Methanobrevibacter: Is it a potential pathogen?

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Methanogens are members of the domain *Archaea*, and fall within the kingdom Euryarchaeota[1]. Interest in methanogens has resulted from their unique ability to produce methane from H_2/CO_2 and in some cases, a limited number of one- and two-carbon substrates like formate, acetate, methanol and methylamine. Methanogenic bacteria are found in various anoxic habitats, where biopolymers are degraded anaerobically. Methanogenic *Archaea* have been isolated from sewage sludge, lake sediments, hot springs, decaying cottonwood trees, anaerobic digesters and the gastrointenstinal tract ecosystems of animals, including humans[2,3]. The name Archaebacteria or *Archaea* (as they are called today) was suggested for methane-producing anaerobic bacteria (methanogens) in 1977, due to their unique properties that distinguished them from bacteria and eukaryotes[4]. Subsequently, halophiles and thermoacidophiles too were included in this branch and the group was generally referred to as extremophiles, owing to natural habitats of majority of the organisms present. However, during the last decade it was realized that there are significant number of mesophiles in this group[5].

It is rather surprising that despite being so ubiquitous, diverse and abundant, no member of this domain has been described as human pathogen. *Methanobrevibacter* is one such major genus of the Methanobacteriaceae family for which majority of the species were isolated from the gastrointenstinal ecosystems like human colon, bovine, rumen, termite hindguts and other animal intestinal tract contents[6–10]. They are known to colonize and have long-term coexistence with endogenous flora of the host and have been detected in human oral cavity as well as the human vagina and colon[2,6,11]. Although the presence of *Methanobrevibacter* spp. in human dental plaques was reported as early as 1988, the identity of the species was unknown till 1994, when Ferrai *et al.*[12] described the species as *Mbr. oralis*[2]. Until recently, there were no studies on the quantitative relation between the archaeal population inhabiting a particular diseased site and its ability in causing the disease. A recent report by Lepp *et al.*[13] revealed the relationship between the severity of chronic periodontitis and the relative abundance of the archaeal population in periodontitis patients. The authors noted an increase in the relative abundance of the archaeal population in relation to the severity of periodontitis disease and a corresponding decrease in the relative abundance of the archaeal population at the treated sites in association with clinical improvement. Although the report may not withstand the criteria of Koch's postulates, it definitely is a step ahead in the direction where the most abundant archaeal population has been hypothesized and significantly proven to correlate with the severity of the disease.

The authors studied the abundance of the population and the diversity of *Archaea* in the human subgingival crevices using the well-established molecular approach of constructing a 16S rRNA library with domain specific primers and then sequencing the clones thus generated, to determine the community structure in phylogenetic terms[13]. It was found that 81% of the 16S rRNA clones (phylotype SBGA-1) showed 99.8% sequence similarity to *Mbr. oralis* (based on 572 nucleotides) and 97.7% sequence similarity to *Mbr. smithii* (based on 998 nucleotides). The remainder (19%) of the cloned sequences (phylotype SBGA-2) showed 99.8% sequence similarity with a *Methanobrevibacter* sequence associated with a ciliate, *Euplodinium maggi*, which inhabits the ovine rumen.

An interesting question that arises here is whether phylotype SBGA-1 is indeed a strain of *Mbr. oralis*, the most abundant bacterium found in dental plaques or is it similar to *Mbr. smithii* found in human faeces? If the answer is no, then is it a novel species of the genus? The answer to these questions could be found in the recent work by Dighe *et al.*[14]. There are more than a hundred 16S rRNA sequences available in the database that are described as belonging to the genus *Methanobrevibacter*, most of which represent clones arising out of libraries generated from PCR-amplified 16S rRNA genes from endosymbionts of protozoans, animal faeces, rumen, etc. Dighe *et al.*[14] studied the correlation between 16S rRNA gene sequence similarity and the extent of DNA hybridization for the genus *Methanobrevibacter* based on the available DNA hybridization data from cultured representatives to organize these sequences in distinct species. Using the available data for the genus, a highly significant relationship was found between DNA–DNA hybridization and percentage 16S rRNA sequence similarity. In the absence of a systematic variation in the error, this analysis suggested that the extent of DNA–DNA hybridization would be less than 70% at least 99% of the time, when percentage 16S rRNA sequence similarity equals 98.4%. It is interesting to note that all the clones of the phylotype SBGA-1, as reported by Lepp *et al.*[13], showed less than 98.4% sequnce similarity when analysed for a longer region. Thus, all these possibly represent a different species of this genus, separate from *Mbr. smithii*.

