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研究报告

Changes in the expression of outer membrane proteins during *Myxococcus xanthus* development

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Abstract: [Objective] The membrane proteins of *Myxococcus xanthus*, a model organism for studying prokaryotic development, remain an under-explored territory. **[Methods]** Putative outer membrane protein (OMP)-encoding ORFs were selected from *Myxococcus xanthus* genome using six computational algorithms. With *lacZ* as a reporter, we monitored the expression profiles of these genes during vegetative growth and development. **[Results]** Eleven putative OMPs have been identified based on bioinformatic analysis. Increased expression during development was found in two genes, MXAN3106 and MXAN3883, which were predicted to encode protein transporters of the secretin family and the fimbrial usher protein (FUP) family, respectively. The remaining nine genes that code for TonB-dependent receptors or efflux proteins exhibited a decreased expression or remained at a very low expression level after initiation of development. **[Conclusion]** Our data suggest that for *M. xanthus*, switching from growth to development is associated with a dramatic change in the expression pattern of membrane proteins.

Keywords: *Myxococcus xanthus*, Membrane proteins, Gene expression

黄色粘球菌发育阶段外膜蛋白表达的变化

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摘 要: 【目的】黄色粘球菌是研究原核发育的一种模式生物，对其膜蛋白的研究仍然十分缺乏。【方法】利用 6 种预测软件，在黄色粘球菌的基因组中筛选编码外膜蛋白(OMP)的基因。根据报告基因 *lacZ*，检测这些基因在营养性生长和发育阶段的表达。【结果】基于生物信息学分析，筛选出 11 个编码外膜蛋白的基因。其中 2 个基因(MXAN3106 和 MXAN3883)在发育阶段表达量上升,它们分别编码 Secretin 家族和 Fimbrial usher protein (FUP)家族转运蛋白。其余 9 个基因在发育起始阶段表达量降低或保持较低水平，它们均编码 TonB 依赖型受体或外排蛋白。【结论】这些数据提示，黄色粘球菌由生长到发育的转换过程，伴随着膜蛋白表达的显著变化。

关键词: 黄色粘球菌，膜蛋白，基因表达

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1 Introduction

Myxococcus xanthus is a Gram-negative bacterium characterized by social behavior and a complex developmental cycle. Under vegetative conditions, *M. xanthus* cells move cooperatively in large swarms; under conditions of high cell density, nutrient starvation, and a solid surface, 10^5 cells aggregate in a coordinated fashion to form a fruiting body[1]. Starting 24 h after starvation, the motile, rod-shaped cells inside the fruiting body differentiate into dormant ovoid spores. Fruiting body formation involves temporally ordered change in the expression of genes which drives the multicellular morphogenesis[2]. Extrapolation to the full genome using Tn5 *lac*, a transposable promoter probe, implies several hundred genes may increase their expression during *M. xanthus* development, however, most of these developmentally regulated genes are not essential for aggregation or sporulation[3].

As interfaces to the outside word and as interfaces between cells, biological membranes fulfill various functions. In particular, membrane proteins play roles in cell signaling, in transport across cell membranes, in energy transduction processes, and in cell motility. So far several membrane proteins in *M. xanthus* have been characterized genetically and biochemically. For instance, cell surface-associated C-signal CsgA acts as a developmental timer to sequentially induce aggregation and sporulation[4-5]. The secretin homolog PilQ, which forms a channel in the outer membrane, is required for type IV pilus biogenesis, social motility and development[6], and assembly of PilQ complexes requires another membrane protein Tgl[7]. In addition, genomic analysis of *Myxococcus* model strain DK1622 revealed the presence of 97 serine-threonine protein kinases[8], some of which (e.g., Pkn1, Pkn8 and Pkn9) function as receptors to receive environmental signals and have been shown to play roles in fruiting body morphogenesis[9]. Despite the importance of membrane proteins in *Myxococcus* life cycle, they remain an under-explored territory. For example, C signal receptor, a key player in C-signalling pathway and is supposed to be membrane associated, has not been identified yet.

