PROCORE - FRANCE/HONG KONG JOINT RESEARCH SCHEME
COMPLETION REPORT

Project Reference Number

F-HK39/10T

Project Title

Regulated expression of multidrug resistance genes in anaerobic E. coli and its physiological implications in host adaptation

Particulars

	Hong Kong team	French team
Name of Project Co-ordinator (with title)	English: Aixin Yan Chinese: 閆愛新	Jost ENNINGA
Name of Co-Investigator (if any)	English: Chinese:	
Institution or Institutional affiliation	[ ] CityU [√] HKU [ ] CUHK [ ] HKUST [ ] HKBU [ ] LU [ ] HKIEd [ ] PolyU	[ ] CEA [ ] INRA [ ] CNRS No. [ ] INRIA [ ] INFREMER [ ] INSERM No. [ ] University of [√] Others: Institut Pasteur
Other project team members (if any)	Miss Xiao, Minfeng	Mr. Laurent Audry

Funding Period

	1st year	2nd year (if applicable)
Start Date	01/01/2011	01/01/2012
Completion Date	31/12/2011	31/12/2012

Objective(s) as per original application

1. Substantiate the transcriptional activation of multidrug efflux transporter genes in anaerobic *E. coli* by *in vivo* and *in vitro* approaches;
2. Examine multidrug resistance phenotype in anaerobic *E. coli* cells by disk and growth curve methods;
3. Characterize the biochemistry and physiological significance of selective anaerobically regulated drug exporters

[Please attach relevant document(s)]

i) Outline of proposed research and results obtained

Objective 1) Substantiate the transcriptional activation of multidrug efflux transporter genes in anaerobic *E. coli* by *in vivo* and *in vitro* approaches:

We have analyzed the transcription of all 20 Efflux genes present in *E. coli* genome using the promoter-lacZ fusions under the anaerobic growth condition of the bacterium and found that transcription of the *mdtEF* efflux genes which are present in the *gadE-mdtEF* operon was significantly up-regulated. Genetic results revealed that deletion of the anaerobic global regulator *arcA* almost completely abolished the up-regulation, suggesting that ArcA primarily regulates the activation of the MdtEF efflux pump when *E. coli* grows under anaerobic condition. Using electrophoretic mobility shift assay (EMSA), we demonstrated that purified and in vitro phosphorylated ArcA indeed binds to the promoter region of the *gadE-mdtEF* operon, substantiating the transcriptional activation of the MdtEF multidrug efflux pump during the anaerobic adaptation of *E. coli*. To further prove this at protein level, we also tried to construct the strain that contains a fluorescence tag iLOV to the C-terminus of *mdtE* and *mdtF*. Unlike GFP and its analogues, the fluorescence of iLOV is independent of the presence of oxygen, allowing the characterization of gene expression using fluorescence tag under anaerobic conditions. This part of work is underway currently.

Objective 2) Examine multidrug resistance phenotype in anaerobic *E. coli* cells by disk and growth curve methods:

After confirming the anaerobic up-regulation of *mdtEF* by the anaerobic global regulation ArcA, we also measured the efflux activity and drug resistance phenotype of the *ΔmdtEF* strain in comparison with the WT under anaerobic growth conditions. Direct efflux assay using the fluorescent drug doxorubicin showed that anaerobically grown *E. coli* exhibited enhanced efflux activity in comparison with that of aerobically grown *E. coli*, and *ΔmdtEF* led to decreased efflux activity of the bacterium under anaerobic condition, suggesting that anaerobically induced MdtEF indeed causes enhanced efflux activity of the bacterium under the anaerobic physiological condition. We also measured the effect of this enhanced efflux activity on the drug tolerance or resistance of the anaerobically grown *E. coli* by growth curve method. It was shown that *ΔmdtEF* strain grew significantly slower than the WT strain in the presence of the antibiotic kanamycin and the antimicrobial agents Rhodamine 6G and SDS, confirming the role of MdtEF in conferring the antibiotic resistance of *E. coli* under its anaerobic growth environment.

