DNA MICROARRAY FOR MOLECULAR EPIDEMIOLOGY OF SALMONELLA S. HUEHN AND B. MALORNY pdf

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Here we describe a DNA microarray comprised of sixty-mer oligonucleotide probes to study the epidemiology of Salmonella enterica subsp. enterica isolates at the genotypic level.

Construction of sub species I specific, core genes of Salmonella enterica and variable gene probes Comparative genome analysis of different serovars of Salmonella, E. Fimbriae, prophage like elements, pathogenic islands and other genes such as LPS encoding genes have also been reported to encode for diversity within serovars of Salmonella. Therefore, gene probes were constructed on core genes of S. The DNA sequences of these genes for S. Typhimurium LT2 and S. Typhi genomes were downloaded from the NCBI database, and the probes 70 mers were designed using Arrayoligoselector http: The gene name, gene accession number, source, probe sequence, are listed in Appendix 1. Autoblank buffer without oligos was used as a negative control. Each slide had triplicate spots of each feature and every experiment was repeated twice. Fluorescence data from the scanned images was extracted by genepix pro 6. For each feature, the background subtracted median foreground intensity value was averaged for every triplicate spot and was log-transformed. Then mean and standard deviation SD of the sub species 1 and Salmonella core gene specific features were calculated. This cut off criteria was selected after testing several cut off thresholds on the reference strains. The above probe classification was carried out using Avadis Avadis software, Strand genomics and Microbial Diagnostic Array Workstation http: For finding the closest serovar match for an unknown sample we developed a Matlab programme that is implemented as follows. Let Pz denote the set of negative probes in the input data sample and each reference has its own set of known negative probes. Let P1, P2, â€¦, P14 for each of the 14 references denote these sets and let the number of probes in each of these sets be denoted N1, N2, â€¦, N Then algorithm finds the Pz probes in each of P1, P2, â€¦ P Let us denote these numbers Pz1, Pz2, â€¦, Pz The maximum value of this ratio points to the reference to which the input sample is closest. This programme is included in appendix 4 as a zip file. The instructions for running this programme, sample input data and reference data set are included in this zip file.

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Huehn S., Malorny B. () DNA Microarray for Molecular Epidemiology of Salmonella. In: Caugant D. (eds) Molecular Epidemiology of Microorganisms. In: Caugant D. (eds) Molecular Epidemiology of Microorganisms.

Softcover reprint of the original 1st ed. Plasmid Replicon Typing Timothy J. Johnson and Lisa K. Hiett and Bruce S. Ecker, Christian Massire, Lawrence B. Hall, and Rangarajan Sampath 8. Savelkoul, and Paolo Visca 9. Friedrich, and Marc J. Full Sequencing of Viral Genomes: Cottam, Jemma Wadsworth, Nick J. Knowles, and Donald P. Jolley Trama The development and rapid implementation of molecular genotyping methods have re- lutionized the possibility for differentiation and classification of microorganisms at the subspecies level. Investigation of the species diversity is required to determine molecular relatedness of isolates for epidemiological studies. Methods for molecular epidemiology of microorganisms must be highly reproducible and provide effective discrimination of epidemiologically unrelated strains. A wide range of techniques has been applied to the investigation of outbreaks of tra- missible disease, and these have been critical in unraveling the route of spread of pat- gens for humans, animals, and plants. The choice of a molecular method will depend on the type of questions to be addressed, on the degree of genetic diversity of the species to be analyzed, and on the mechanisms responsible for generation of the diversity. The applications of molecular methods, singly or in combination, have greatly contributed in the past two decades to basic microbial science and public health control strategies. Molecular Epidemiology of Microorganisms: Methods and Protocols brings together a series of methods-based chapters with examples of application to some of the most important microbes. Both traditional and novel techniques are described, and the type of information that can be expected to be obtained by their application is indicated.

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3: Microarray for molecular typing of Salmonella enterica serovars - Europe PMC Article - Europe PMC

DNA MICROARRAY FOR CHARACTERISZATION AND TYPING OF SALMONELLA ENTERICA SUBSP. ENTERICA: A NOVEL TOOL FOR RISK ANALYSÃ•S S. HÃ¼hn1, C. Bunge2, B. Guerra2, M.