We are interested in changes in cell surface proteins when *M. xanthus* cells shift their life cycle from expansive vegetative swarming to aggregation into multicellular fruiting bodies. It is reasonable to think that changes in cell behavior and morphology depend on physiological and metabolic changes that occur during conversion between two separate cycles of growth and development. Since *M. xanthus* cell membrane is directly involved in cell-cell communication, cell motility and the transport of nutrients, ions, and excretory substances, expression pattern of various membrane associated proteins should be changed upon initiation of development. Work by Orndorff and Dworkin indicated that during aggregation there were six individual membrane proteins whose synthetic rates were drastically altered[10]. Earlier, Inouye et al. showed that five membrane-bound proteins (designated proteins 1 to 5) were differentially expressed during fruiting body and spore morphogenesis[11]. However, genes for these proteins have never been identified or characterized.

Initially we tried to resolve Myxococcus membrane proteins by two-dimensional electrophoresis but failed due to problems of reproducibility. Here we monitored the expression profiles of some genes whose products are predicted be localized in the outer membrane. We first utilized six independent bioinformatic softwares to predict outer membrane proteins (OMPs) from translated ORFs of *M. xanthus* DK1622 genome. Eleven hypothetical genes were predicted to encode OMPs by every program we used. The expression profile of one gene, MXAN3883, has been described previously. In this report, we showed the expression pattern of the remaining ten genes during *M. xanthus* development.

2 Materials and Methods

2.1 Bioinformatic analyses

Six computational algorithms were utilized to identify signatures characteristic of OMPs from genome of *M. xanthus* DK1622 (GenBank accession No. CP000113): PSORTb bacterial protein subcellular localization prediction tool (http://www.psort.org/psortb/); TMB-Hunt amino acid composition based transmembrane beta barrel prediction tool (http://bmbpcu36.leeds.ac.uk/~andy/betaBarrel/AACompPred/aaTMB_Hunt.cgi); TMBETA-NET amino acid composition based transmembrane beta strand prediction tool (http://psfs.cbrc.jp/tmbeta-net/); TMBETA-SVM amino acid composition and residue

pair information-based prediction tool (http://tmbeta-svm.cbrc.jp/); PSLpred hybrid approach-based bacterial protein subcellular localization prediction tool (http://www.imtech.res.in/raghava/pslpred/); PA-SUB machine learning based protein subcellular localization prediction tool (http://www.cs.ualberta. ca/~bioinfo/PA/Sub/). TMBETA-SVM picks up OMPs using support vector machine (SVM), TMB-Hunt and TMBETA-NET predict OMPs based on the presence of membrane-spanning beta-strand segments, while other tools including PSORTb, PSLpred and PA-SUB predict subcellular localization of proteins by integrating several approaches. These six tools were selected at random and combined use of different algorithms would maximize the accuracy of bioinformatic analyses. Totally, 11 ORFs were simultaneously identified as OMP-coding genes by all the six programs.

2.2 Cell growth and development

Escherichia coli JM83 was grown in LB broth in the presence of relevant antibiotics. *M. xanthus* strains were grown in CTT media as previously described[12]. Kanamycin or oxytetracycline was used for selection at concentrations of 40 mg/L and 12.5 mg/L, respectively. *M. xanthus* fruiting body development was induced on TPM agar (10 mmol/L Tris-HCl (pH 7.6), 1 mmol/L KH_2PO_4, 8 mmol/L $MgSO_4$, and 1.5% Difco Bacto-Agar). Specific β-galactosidase activities were determined by the protocol of Kroos et al[3]. Protein concentrations were measured by BCA protein assay (Pierce).

2.3 Strains

DK1622 was used as the parent wild-type strain for all *M. xanthus* strains throughout this study. All strains constructed were confirmed by polymerase chain reaction (PCR).

