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Systems Biology of Drug Resistance Evolution

Posters

Predicting functional SNPs in DEFB1 and CAMP promoters in Tuberculosis and/or HIV/AIDS.

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Single nucleotide polymorphisms (SNPs) in transcription factor binding sites (TFBSs) could modify the transcription rate of genes related to complex diseases. Recent projects, such as the 1000 Genomes Project (1kGP) and ENCODE data have revealed indispensable information that have been used for in silico analysis of the molecular and biological repercussion of TFBS SNPs. Several approaches have been proposed, however most of them are very limited, because they study only SNPs within genes and do not integrate important biological data (TFBSs, expression profiles, pathway analysis, polymorphisms, chromatin accessibility) that could make a more accurate prediction. Our work aims to integrate the biological data in a coherent methodology to predict the functional SNPs in the promoter of DEFB1 and CAMP associated with tuberculosis (TB) and/or HIV/AIDS. Our methodology coherently integrates multiple databases to identify transcription factors (TFs) whose binding site is affected by a SNP in the promoter of DEFB1 and CAMP. Our results identified 16 TFs that have been previously identified as TB and/or HIV/AIDS related in expression profiles and four of them are present in the diseases infection pathways which verifies our method. This novel method could be applied to other promoters and other complex diseases in order to design new treatment drugs.

The possibility of impossibility: when pA/C found the pX1

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We previously describe that in the Mexican Salmonella Typhimurium population none of the ST19 and ST213 strains harbored both the Salmonella virulence plasmid (pSTV) and the prevalent IncA/C plasmid (pA/C). This observation led us to hypothesize that a restriction in the horizontal transfer of these plasmids existed. The aim of this study was to determine if the genetic background of the different recipient strains affected the transfer frequencies of pA/C. We designed a conjugation scheme using ST213 strain YU39 as donor of the bla_{CMY-2} gene (conferring resistance to ceftriaxone; CRO) carried by pA/C, and two E. coli lab strains (DH5α and HB101) and two Typhimurium ST19 strains (SO1 and LT2) carrying pSTV as recipients. As a result we observed that YU39 was able to transfer CRO resistance,

via a novel conjugative mechanism, to all the recipient strains although at low frequencies (10^{-7} to 10^{-8}). The presence of pSTV in the recipients had little effect on the conjugation frequency. Unexpectedly, successful CRO-resistant transconjugants showed four different phenomena: 1) the co-integration of pA/C with a co-resident IncX1 plasmid (pX1), 2) the transposition of the bla_{CMY-2} gene from pA/C to pX1, 3) the transfer of pA/C displaying genetic re-arrangements, or 4) the co-mobilization of a small ColE1-like plasmid. These interactions were recorded in a recipient-dependent manner. The transconjugant plasmids involving pX1 re-arrangements (either via co-integration or ISEcp1-mediated transposition) obtained the capacity to conjugate the bla_{CMY-2} gene at very high levels, similar to those found for pX1 (10^{-1}). Two versions of the region containing bla_{CMY-2} were found to transpose to pX1: the large version was inserted into an intergenic region located where the “genetic load” operons are frequently inserted into pX1, while the short version was inserted into the stbDE operon involved in plasmid addiction system. This is the first study to report the acquisition of an ESC-resistance gene by an IncX1 plasmid.

Conclusions: We showed that the transfer of the bla_{CMY-2} gene harbored on a non- conjugative pA/C requires the machinery of a highly conjugative pX1 plasmid. Our experiments demonstrate the complex interactions a single strain can exploit to contend with the challenge of horizontal transfer and antibiotic selective pressure.

Wiesner, M., et al. (2013). "Conjugative transfer of an IncA/C plasmid-borne blaCMY-2 gene through genetic re-arrangements with an IncX1 plasmid." BMC Microbiol. **13**: 264.

