth and inevitably lead to changes of gene expression in protein synthesis. This results showed large increase in *fus*A expression, which indicated that it mainly participated in the synthesis of cell growth and flagella multiplication proteins. While the expression of *tuf*B was significantly raised in consolidation phase, it showed that *tuf*B participated in the synthesis of bacterial reproduction proteins. We demonstrated that *fus*A and *tuf*B can positively regulate *P. mirabilis* swarming behavior whose mechanisms need to be further clarified.

In conclusion, *P. mirabilis* swarm colony development was closely related to the proteins of EF-Tu, EF-G, flagellin 1, fructose bisphosphate aldolase, isocitrate dehydrogenase and alkyl hydroperoxide redutase in these five phases. The movement and metabolism mode of *P. mirabilis* was diverse in different growth phase. These genes related with motion and metabolism played a key role in regulating *P. mirabilis* swarm colony development.

Conflict of interests

The author(s) have not declared any conflict of interests.

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