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The 11th International Symposium on *Yersinia*: Crowdsourcing and Multilateral Cooperation – the Way to Address Bacterial and Data Challenges

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The 11th international Symposium on *Yersinia* was held in Suzhou, China June 24 – June 28, 2013. The meeting was organized by Cold Spring Harbor Asia and arranged by Ruifu Yang, Beijing Institute of Microbiology and Epidemiology, China; Elisabeth Carniel, Institut Pasteur, France; Paul Keim, Northern Arizona University, U.S.A.; and Andrey Anisimov, State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russia. The Symposium covered different aspects of *Yersinia* physiology, epidemiology, diagnostics, infection and virulence, genomics and other Omics-driven studies. The keynote presentation by William Goldman from the University of North Carolina, U.S.A., briefly summarized the present state of *Yersinia* research and the impact of some outstanding scientists. The special lecture by Nils Christian Stenseth discussed the interplay between ecology and evolution in triple host-vector-pathogen systems using the plague agent, *Y. pestis*, as an example.

One of the most fascinating lectures was done by Joe Hinnebusch who presented new data on possible mechanisms of *Y. pseudotuberculosis* conversion to a flea-borne transmission on its way to the plague agent. He retraced the evolutionary progression of genetic changes that might enable *Y. pseudotuberculosis* to build up a biofilm not in the hindut but rather in the proventriculus of the flea, ability that is important for *Y. pestis* midgut blockage. Addition of the Ymt phospholipase D by acquisition of the *ymt* gene on a transmissible pFra plasmid is sufficient to allow *Y. pseudotuberculosis* to colonize the midgut. However, modification of at least three other genes (*rcsApe*, PDE2-pe and PDE3pe) was necessary to establish stable *Y. pseudotuberculosis* infection in the flea's midgut. Another enzyme, the urease, demonstrated an acute toxicity for *X. cheopsis* fleas by the enteropathogenic *Yersinia* species, *Y. pseudotuberculosis* and *Y. enterocolitica*. However the gene is silenced in the plague bacillus. Thus, silencing of the insecticidal toxicity of the urease might be an important step in *Y. pseudotuberculosis* adaptation to the flea-borne transmission and a significant step on its road to *Y. pestis*. Such data were presented by Imart Chokha from Joe Hinnebusch's group.

Elisabeth Carniel (Institute Pasteur, Paris, France) continued with the characterization of the impact of genomic sequences differently represented in *Y. pestis* and the enteropathogenic *Y. pseudotuberculosis*. This time she addressed the role of a conserved RfaH regulator that activates known virulence associated genes like hemolysin and O antigen in *Escherichia coli* and *Salmonella* Typhimurium. However, deletion of the *rfaH* gene in the virulent *Y. pestis* CO92 did not alter its ability to resist against actions of the deoxycholate, sodium dodecal sulfate and polymixin B. Moreover, the mutant retains its ability to withstand the bactericidal activity of the human serum at a same degree as the wild type strain. This greatly suspects the importance of RfaH in *Y. pestis* virulence. Anne Debrise from the same group applied bioluminescence imaging to trace the course of plague infection in live animals. Using the luciferase reporter and the Tn7-based transposon delivery system, they monitored the expression of six different genes (*pla, yopE, ail, caf1M, psaA* and *fyuA*) in the murine infection model of bubonic plague. They observed the in vivo sequential expression of *pla*, *yopE, ail, caf1M*, and *fyuA* but not of the *psaA* promoters from the site of subcutaneous injection to the spleen and liver, and up to terminal septicemic phase. These data once more emphasize the importance of the established virulence genes encoded by the three virulence associated plasmids (pFra, pYV and pPla), and by the High-pathogenicity island, encoding the Yersiniabactin iron acquisition system, and argue against a direct role of *psaA* in *Y. pestis* virulence. The same author reported on the construction of a live attenuated *Y. pseudotuberculosis* strain with an operon encoding the fraction 1 antigen stably integrated into the host chromosome. This strain V674TnF1 stably produces the Fra1 antigen and confers 100 % protection against the bubonic plague after a challenge with 10^3 CFU (100 x LD_{50}) of *Y. pestis*. Even a single subcutaneous injection of 10^7 CFU of the recombinant strain protected 100 % animals against the bubonic plague (challenged with 10^5 CFU).