2.4 Construction of *lacZ* reporter fusions

The *lacZ* fusion plasmids were constructed using the vector pMP220[13]. For each fusion, a DNA fragment containing the putative promoter region and some 5′-terminal codons was generated by PCR using DK1622 genome as a template. The PCR primers are listed in Table 1 except the primers for MXAN3883 which were described previously[14]. The amplified product was digested with *Xba* I and *Pst* I or *Sph* I; in addition, a 2.9-kb *Eco*R I-*Xba* I myxophage MXAN8 *attP* fragment was recovered from pUC18-attP. These two fragments were ligated with pMP220 that had been digested with *Xba* I and *Pst* I or *Sph* I, resulting in transcriptional *lacZ* fusion vector pMP-MXAN (Table 2). The plasmids were introduced into wild

Table 1 Primers for generating promoter-containing fragments by PCR
表 1 PCR 扩增引物

Name	Sequence (5′→3′)[a]	Location of amplicon[b]
1316	F: GCTCTAGAAAGCACCACTGCCAGTCGGAACATG R: TTCTGCAGCACGAAGAGCAAACACCCACGCCAC	From –1 185 to 30 of MXAN1316
3106	F: GCTCTAGAGAGGCCGAGGATCCGGGCCTCATGG R: CATGCATGCGTGAACATCGTCCGTGTCCCCTCTT	From –1 054 to 8 of MXAN3106
3905	F: GCTCTAGACTCATCACAGTCCGTGTTGTCCTCC R: TTCTGCAGGGTGATGGCGTGACGGGGTCTATGC	From –1 142 to 27 of MXAN3905
4176	F: GCTCTAGAACGGCGATGAGCAGGTGGAGGCAGT R: TTCTGCAGCCAGGGCGACCGCGATCACGAAGGC	From –1 214 to 31 of MXAN4176
4198	F: GCTCTAGACAACACCAGGATGCCCACGCACGAG R: TTCTGCAGCCGTCACCAGCCCGAGGAAGCCCAG	From –1 072 to 40 of MXAN4198
4365	F: GCTCTAGAGGTTCAGCGACAGTGACGCCAGGTA R: TTCTGCAGATGACGAGGACCAGGCTGAGGAGGG	From –1 189 to 50 of MXAN4365
5030	F: GCTCTAGAGGCTGGAGCAGGCGGCGAACAAGGA R: TTCTGCAGGTCGACGCTGCGACGAGCGCCAAGA	From –1 079 to 47 of MXAN5030
6044	F: GCTCTAGAGCTCGAAGTTCGCGTCCAGGAACGA R: TTCTGCAGGAATGGTGGCGCTCCCCACGGGCAT	From –2 779 to 25 of MXAN6044
6579	F: GCTCTAGATGAAAGGTGGCCGCCCGATGAAC R: TTCTGCAGACACGGCAGGCCCAGCGAGGAAG	From –1 241 to 34 of MXAN6579
6911	F: GCTCTAGAAGGAGATGGCGGTGGACCTGGCG R: TTCTGCAGTCCTGCGCCCAGGCGGGCGTCAT	From –1 276 to 23 of MXAN6911

Note: [a]: Underlined nucleotides indicate restriction enzyme sites; [b]: +1 indicates translation initiation site of each OMP gene.
注：[a]：下划线为限制酶位点；[b]：+1 表示翻译起点.

Table 2 Plasmids used in this study
表 2 本研究中所用的质粒

Plasmids	Relevant characteristics	Reference
pUC18-attP	pUC18 with a 2.9-kb MXAN8 *attP* fragment, Ap^R	26
pMP220	*lacZ* transcription fusion vector, Tc^R	13
pMP-MXAN1316	pMP220 with *attP* and regulatory sequence upstream of MXAN1316, Tc^R	This work
pMP-MXAN3106	pMP220 with *attP* and regulatory sequence upstream of MXAN3106, Tc^R	This work
pMP-MXAN3905	pMP220 with *attP* and regulatory sequence upstream of MXAN3905, Tc^R	This work
pMP-MXAN4176	pMP220 with *attP* and regulatory sequence upstream of MXAN4176, Tc^R	This work
pMP-MXAN4198	pMP220 with *attP* and regulatory sequence upstream of MXAN4198, Tc^R	This work
pMP-MXAN4365	pMP220 with *attP* and regulatory sequence upstream of MXAN4365, Tc^R	This work
pMP-MXAN5030	pMP220 with *attP* and regulatory sequence upstream of MXAN5030, Tc^R	This work
pMP-MXAN6044	pMP220 with *attP* and regulatory sequence upstream of MXAN6044, Tc^R	This work
pMP-MXAN6579	pMP220 with *attP* and regulatory sequence upstream of MXAN6579, Tc^R	This work
pMP-MXAN6911	pMP220 with *attP* and regulatory sequence upstream of MXAN6911, Tc^R	This work

type strain DK1622 through electroporation[15]. Transformants were selected by plating the cells onto CTT plates containing oxytetracycline. None of the plasmids used in this study could independently replicate out of the chromosome in *M. xanthus*, thus, all transformants that were resistant to oxytetracycline were the result of integration of the plasmid into the chromosome either by homologous recombination or by site-specific recombination between *attP* on the plasmid and the *attB* site on the chromosome. PCR was used to screen antibiotic resistant colonies for proper integration of each plasmid at the *attB* site.