Objective 3) Characterize the biochemistry and physiological significance of selective anaerobically regulated drug exporters:

The fact that the efflux pump MdtEF was up-regulated under the anaerobic growth environment of *E. coli* without the pre-exposure of antibiotics suggested that this efflux pump has physiological functions, i.e. export of certain metabolic by-products accumulated under this physiological condition. We searched literatures and found a previous study which reported that considerable amount of indole nitrosative metabolic by-products were accumulated in *E. coli* grown during anaerobic respiration of nitrate. To examine whether the up-regulated MdtEF pump functions to export these metabolic by-products, we synthesized indole red, a 3-nitro derivative of indole, and analyzed a) whether the compound indeed is toxic to *E. coli* cells grown anaerobically; and b) whether*ΔmdtEF* strain is more susceptible to this compound. It was shown that indole red indeed was toxic to *E. coli* cells since *E. coli* cells grew slower in the presence of the compound than in the absence of the compound. Furthermore, *ΔmdtEF* strain grew significantly slower than the WT in the presence of this compound, suggesting that MdtEF indeed contribute to the efflux of this metabolic by-product. Recently, we also confirmed this notion by metabolic profiling combined with the LC-MS analysis which showed that less indole nitrosative products were accumulated in *ΔmdtEF* strain than the WT.

ii) Significance of research results

Using the integrated approaches of genetics, biochemical, chemical, and metabolomics, this research demonstrated the up-regulation of bacterial multidrug efflux pumps, which constitutes an important mechanism of antibiotic and multidrug resistance, under the anaerobic physiological condition of bacteria. Since the primary colonization and infection site of enteric bacteria in human host is an anaerobic environment, this finding has direct relevance to the development of antibacterial interventions and control of antibiotic resistance in clinics. The finding also proves the increasingly compelling concept of the role of bacterial multidrug efflux pumps in bacterial stress response which proposed that the antibiotic or multidrug resistance caused by the up-regulation of efflux pump genes is merely a side-effect of their roles in facilitating bacteria to the adverse and changing environments of their ecological niches and in human host. Recognition of this concept has direct and significant values in both academic and applied research of combating the drug resistance.

iii) Research output

A research paper published in an internationally peer-reviewed major journal in molecular biology and biochemistry and two conference abstracts:

Research article:

Yiliang Zhang, Minfeng Xiao, Tsukasa Horiyama, Yinfeng Zhang, Xuechen Li, Kunihiko Nishino, **Aixin Yan***, The multidrug efflux pump MdtEF protects against nitrosative damage during the anaerobic respiration in *Eschericia coli*. 2011, ***J. Biol. Chem.***, *286*, 26576-26584

Conference abstracts:

1) Yiliang Zhang, Minfeng Xiao, Tsukasa Horiyama, Yinfeng Zhang, Xuechen Li, Kunihiko Nishino, **Aixin Yan**, Novel functions of the multidrug efflux pump MdtEF in bacterial stress response to oxygen limitation *Gordon Research*

Conference on Multi-Drug Efflux Systems **2011**, June 12-17, Les Diablerets, Switzerland

2) Ka-Chun Fung, Yiliang Zhang, Kunihiko Nishino, **Aixin Yan,** Up-regulation of multidrug efflux pumps during the bacterial stress response to host physiological conditions. *4th ASM conference on Antimicrobial ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Human, and Environment*, June 25-29, **2012**, Aix en Provence, France

iv) Potential for or impact on further research collaboration

To substantiate the up-regulation of the multidrug efflux gene *mdtEF* in the anaerobic physiological condition of *E. coli*, we (the HK and the French team) integrated the expertise and strength of both sides and developed the promoter-iLOV and protein-iLOV tagged strains to study the gene expression and protein expression during the transition from aerobic to anaerobic condition using fluorescence microscopy. Currently we are extending this analysis to a methodology that can be utilized for the general analysis of gene expression and protein expression under the anaerobic physiological condition of bacteria. To this end, a series of promoter-lacZ constructions of genes which expression displays different patterns in response to oxygen limitation, i.e. activated, repressed, constitutive, or having the maximal expression under microaerobic condition, were constructed. Transcription of these fusions in comparison with the corresponding lacZ fusions is underway. Once it is confirmed that transcription pattern from the iLOV fusion is similar to that from the lacZ fusion, we will apply the methodology to test genes with unknown functions. The long term goal of this extended study is to dynamically monitor the gene expression changes of virulence genes during the anaerobic environment of their infection in pathogenic strains, such as *Shigella*, *Salmonella etc.* There are certainly great potential for further research collaboration which requires the integration of the expertises from both sides, i.e. the anaerobic bacterial cultural and biochemical techniques on the HK side and the in situ fluorescence microscopy techniques on the French side.