Evolutionarily Conserved Essential Genes from Arctic Bacteria: A Tool for Vaccination

Sir,

Due to advancement in various molecular biological techniques, several indispensable evolutionarily conserved essential genes (genes that are necessary for viability of the living organisms) have been identified over the past decades among various groups of bacteria.[1-3] Moreover, the amino acid sequences of some of these essential genes were highly conserved across various classes of bacteria such as thermophiles (heat-loving) and psychrophiles (cold-loving). However, some of the product of essential genes of psychrophiles have been adapted to the cold environments for millions to billions of years[4-6] thus making these bacteria temperature-sensitive (TS), reduced viability at higher temperatures. In accordance with the above mentioned facts, the human body temperature varies depending upon the body parts, temperature of the skin varies between 32 and 36°C whereas the body core is about 37–42°C.[7,8] Over decades, attempts have been made to develop TS bacterial strains and viral strains,[9-12] yet bacterial strains were not adapted for human vaccination,[13] for example, the TS version of *Yersinia pestis*[14] and because of their reversion this strain was not further developed.[13] Further, small numbers of TS bacterial strain vaccines (developed by chemical mutagenesis) were developed for veterinary use; but it is uncertain whether the TS nature is primary attenuating phenotype or merely a coincidental phenotype.[13] In the following work, Duplantis and colleagues have successfully transformed mesophilic pathogenic bacteria to a TS bacterial strain using essential genes from psychrophile and provide evidences for a successful vaccination strategy developed using such TS bacterial strain. The highlight, in the following work, is provided by the concept of using temperature adapted essential orthologue genes from psychrophile to develop the TS non-pathogenic version of bacterial strains.

In this study, the authors chose pathogenic mesophile *Francisella tularensis* subsp. *novicida* (*F. novicida*), maximum growth temperature is about 45°C, as host to transfer essential genes from a psychrophile *Colwellia psychrerythraea*, maximum growth temperature is about 19°C. Strains of *F. tularensis* is highly virulent to mice but also known to cause zoonotic disease in humans.[15] Here, the selection of host and donor species was based on the similar G + C contents of these species.[16,17] In order to maximize the chance that the foreign gene is expressed identically to the homolog that it replaced, the authors engineered the foreign psychrophilic gene under the control of a host promoter and expressed it using host machinery [Figure 1]. The rate of mutation that will revert back the TS strain to a temperature-resistant strain was also assessed by the authors on essential gene such as $ligA_{Sf}$, $ligA_{Cp}$, $ligA_{Ph}$, $hemC_{Cp}$, $pyrG_{Cp}$, $dnaK_{Cp}$, $murG_{Cp}$, $dnaK_{Sf}$, fmt_{Cp}, $ftsZ_{Cp}$, cmk_{Cp}, and $tyrS_{Cp}$.[18]

To check the TS viability of the TS *F. novicida* carrying either $ligA_{Cp}$, $ligA_{Ph}$ or $dnaK_{Cp}$ essential genes, the strains were broth cultured at restrictive temperatures. Results showed an identical growth rate to that of wild-type *F. novicida*. Since *F. novicida* is a facultative intracellular pathogen, the growth rate of TS *F. novicida* in infected macrophages-like cell line (J774 cell line) was examined to check the TS viability. The results showed a decline in the number of viable TS *F. novicida* in the J774 cell line at restrictive temperature, indicating the temperature sensitivity of TS *F. novicida* carrying either $ligA_{Cp}$, $ligA_{Ph}$ and $dnaK_{Cp}$ essential genes. Moreover, TS *F. novicida* carrying either $ligA_{Cp}$ or $ligA_{Ph}$ showed a growth rate similar to wild-type *F. novicida* at permissive temperatures within infected J774 cells, whereas strain carrying $dnaK_{Cp}$ showed a poor growth indicating the gene-specific variability among the TS strains created.[18]

To determine the pathogenic effect caused by TS *F. novicida* on infected cultured J774 cells, the cells were microscopically examined for their viability. It was found that the J774 cell infected with TS *F. novicida* remained viable on shifting to restrictive temperature when compared to control (J774 cells infected with wild-type *F. novicida*), indicating the nonpathogenic nature of engineered TS *F. novicida* strains.[18]

In order to test the capacity of TS *F. novicida* to grow only in cooler body regions and not in warmer body core, rats and mice were infected with the TS *F. novicida* at the base of tail. It was observed that TS *F. novicida* with restrictive temperatures at or below 37°C were not found in spleen