Dynamical models for differentiation in bacteria

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Organisms perform processes to respond to fluctuating environments, one of this is the differentiation in which a cell develop specific characteristic through some morphogenetic process. In bacteria is observed in organisms like C. crescentus and B. subtilis. By the asymmetric division C crescentus generates two cell types: swarmer and stalked cells, and had a cell cycle, where the swarmer differentiate into the stalked, ones is formed begins to divide. These processes are globally mediate by the transcription factor (TF) CtrA. While for B. subtilis when is under environmental stress, some kinases phosphorylates and through some intermediary kinases activates specifics TFs for a given cell type. One of this TF is Spo0A, which control the expression of the pathways for spore forming and the extracellular matrix producer’s. Other is ComK that control the competence cell type. The miner phenotype is facilitated by the action of DegU in its phosphorylated state. The aims of this work is to found out the regulatory networks of the control of the cell fates and the differentiation processes in the two mentioned organisms and by a mathematical model to the networks explain the dynamical events of differentiation. The networks (G) were reconstructed from literature. Then was performed a discrete modeling to the G, assigning the Boolean functions (Fv) and applying $\sigma v(t+1) = Fv(\sigma v_1(t),\ldots,\sigma v_k(t))$, which is the rule on how the state of the nodes (genes/proteins) will change to the next time (t+1). By the modeling results of some stable steady states, which had some correspondence of the expression of the TFs in each cell type mentioned above for each organism. Also could analyze that the G are robust to perturbations, function in a flexible manner and operates near the critical regimen, all of this features are important as the organisms had to be adapted to a changing environment.

A phylogenetic and comparative genomic analysis to develop a Multiplex-PCR probe system for the proper identification of *Klebsiella variicola*

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Klebsiella variicola was very recently described bacterial species and is very closely related to Klebsiella pneumoniae; as a matter of fact, K. variicola isolates were at first identified as K. pneumoniae. This work describes the development of a multiplex-PCR method to identify K. variicola; notably this development was based on sequencing a K. variicola clinical isolate (801) and comparing it to other K. variicola and K. pneumoniae genomes. The K. variicola isolates cluster together but also K. pneumoniae 342 is included in this group and all the K. pneumoniae isolates form a group. The phylogenetic analysis of rpoB further supports the differentiation of K. variicola isolates from the K. pneumoniae isolates. The multiplex-PCR (M-PCR-1 to 3) probes system capable of identifying K. variicola with high accuracy, assays using the unique genes of K. variicola and K. pneumoniae genomes. The M-PCR-1 was assayed on a collection of multidrug resistant (503) and antimicrobial sensitive (557) K. pneumoniae clinical isolates; of K. variicola was found with a prevalence of 2.1% (23/1,060). Concerning the multidrug resistant isolates, 2.5% (13/503) isolates were K. variicola and 1.8% (10/557) K. variicola isolates were antimicrobial sensitive. A low but significant prevalence of K. variicola isolates was found, which implies that misclassification is not that unlikely. In a broad sense, this study exemplifies how a compound strategy of phylogenetic analyses of carefully selected loci and comparative genomics can be used to develop molecular biology tools that could help to elucidate other divisive cases of misclassification for other bacterial pathogens.

Outbreak of OXA-72 carbapenemase-producing Acinetobacter spp. in Mexico.

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Carbapenem resistance due to OXA-type carbapenemases seriously limits therapeutic options in nosocomial infections caused by Acinetobacter spp., and is an emerging problem in Mexico. We investigated the molecular epidemiology and carbapenem-hydrolyzing class D β-lactamases (CHDLs) genes of 32 nonrepetitive imipenem-resistant clinical isolates of Acinetobacter spp. collected from 2009 to 2010 at Ignacio Morones Prieto Hospital, San Luis Potosi, Mexico. Genotyping by pulsed-field gel electrophoresis (PFGE) found a major clone with 8 subtypes (A to A8). Multi Locus Sequence Type

