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A Unique Genome Wide Approach to Search Novel Markers for Rapid Identification of Bacterial Pathogens

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Introduction

The bacterial ability to survive in a range of environments, including extremes of conditions has been a subject of intense research, since their discovery. The interactions between bacteria and human beings have been very intricate, ranging from symbiotic to pathogenic [1,2]. The first category of bacteria stays within and on the human body on a permanent basis from birth till death. On the other end of this spectrum, are interactions responsible for the deterioration of human health on temporary and/or long term basis. Bacteria seem to enjoy this niche provided by the human body. Certain pathogenic bacteria invade the human body and persistently damage it by causing infectious diseases, such as tuberculosis [3]. These bacterial pathogens stay in the human body for a very short period, such as in diarrheas [4,5]. Here, they multiply vigorously and after having achieved a large population and extensively damaging human body, pretend to leave it in a hurry. A few such notorious bacteria, responsible for various epidemics which have extensively wiped out human populations are: Clostridium, Helicobacter, Salmonella, Shigella, Streptococcus, Vibrio, Yersinia, etc. [4-6]. Attempts to control these pathogens through the utilization of antibiotics have been quite successful till recent times. Yet, each time the success of novel antibiotics has been short lived [7]. Within a span of ten years or so after the discovery of 'novel' antibiotics, bacteria have been able to evolve and become resistant [8,9]. Bacterial drug resistance has been fueling the ongoing search for novel drugs and drug targets [3,10]. Another feature, which shields bacteria against antibiotic attack, has been their ability to form biofilms through a cell density dependent phenomenon termed as quorum sensing [11-13]. The biofilm provides a protective and resilient structure to the bacteria, where antibiotic infiltration is largely prevented. Thus, the need in case of a pathogenic scenario is to identify the bacteria in a shortest possible time and diagnose the disease. This will obviously allow the physician to take swift measures to control and reverse the damage.

The trouble with rrs gene

Unlike conventional methods of identifying bacteria using biochemical assays, which are time consuming and not very accurate, molecular techniques have been quite effective. The advent of Molecular biology and Bioinformatics, has yielded a new lease to the bacterial taxonomy. Among a host of bacterial genes, which are highly conserved throughout the diverse taxa, 16S rRNA (rrs) is prominently used for identifying them [14-17]. The popularity of rrs can be judged from the fact that, RDP has 3,224,600 rrs entries (http://rdp.cme.msu.edu/index.jsp). The major limitations of rrs are: (i) its use to identify bacteria only up to the species level, (ii) in pathogenic bacteria having its multiple copies, leads to overestimation of bacterial populations, and (iii) high similarity between rrs copies from different species, leads to mislabeling of the organism. However, there have been a few other highly conserved - housekeeping genes (HKGs): recA, gyrB, rpoB, etc., which can be used either independently or supplementarily for distinguishing closely related organisms [14]. In spite of being quite effective, these genes have not gained the requisite popularity, they deserve. Effectively, a combination of up to 8 HKGs have been shown to distinguish bacterial strains in an unambiguous manner [18]. This amounts to marking them as uneconomical and time consuming. The need is to look for supplementary genes to be used as markers for identifying organisms with high specificity. Hence, rather than looking for a universal gene, we may resort to a genus specific molecular approach.

The dangerous pathogens

An extremely lethal Clostridium is a "Category A agent" with the highest risk, especially for use as a bioweapon. The major hurdle in identifying them is high heterogeneity of rrs: 9-22 copies of per genome [4,18]. Another dangerous bacterium is Yersinia, a gastrointestinal pathogen which contaminates food, water, and blood during transfusion. Similarly, Vibrio species generally found in marine environments are also responsible for life threatening diseases in humans. The identification of Yersinia and Vibrio by rrs gene sequences is hindered by the presence of 6-11 copies of this gene per genome, which also show high similarity among themselves [5,6]. Streptococcus, another organism with multiple rrs gene copies, is among those bacteria which can cause severe epidemics leading to high morbidity and mortality [19]. Environmental and Health Departments are struggling to develop assays to detect the infection causing organisms.

Genome wide search for unique markers

A novel approach has been developed recently for searching novel markers in these pathogens. It primarily involves searching genes common to all the completely sequenced genomes of species of a genus. From this pool of common genes, around 30 genes (200-4000 nucleotides) are selected, which represent sizes ranging. In silico digestion of each of these genes is carried out by cleaving them with 10 type II Restriction Endonucleases (RE) (4-6 base cutters). Unique RE digestion patterns are selected out and can be used for identification of particular organism with high precision. Novel markers for identifying strains have been deduced by using combinations of REs (AluI, BfaI Tru9I) and common genes: (a) Clostridium - (i) recN, dnaJ and secA and mutS, grpE, (b) Yersinia - aceE, gyrB, malE, rpoB, (c) Vibrio -

dapF, fadA, hisD, ilvH, lpxC, recF, recR, rph and ruvB, and (d) Streptococcus - purH [4-6,19].

The unique feature of this strategy is that it relies on genes, which are found in all the species within a genus. Hence, in a population of bacteria of diverse origins, the likely hood of detecting the bacteria suspected to be the causal organisms is high. The proposed strategy is anticipated to be effective in detecting pathogens with more precision and economy in comparison to the presently available methods.

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