The group of Petra Dersch from the Heimholtz Centre for Infection Research, Brunswick, Germany, prepared interesting presentations on virulence regulators in *Y. pseudotuberculosis*. Aaron Nuss monitored temperature-dependent bistability of the pivotal virulence transcriptional global regulator RovA. Barbara Waldmann, using RNA deep sequencing, has done *Y. pseudotuberculosis* transcriptional profiling in different temperature conditions, at environmental (25 °C) and host (37 °C) temperatures. She identified a large number of new putative small RNAs and validated some of them by Northern blotting. Petra Dersch summarized the data in a complex model in which the nucleotide-associated thermosensitive protein YmoA, together with the Csr system (including the small regulatory RNAs CsrB and CsrC and RNA-

binding protein CsrA), represents the central part of a composite regulatory network. This network controls the switching from a RovA-dependent early colonization phase to a pYV virulence plasmid infection phase in order to escape host defenses.

Biofilm formation in *Y. pestis* was addressed in several remarkable presentations. Joanne Purves from the University of Nottingham, United Kingdom, investigated quorum-sensing controlled genes and found that the histidine metabolic regulator HutC acts as a biofilm and motility regulator and provides a link between quorum sensing, virulence and central metabolism in *Y. pseudotuberculosis*. Stephan P. Willias from the University of Texas, Galveston U.S.A. showed that biofilm formation in *Y. pestis* was differentially modulated by the available carbon sources. While glucose inhibited biofilm production, alternative carbon sources supported the production of biofilms. The authors concluded that cAMP receptor protein CRP acts as a critical facilitator of *Y. pestis* and affects biofilm formation. Yi-Cheng Sun from Chinese Academy of Medical Sciences and Peking Union Medical College, China, addressed the role of two diguanylate cyclases, HmsT and Y3730, responsible for the synthesis of c-di-GMP, a cyclic diguanylate that controls biofilm formation in *Y. pestis*. They proposed that Y3729, that resides in the periplasm and interacts directly with the sensor domain of Y3730, senses the environmental signals and regulates the enzymatic activity of diguanylate cyclase Y3730 and c-di-GMP synthesis and thus biofilm formation.

Mikael Skurnik from the University of Helsinki, Finland, discussed the role of the surface located environmentally-exposed virulence factors: the trimeric autotransporter adhesion protein YadA, the integral adhesion and invasion outer membrane protein Ail and lipopolysaccharide LPS with structural and endotoxin activity. He described the roles of these surface located structures in complement resistance and sensitivity to phages. Motohiro Matsuura from Kyoto University Graduate School of Medicine, Kyoto, Japan, addressed modifications of lipid A structure in *Y. pestis* lipopolysaccharide that facilitate bacterial evasion of the human innate immunity. The temperature changes during infection cycles from lower temperatures (external environments or in fleas) to higher temperatures (mammal host) are pretty similar in all pathogenic *Yersinia*. This suggests that the production of a less immunostimulatory form of the LPS during the entry into the mammalian host is conserved in *Yersinia* genus.

Several lectures covered different aspects of metal acquisition systems and transporters in *Yersinia*. Alexander Rakin from Max von Pettenkofer Institute, Munich, Germany, argued against singularity of the yersiniabactin (Ybt) iron uptake system in highly pathogenic *Yersinia* species. While most of *Y. pseudotuberculosis* O1 strains carry the functional *ybt*-cluster, the "outbreak" O1 strains (responsible for the Far East Scarlet-like Fever, FESF), as well as serotype O2, O4 and O5 strains are devoid of these genes, but still demonstrate a siderophore activity. Two iron acquisition gene clusters are present in *Y. pestis / Y. pseudotuberculosis* group: *ynp* and *ysu*, able to encode two additional siderophores, pseudochelin and yersiniachelin, correspondently.

Jacqueline D. Fetherston from University of Kentucky, Lexington, U.S.A., addressed the role of the *yfe, feo* and a putative fetMP ferrous iron transporters in *Y. pestis*. The authors demonstrated that the *yfe, feo* double mutants have a more severe defect in strains with deleted *pgm* locus than in strains containing genes both for the *ybt* synthesis and *fetPM*. They speculate that the Ybt system moderately contributes to iron acquisition under microaerobic growth conditions but does not account for all of it, while the *fetMP* seems to pose a stronger impact on growth in these conditions.