This article has been cited by other articles in PMC. Abstract Background Salmonella enterica subsp. Outcome of human Salmonella serotype Typhimurium infections ranges from mild self-limiting diarrhoea to severe diarrhoea that requires hospitalization. Increased knowledge of the mechanisms that are responsible for causing infection and especially the severity of infection is of high interest. Strains were analyzed on a DNA-DNA microarray for presence or absence of genes covering marker groups of genes related to pathogenicity, phages, antimicrobial resistance, fimbriae, mobility, serotype and metabolism. Strains showed highly similar profiles when comparing virulence associated genes, but differences between strains were detected in the prophage marker group. A dendrogram clustered strains into four groups. Clustering confirmed DT as being a clonal phagetype. Clustering of the remaining strains was mainly correlated to presence or absence of the virulence plasmid and mobile elements such as transposons. Each of the four clusters in the tree represented an almost equal amount of strains causing severe or mild symptoms of infection. Conclusions We investigated clinical significance of known virulence factors of Salmonella serotype Typhimurium strains causing different disease symptoms, and conclude that the few detected differences in Salmonella serotype Typhimurium do not affect outcome of human disease. Background Globally, Salmonella enterica subsp. For example in in the United States, Salmonella enterica subsp. Furthermore the actual number of infections is estimated to be 38 times higher [2]. Salmonella serotype Typhimurium, denoted S. The outcome of human infection ranges from mild self-limiting diarrhoea to severe diarrhoea that requires hospitalization. In rare cases, often among immunocompromised patients, salmonellosis can be fatal. Several factors in both the host and the bacteria influence the outcome of an infection. Clearly an important aspect of human infection is the immune state of the patient. It has been shown that immunocompromised patients are more prone to develop a severe infection [4]. Another important aspect of human infection is the intestinal microbiota of the host. Ingestion of antibiotics is known to affect the intestinal microbiota leaving the host more prone to infection and disease caused by S. Typhimurium [5]. Significant bacterial factors for the outcome of infection are encoded by a wide range of genetic elements, including plasmids, prophages and Salmonella Pathogenicity Islands SPIs. A total of 14 SPIs have been described so far [6]. SPI-1 encodes type 3 secretion system 1 T3SS-1 that causes secretion and translocation of a range of bacterial proteins to the host cell. The detected variation within S. Typhimurium is primarily represented by the prophages in the genome [9]. Variation in phenotype has also been demonstrated as there are different phagetypes of S. Typhimurium strains, and some of them can even show a high degree of variation in host adaptation [10]. Intra-serotype variability is also caused by the plasmids carried by S. Typhimurium, in particular, the Salmonella Virulence Plasmid pSLT which was observed more frequently in the strains isolated from blood than the strains isolated from faeces [11]. It has been proposed that this plasmid is significant in the spreading of an infectious strain from the intestine [12]. The recent development of microarray technology has allowed an extensive screening of many S. Typhimurium strains [13 - 15], but to our knowledge, no study has been able to link the molecular data obtained by microarray analysis of the strains to detailed epidemiological and clinical patient data. We analyzed a collection of S. Typhimurium strains by DNA microarray analysis. These strains were selected on the basis of a previous epidemiological study where clinical data were obtained by means of patient interviews. The strains were selected from patients with mild infections and from patients with severe infections, and clinical data allowed us to correct for known underlying diseases and patient age. Strains were analyzed for presence or absence of genes covering marker groups of genes related to pathogenicity, phages, antimicrobial resistance, fimbriae, mobility, serotype, and metabolism. We show that S. Typhimurium strains causing very different symptoms in patients had little genomic variation, and the observed variation does not

correlate to the severity of disease. In general, the PFGE types of the strains correspond to the phagetype. The remaining phagetypes showed different PFGE profiles see additional file 1: The MLVA types of the strains were all different. Some strains did not contain the STTR allele at all, corresponding well to the fact that these strains were not carrying the pSLT see additional file 2: Typing results of all strains. DNA microarray marker groups Resistance and Serotyping The DNA microarray included 49 probes that targeted 10 different resistance genes and some of their known variants. The phenotypic resistance profiles all corresponded to the results obtained by the array see additional file 3: Microarray results of all markers. Typhimurium serotype was confirmed by the array see additional file 3: All strains on the array possessed 18 of the 20 metabolism genes. The gene SEN, which has been observed in serotypes S. Gallinarum and the gene STY, which has been found in serotypes S. Paratyphi A, were not detected in any of the S. The DNA microarray contained 10 probes targeting different genes in the prophage marker group. All DT strains displayed identical profiles within the prophage marker group. The prophage genes sb10 and sb54 were present in all DT strains and some other strains. The DNA microarray contained 57 probes targeting different genes in the mobility marker group which also included plasmid incompatibility markers and IS-element markers. The variability correlated well with known properties of strains, e. Only the DT strains showed variation, as they lacked the gipA gene encoded by the Gifsy-1 prophage but harboured the two other genes encoded by Gifsy The DT strains also possessed two other prophage-related genes which no other strains possessed. All strains harboured prophage Gifsy-2 and lacked prophages Gifsy-3 and Fels Five strains of five different phagetypes lacked the pSLT and, therefore, lacked the virulence genes encoded in the plasmid. The same percentage is observed when comparing all of the S. Typhimurium strains detected in Denmark between and data not shown.

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