(MLST) was performed in four isolates and revealed only one sequence type (ST), namely, ST417. All isolates were susceptible to colistin and tigecyclin, and resistant to cefepime, ceftazidime, ceftriaxone, imipenem and meropenem. The blaOXA-51-like and blaOXA-24-like genes were detected in all 32 isolates, blaOXA-58-like was found in 16/32 (50%) of isolates. Sequencing performed on 8 representative isolates confirmed the presence of the blaOXA-72 carbapenemase gene. Analysis of the genetic context of blaOXA-72 showed one copy of this gene, flanked by XerC/XerD-like binding sites. Genes coding for metallo-β-lactamases were not detected. These findings indicated clonal spread of imipenem-resistant Acinetobacter spp. and wide dissemination of the OXA-72 carbapenemase in Mexico. Furthermore, the factors responsible for dissemination of such isolates need to be identified, controlled, and prevented to avoid major outbreaks.

Prevalence of Extended-Spectrum-B-lactamases (ESBLs) among Enterobacteriaceae clinical isolates in Mexico: A Multicenter Study

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The extended spectrum ß-lactamases (ESBL) are enzymes with a capacity to hydrolyze a wide variety of β-lactam antibiotics including third generation cephalosporins and aztreonam. This is one of the most common mechanisms of resistance in Gram-negative bacteria. The three major families of ESBL (SHV, TEM and CTX-M) have been described worldwide and the first two were derived from wild β-lactamases TEM. This occurred for one or more amino acid substitutions which subsequently gave rise to the emerging family of CTX-M. The genes coding for the ESBL are mobilized usually by plasmids, which facilitate its spread among different species of Gram-negative bacteria. The aim of the study was to identify the frequency of ESBL producing Enterobacteriaceae clinical isolates causing nosocomial infections in multicenter study during the period 2005-2009 in Mexico.

Methods: A total of 648 clinical isolates of ESBL producers (E. coli 66% 427/648, K. pneumoniae 26% 170/648, E. cloacae 5% 35/648, others 2% 16/648) were collected from 12 hospitals in Mexico. Bacterial identification and susceptibility was determined by MicroScan (Dade MicroScan Inc. Sacramento, CA). Detection of ESBL production was performed using the method of double disk synergy (CLSI). The identification of the ESBL TEM, SHV, CTX-M, GES and TLA genes were performed by the standard PCR technique with specific primers and products were sequenced and analyzed using informatics programs. A representative sample of 175 (27%) isolates (selected based on the type of ESBL, site of infection and hospital) were sequenced and analyzed to determine the corresponding allelic variant.

Results: Frequency of ESBL families from 648 clinical isolates were CTX-M 56% (363), SHV 18% (120), CTX-M/SHV 13% (85), ESBLs not identified (62), SHV/GES 0.9% (6), CTX-M/TLA 0.7% (5) 0.4% GES (3), CTX-M/GES 0.3% (2), TLA 0.1% (1) and CTX-M/SHV/TLA 0.1% (1) Of the selected sample of 175 isolates, the species identified were Escherichia coli 96 (55%), Klebsiella pneumoniae 48 (27%), Enterobacter cloacae 22 (13%) and other minor species 9 (5%). In the 175 isolates the frequency of the 208 ESBL identified were distributed as follows: CTX-M-15 122 (54%), SHV-12 27 (12%), SHV-5 15/208 (7%), SHV-2 11/208 (5%), GES-20 9/208 (4%), GES-19 8/208 (4%), TLA-1 5/208 (2%), SHV-2a 3/208

(1%), SHV -36 2/208 (1%), GES-17 2/208 (1%), while 4/208 (2%) corresponded to the remaining variants CTX-M-1 CTX-M-55, SHV-26 and GES-1.

Conclusion: ESBL-producing E. coli was the most frequently bacterial species in hospitals, followed by K. pneumoniae, ESBL families associated with these bacteria were CTX-M-15 and SHV-derived, respectively. It should be noted that currently the ESBL CTX-M-15 has been the most frequently identified than SHV as have been reported in previous studies.