3 Results

3.1 Bioinformatic analyses

We used six bioinformatic algorithms to identify proteins localized at the *M. xanthus* outer membrane. Combining the total predictions from these six programs revealed that 11 hits were shared by all predictors. According to BLAST analysis and TIGR annotation, the name and function of the 11 genes are as follows: MXAN1316, MXAN4365, MXAN6044, MXAN6579 and MXAN6911, TonB-dependent receptors; MXAN3905, MXAN4176, MXAN4198 and MXAN5030, outer membrane efflux proteins; MXAN3106, protein transporter of secretin family; MXAN3883, protein transporter of fimbrial usher protein (FUP) family. All of the 11 OMPs seem to be involved in transporting molecules in and out of cells. Several previously characterized OMPs, like PilQ[6-7], failed to be recognized by every predictor used in this study. However, it should be mentioned that PilQ was indeed identified as an OMP by three of the six algorithms: PSORTb, TMBETA-SVM, and PSLpred. Considering limited accuracy of the computational programs, the proteins that failed to be hit by every predictor were excluded from this study. This may result in decreased number of possible OMPs, but the accuracy of the bioinformatic analyses should be greatly increased. Furthermore, cellular localization of certain proteins depends on post-translational modification. For instance, *M. xanthus* CsgA does not adopt a β-barrel structure typical of OMPs and its amino acid sequence does not contain putative membrane-spanning regions, yet both full-length and truncated forms of CsgA were detected in the outer membrane fraction as revealed by biochemical fractionation[5]. Current OMP prediction programs are unable to identify this kind of OMPs.

3.2 Expression analyses of the OMP-encoding genes

Expression of the OMP-encoding genes was examined by constructing their respective transcriptional MXAN-*lacZ* fusions (Table 2). Each

MXAN-*lacZ* fusion was designed to contain a short 5′-terminal coding region together with its potential upstream regulatory sequence. Development of *M. xanthus* cells carrying various MXAN-*lacZ* fusion at chromosomal *attB* was induced by spotting them on TPM starvation agar plates. Cells were collected at different time points and then their β-galactosidase activity was measured (Figure 1). Based on *lacZ* expression profiles, the 11 OMP genes can be divided into 3 groups: (1) β-galactosidase activity could hardly be detected during both vegetative growth and development. This group includes MXAN3905, MXAN4198, MXAN4365, and MXAN6044. When *lacZ* was fused to these genes, less than 4 units of β-galactosidase activity were produced within the first 18–24 h of *Myxococcus* development. (2) β-galactosidase activity could be detected during growth, but the activity decreased upon the initiation of development. This group includes MXAN1316, MXAN4176, MXAN5030, MXAN6579, and