Robert Perry from the same University addressed the role of another metal cofactor required for the enzymatic activity of bacterial and eukaryotic cells, zinc. Although zinc is toxic at high concentrations, maintaining zinc homeostasis is essential for survival and defense of bacteria in mammalian hosts. Previously they have reported on a *Y. pestis znu* mutant that lacks the ZnuABC high-affinity zink uptake system. However, a deletion *pgm, znu* double mutant demonstrated a more pronounced growth defect in a low Zn containing medium than a *znu* parent strain. Moreover, the authors revealed that the double mutant is seriously attenuated in septicemic mouse model of plague compared to the parent strain with a deleted *pgm* locus. From this the authors concluded that the chromosomal pgm locus might encode an additional high-affinity Zn uptake system.

Virginia Miller from the University of North Carolina at Chapel Hill, U.S.A., studied the early events in bubonic plague post inoculation of *Y. pestis* into the skin and trafficking to the draining lymph nodes and spleen. They applied a dissemination assay with 9 nucleotide oligo tagged strains to outline a window of opportunity for plague bacillus dissemination and define whether *Y. pestis* passes through a bottleneck on its pathway to the spleen. They determined that only few tagged strains could be found in the spleen of the infected animals. Thus, *Y. pestis* indeed passes through a bottleneck on its peripheral route from skin through lymph nodes to the spleen. However, the state of *Y. pestis* population tagged with 9 nucleotide oligos and possible influence of such manipulation on the final results cannot be excluded.

An Israel group of Emanuelle Mamroud was interested in the cross talk between the lung and the bone-marrow at early stages of another plague form, a pneumonic plague. They showed that intranasal infection of mice with a fully virulent *Y. pestis* strain was sensed early after infection by the bone-marrow compartment, indicating a potential cross-talk between the lung and the bone-marrow already at the early stages of pneumonic plague. Both *Y. pestis* and soluble antigens were detected in the late stages in bone-marrow.

The group of Deborah Anderson from the University of Missouri, Columbia, U.S.A., established a pneumonic

plague model in Brown Norway Rats that closely mimics a human disease. In such model they compared the natural disease history caused in animals challenged with low ($5x10^3$) and high ($5x10^5$) *Y. pestis* doses within 3 days. The authors defined very high frequency of hydronephrosis, which is a common incidental finding in aged Brown Norway Rats and founded that bacteremia was not always present and nearly 40% of rats in high dose group demonstrated no clinical signs before being found dead.

Juergen Heesemann has presented interesting data on different murine innate immunity systems mechanics to *Y. enterocolitica* ssp. enterocolitica infection. They demonstrated three different levels of susceptibility to *Yersinia* infection in a set of knock-out mice: highly-resistant group, wild-type resistant group and high-susceptibility group. The degree of *Yersinia*-resistance correlated with the immigration rate of neutrophils and concentration of G-CSF, KC, IL-1a and MIP-2 within the first 24 hours post infection. The highly sensitive mice responded with a delay of 24 hours post infection compared with the highly resistant and wild-type resistant groups. Also, *Yersinia* invasion of the spleens of highly sensitive mice resulted in overwhelming *Yersinia* growth with evident destruction of the tissue architecture.

James Bliska from the Stony Brook University, NY, U.S.A., described their recent progress in understanding a dual nature of the virulence factors by studying the type 3 secretion system. They concentrated on the role of the protective epitope of the GTPase-activating protein YopE and its contribution to adaptive immunity against *Y. pseudotuberculosis* and possible interactions with other effectors.

Ake Forsberg from the Umea University, Sweden, described the intracellular targeting of virulence type 3 effectors in the absence of pore formation in his presentation. He presented further evidences against an out-of-date simplified model describing the T3SS mediated targeting exclusively by pore formation in the host cell membranes. He also further demonstrated the involvement of the protective LcrV antigen in the early delivery of the T3SS effectors to their respective targets.

There were multiple presentations concerning epidemiology aspects of *Yersinia* and *Yersinia*-caused diseases. Interesting data on genetic diversity, epidemiological features and distribution of pathogenic *Yersinia* were presented by Andrey Anisimov, from the State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russia; Mark Achtman from the University College Cork, Republic of Ireland; Alzira M.P. Almeida from the Centro de Pesquisas Aggeu Magalhaes, Brazil; Minoarisoa M. Rajerison from the Institut Pasteur de Madagaskar, Madagaskar; Waldemar Rastawicki from the National Institute of Public Health-National Institute of Hygiene, Poland; Huaiqi Jing from the Chinese Center for Disease control and Prevention, Beijing, Chaina; Alan McNally from the Nottingham Trent University, United Kingdom, David M. Wagner from the Northern Arizona University, U.S.A. and in numerous poster presentations.