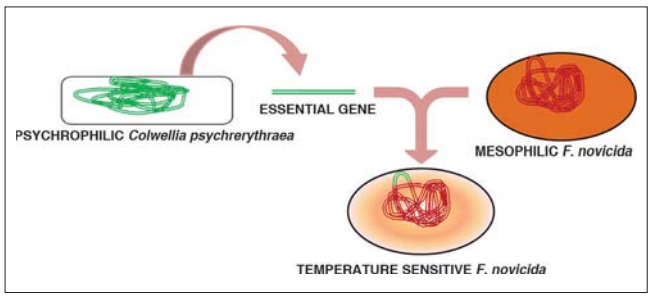

Figure 1: Flowchart representing the transfer of essential gene from a psychrophilic bacterium to a mesophilic bacterium resulting in formation of temperature sensitive mesophilic host bacterium

whereas *F. novicida* with restrictive temperature above 37°C were detected in spleen (control), indicating the inefficiency of TS *F. novicida* strains to proliferate at body core organs. Moreover, viable TS *F. novicida* were found at the site of infection even after 3 days of injection, indicating the capacity of TS *F. novicida* to survive at cool body parts. These results were further validated by injecting the TS *F. novicida* into the fleshy part of mouse ears.[18]

To check whether the persistence of TS *F. novicida* at cool body parts induce protective immunity, mice were challenged with intranasal infection of wild-type *F. novicida* after 21 days of vaccination (injection of TS *F.* novicida carrying either $ligA_{Cp}$, $ligA_{Ph}$ or $dnaK_{Cp}$) at the base of the tail. Using organ bacterial burden, morbidity and weight loss as measures of protection, it was found that TS *F. novicida* protected the host animal against the challenge caused by wild-type *F. novicida* [Figure 2]. It was also found that strains carrying $dnaK_{Cp}$ showed greater dissemination and least protection, indicating the null role of dissemination in immune stimulation.[18]

Furthermore the effect of psychrophilic *ligA* was also examined in another Gram-negative bacterium, *Salmonella enterica*. It was found that psychrophilic *ligA* rendered the organism TS, indicating that this methodology can be applied to numerous important pathogens such as *Salmonella thyphi, Escherichia coli, Yersinia pestis* etc. Moreover, a codon-optimized version of $ligA_{Cp}$ (deleting most of the $ligA_{Cp}$) also made *Mycobacterium smegmatis* TS, indicating the functional ability of $ligA_{Cp}$ product in a Gram-positive lineage bacterium.[18]

The technology described in this work by the authors holds good not only to make live TS bacterial vaccines (known to be efficacious for protection against diseases that need cell-mediated immunity) but also in development of killed whole-cell or subunit vaccine and in development of TS version of dangerous bacterial pathogens which can be used to study their biochemical and pathological characteristics without physical containments.[18] Eventhough the Mother Nature has provided with hundreds of evolutionary conserved essential genes, careful selection of the essential gene from psychrophilic species is required to prevent the transformation of genetically engineered TS strains back to temperature-resistant strains through mutations. Technology developed by Duplantis *et al.* provides the way for engineering other pathogenic bacteria, so that they can be used as vaccines. However successful clinical trials, using TS bacterial strain for protection against bacterial pathogens, and experimentation will provide the way for the use of this technology for the welfare of humanity.

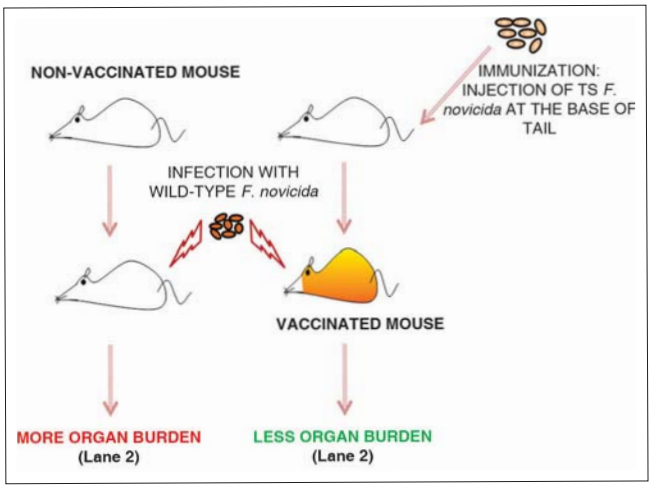

Figure 2: Flowchart indicating the process of vaccination and subsequent protection against wild-type *F. novicida* (lane 2) in mouse, whereas showing more organ burden caused in non-vaccinated mouse (lane 1), due to infection with wild-type *F. novicida*.

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