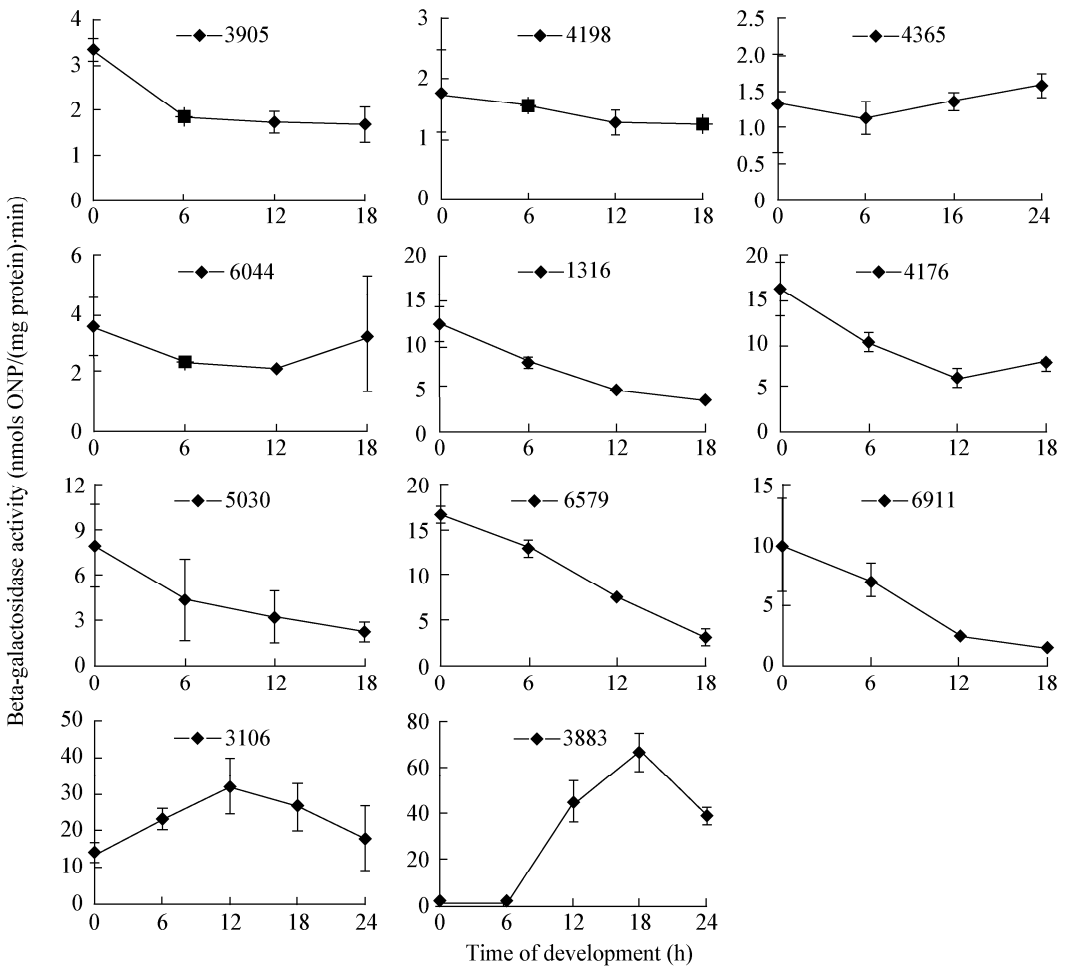

Figure 1 Expression patterns of 11 OMP-encoding genes during *M. xanthus* development

图 1 黄色粘球菌发育阶段 11 个外膜蛋白编码基因的表达模式

Note: *M. xanthus* DK1622 derivatives harboring *lacZ* fused to various OMP-encoding genes were starved on TPM agar for indicated periods of time, and samples harvested and tested for specific activity of β-galactosidase. Data points are presented as values (mean and SD) of at least two independent experiments.

注：将黄色粘球菌 DK1622 的不同衍生菌株在缺乏营养的 TPM 固体培养基上诱导发育，这些衍生菌株包含融合了 *lacZ* 的不同外膜蛋白编码基因，然后在不同的发育时间点分别收集细胞，测定 β-Galactosidase 的活性，所有数据(平均值和标准差)是至少 2 次重复试验所得.

MXAN6911. (3) The amount of β-galactosidase increased in early development. Two genes, MXAN3106 and MXAN3883, fall into this group. For MXAN3106-*lacZ* fusion, the peak amount of β-galactosidase was approximately three-fold compared to that at 0 h; by 24 h, the amount of β-galactosidase decreased but stayed at a level comparable to that at 0 h. The expression pattern of MXAN3883-*lacZ* is consistent with the data in a previous report[14], where the timing of MXAN3883 expression was confirmed further by the accumulation profile of MXAN3885 protein, another component encoded by the MXAN3885-3883 operon. This also implies that the expression patterns of the 11 *lacZ* fusions presented in Figure 1 are reliable.