Several presentations dealt with paleogenetics. Holger Scholz from the Bundeswehr Institute of Microbiology, Munich, Germany, in cooperation with scientists from Germany, U.S.A. and Norway, tried to answer the question whether *Y. pestis* was the real agent of the Justinianic Plague of the 6^{th}-8^{th} centuries 541 – 767 A.D. They were able to confirm the presence of the *Y. pestis* sequences in the tooth samples of the human skeletal remains from the Early Medieval cemetery 6^{th} – 7^{th} century in Aschheim near Munich. The findings appear to confirm that *Y. pestis* might be really responsible for the Justinianic Plague. However, the question about how the plague arrived in southern Germany remains open.

Also Kirstin Bos from the University of Tuebingen, Germany, address a genomic reconstruction of *Y. pestis* from archaeological populations using next generation sequencing biased against longer fragments compared to PCR. They coupled whole genome array-based target enrichment with next generation sequencing and reconstructed *Y. pestis* draft genome from London, England, 1348-1350. Because the authors reconstructed the ancient genome using an extant *Y. pestis* CO92 they were not able to identify the regions of differences that may be existed in the ancient isolate bur later lost in modern strains. However even the draft genome sequence let the researchers recognize the lack of nucleotide differences between the medieval and modern bugs. Thus the question about what contributed to high mortality of the Black Death still remains. Whether it was climate, changes in vector population, different genetic susceptibility of the host population, or con-current disease and human behavior is not known and these questions remain till now without answers. To mention, de novo reconstructed ancient *Mycobacterium leprae* genomes did not significantly differ from modern *M. leprae* strains from patient biopsies (Schuenemann V.J., *et al*., 2013). Thus such questions whether the ancient strains were more or less virulent or a simple improvement in sanitation results in eradication of these deadly diseases remain unanswered.

Sandra Reuter from the Wellcome Trust Sanger Institute, Cambridge, United Kingdom, together with her collaborators from Finland, France, Ireland and U.K., used whole genome sequencing approach to delineate the gene complement of *Yersinia* genus and define the patterns of virulence evolution. They determined genomic sequences of more than 100 strains of both pathogenic and environmental *Yersinia* and analyzed 85 housekeeping genes that allowed them to define more than 6000 SNPs. The authors concluded that, contrary to a popular view on pathogenic *Yersinia* species sharing a common pathogenic ancestor, *Yersinia*e have evolved independently following parallel evolutionary paths in acquiring the same virulence determinants (like independent acquisition of the pYV virulence plasmid happened on multiple occasions) and limiting their metabolic repertoire (e.g. mutations in *cob/pdu*, *cel* and *ttr* operons

known to be important in evolution of host specificity in *Salmonella*). However, a possible origin and donor of the pYV plasmid remains unclear.

The motto of the 11th international Symposium on *Yersinia* can be defined as "crowdsourcing and multilateral cooperation to react to the challenges of today". This was clearly expressed by Ruifu Yang from Beijing Institute of Microbiology and Epidemiology, China. He attracted the attention of the Symposium to the necessity of creating an international consortium that will practices Open Source genomics and Crowdsourcing analysis in order to address the big data challenge by modern biological science. He mentioned that it is the right time to establish a comprehensive open database for *Yersinia* that includes the increasing number of genomic, transciptomic, proteomic, metabolomics, phenomic and interactomic data. Only cooperation of different institutions and Genome Analysis Crowdsourcing allowed fast identification and characterization of the new rapidly evolved *E. coli* O104:H4 clone responsible for the deadly outbreak in Germany (https://github.com/ehec-outbreak-crowdsourced/BGI-data-analysis/wiki). Emergence of such a strain once more demonstrates the constant flexibility of bacterial genomes and necessity of wide international cooperation in combating the challenges of Mother Nature.

The next, 12th Symposium on *Yersinia* is supposed to take place in Tbilisi, Georgia, in three years. Looking forward to meet you in Tbilisi!

References

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