4 Discussion

Computational algorithms are sometimes not unequivocal for predicting OMPs, therefore it can be helpful to utilize several programs in combination to increase the reliability of the predictions. A recent study in the Gram-negative bacterium *Actinobacillus pleuropneumoniae* revealed that, of the 45 predicted OMPs that were shared by at least two of the three predictors (Proteome Analyst, PSORTb, BOMP), 27 were ultimately confirmed as OMPs by gel-based analysis of the NBSC outer membrane sample coupled to mass spectrometry[16]. Other in silico identified OMPs that failed to be uncovered by mass spectrometry may be ascribed to incomplete representation of the membrane proteome. In the present report, the ORFs that were predicted to code for OMPs by every program were selected for further investigation. We think that finding an OMP by six independent programs should provide a higher degree of confidence for the prediction.

Among the 11 hits shared by the six predictors, 9 genes in groups 1 and 2 code for putative efflux transporters or TonB-dependent receptors. Efflux proteins pump out unwanted toxic substances outside the cell, and TonB-dependent outer membrane receptors are involved in transport of valuable nutrients larger than 600 Da, mainly including iron-siderophore complexes and vitamin B12[17]. Results of *lacZ* reporter assay suggest that OMP genes in group 1 might be dispensable throughout *M. xanthus* life cycle. An alternative explanation is that these 4 genes in group 1 are silent when cells are cultured in standard laboratory media, yet they may be activated in environmental conditions different from standard ones. *lacZ* fused to OMP genes in group 2 produced certain amount of β-galactosidase at t=0, implying these OMP genes may be functional in importing and exporting substances during vegetative growth. After initiation of development, expression of these genes decreased. The decreased expression may be ascribed to nutrient exhaustion and cease of growth during development, for cells do not need to make these OMPs to transport biochemicals that are necessary to maintain growth. The prevalence of TonB-dependent receptor homologs within group 2 underscores the significance of iron or vitamin B12 uptake for the vegetative growth of *M. xanthus*. However, some of TonB-dependent receptors (i.e. MXAN4365 and MXAN6044) fall into group 1, probably reflecting functional redundancy of this type of proteins.

We are particularly interested in MXAN3106 and MXAN3883, both of which increased their expression in early development. MXAN3883 encodes an usher protein of the chaperone-usher pathway that in *M. xanthus* functions in the secretion of spore coat proteins[14,18]. This is a new function of the chaperone-usher pathway that in most cases is involved in the pilus assembly of Gram-negative bacteria. For MXAN3106, work by Pan et al[19] indicated that this gene plays a role in the osmotic regulation of the cell membrane. MXAN3106 protein belongs to the secretin family which form homomultimeric outer membrane complexes that serve as channels for type IV pilus biogenesis, type II and type III protein secretion, and for release of filamentous phages[20]. The C-terminal region of the secretin family is highly conserved, which is believed to play an important role in the assembly of secretin; while the N-terminal region is much less conserved, which is thought to regulate substrate recognition[21-22]. We speculate that MXAN3106 protein may not be a functional analog of *M. xanthus* secretin PilQ because except for MXAN3105, which is similar at amino acid level to *pilD*, genes flanking MXAN3106 do not show amino acid similarity to the *pil* genes[23]. A second line of evidence for functional difference between MXAN3106 and PilQ comes from the observation that no significant sequence similarity was found in the N-terminal part of the two proteins. Since the

N-terminal half of secretins is conserved only in proteins from related secretion pathways and is presumed to be involved in substrate recognition[21-22], the substrates secreted by MXAN3106 may be structurally different from those by PilQ (e.g. pilus subunit). The exact role of MXAN3106 awaits future studies.

REFERENCES

[1] Dworkin M. Recent advances in the social and developmental biology of the myxobacteria[J]. Microbiological Reviews, 1996, 60(1): 70-102

[2] Søgaard-Andersen L, Overgaard M, Lobedanz S, et al. Coupling gene expression and multicellular morphogenesis during fruiting body formation in *Myxococcus xanthus*[J]. Molecular Microbiology, 2003, 48(1): 1-8

[3] Kroos L, Kuspa A, Kaiser D. A global analysis of developmentally regulated genes in *Myxococcus xanthus*[J]. Developmental Biology, 1986, 117(1): 252-266

[4] Kruse T, Lobedanz S, Berthelsen NM, et al. C-signal: a cell surface-associated morphogen that induces and co-ordinates multicellular fruiting body morphogenesis and sporulation in *Myxococcus xanthus*[J]. Molecular Microbiology, 2001, 40(1): 156-168

[5] Lobedanz S, Søgaard-Andersen L. Identification of the C-signal, a contact-dependent morphogen coordinating multiple developmental responses in *Myxococcus xanthus*[J]. Genes & Development, 2003, 17(17): 2151-2161

[6] Wall D, Kolenbrander PE, Kaiser D. The *Myxococcus xanthus* pilQ (sglA) gene encodes a secretin homolog required for type IV pilus biogenesis, social motility and development[J]. Journal of Bacteriology, 1999, 181(1): 24-33

[7] Nudleman E, Wall D, Kaiser D. Polar assembly of the type IV pilus secretin in *Myxococcus xanthus*[J]. Molecular Microbiology, 2006, 60(1): 16-29

[8] Goldman BS, Nierman WC, Kaiser D, et al. Evolution of sensory complexity recorded in a myxobacterial genome[J]. Proceedings of the National Academy of Sciences of the United States of America, 2006, 103(41): 15200-15205

[9] Nariya H, Inouye S. Identification of a protein Ser/Thr kinase cascade that regulates essential transcriptional activators in *Myxococcus xanthus* development[J]. Molecular Microbiology, 2005, 58(2): 367-379

[10] Orndorff PE, Dworkin M. Synthesis of several membrane proteins during developmental aggregation in *Myxococcus xanthus*[J]. Journal of Bacteriology, 1982, 149(1): 29-39

[11] Inouye M, Inouye S, Zusman DR. Gene expression during development of *Myxococcus xanthus*: pattern of protein synthesis[J]. Developmental Biology, 1979, 68(2): 579-591

[12] Hodgkin J, Kaiser D. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): two gene systems control movement[J]. Molecular Genetics and Genomics, 1979, 171: 177-191

[13] Spaink HP, Okker RJ, Wijffelman CA, et al. Promoters in the nodulation region of the Rhizobium leguminosarum Sym plasmid pRL1JI[J]. Plant Molecular Biology, 1987, 9(1): 27-39

[14] Leng X, Zhu W, Jin J, et al. Evidence that a chaperone–usher-like pathway of *Myxococcus xanthus* functions in spore coat formation[J]. Microbiology, 2011, 157: 1886-1896

[15] Kashefi K, Hartzell PL. Genetic suppression and phenotypic masking of a *Myxococcus xanthus* frzF- defect[J]. Molecular Microbiology,1995, 15(3): 483-494

[16] Chung JW, Ng-Thow-Hing C, Budman LI, et al. Outer membrane proteome of *Actinobacillus pleuropneumoniae*: LC-MS/MS analyses validate in silico predictions[J]. Proteomics, 2007, 7(11): 1854-1865

[17] Postle K, Kadner RJ. Touch and go: tying TonB to transport[J]. Molecular Microbiology, 2003, 49(4): 869-882

[18] Zhu W, Wu M, Cao S, et al. Characterization of McuB, a periplasmic chaperone-like protein involved in the assembly of Myxococcus spore coat[J]. Journal of Bacteriology. 2013, 195(13): 3105-3114

[19] Pan HW, Liu H, Liu T, et al. Seawater-regulated genes for two-component systems and outer membrane proteins in myxococcus[J]. Journal of Bacteriology, 2009, 191(7): 2111

[20] Bayan N, Guilvout I, Pugsley AP. Secretins take shape[J]. Molecular Microbiology, 2006, 60(1): 1-4

[21] Genin S, Boucher CA. A superfamily of proteins involved in different secretion pathways in gram-negative bacteria: modular structure and specificity of the N-terminal domain[J]. Molecular Genetics and Genomics, 1994, 243(1): 112-118

[22] Shevchik VE, Robert-Baudouy J, Condemine G. Specific interaction between OutD, an Erwinia chrysanthemi outer membrane protein of the general secretory pathway, and secreted proteins[J]. The EMBO Journal, 1997, 16(11): 3007-3016

[23] Spormann AM. Gliding Motility in Bacteria: Insights from Studies of *Myxococcus xanthus*[J]. Microbiology and Molecular Biology Reviews, 1999, 63(3): 621-641