Finally, there is a scientific basis in regarding the *Archaea* as potential human pathogens. Ample access to the host along with the capability to colonize the human host definitely indicates their capacity to evade the human immune system. Also, their relative abundance as compared to the other mesophiles in the clonal populations directly indicates their capacity to out compete the other human microbiota. Moreover, their ability to stay syntrophically with the *Treponema* populations in the severe periodontitis cases as reported by Lepp *et al.*[13], clearly indicates that methanogens not only participate in syntrophic relationships in the subgingival crevice promoting colonization by secondary fermenters during periodontitis, but possibly also in other anaerobic communities within the human host as well. Furthermore, recent research indicates that the unique archaeal polar lipids serve as potent immune adjuvants both *in vitro* and *in vivo*[15]. Also, the *S*-layer of many archaea might help the organism in evading the host immune response in a similar manner as few of the Gram-negative bacteria do. Thus, in the near future, *Archaea* may be implicated as causative agents for some diseases. The study by Lepp *et al.*[13] can be regarded as a first step in this direction.

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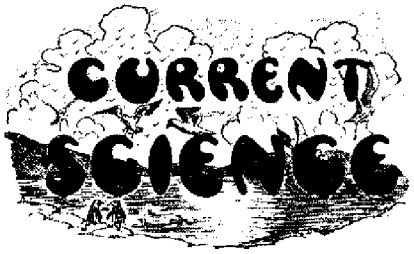

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'Patulin' – A remedy for common cold

Common cold is an ailment costing the nation a heavy price in sickness, un-employment and loss of several man-hours. It often leads to pneumonia, bronchitis and other respiratory complications, and weaken the system and render the body susceptible to other infections. A 100 per cent cure for cold has been sought unsuccessfully for many years and although numerous palliative drugs have been tried from time to time, the results were uniformly disappointing. But of late, we have all read with great interest the announcement of the success of 'Patulin' in the treatment of common cold. If this achievement passes the extensive tests, it will constitute a great contribution.

Prof. Raistrick and his colleagues have isolated a metabolic product of *Penicillium notatum* Bainier and shown its antibacterial properties for both gram-positive and gram-negative organisms. This active inhibitor has now been identified as anhydro-3-hydroxy-methylene-tetrahydro-γ-pyrone-2-carboxylic acid and named 'Patulin'.

The story of 'Patulin' as a remedy for cold is very interesting. An almost accidental observation by Prof. Gye of the Imperial Cancer Research Fund Laboratories suggested that 'Patulin' might be useful in the treatment of common cold. When this new drug was sent to him for study, Prof. Gye had a severe common cold. Knowing its antibacterial properties Prof. Gye tried it on himself. The outcome was encouraging and he repeated the experiment on other members of his staff. Further experiments were conducted by Surgeon-Commander Hopkins at a Naval Establishment in the South East of England. The response to treatment has been encouraging. Hopkins showed that 'Patulin' has no effect in the early period of cold, probably caused by virus infection; but secondary stage which constitutes invasion with gram-positive and gram-negative organism can be entirely prevented. These trials, spread over a period of months, gave good results and a strong balance of evidence in favour of treated group; 57 per cent of treated cases recovering completely within 48 h as compared with only 9.4 per cent of the controls.

The exact mode of action of 'Patulin' in cold is not yet known. The action *in vitro* of 'Patulin' against a number of pathogenic aerobic organisms has been studied; the results show that it possesses bacteriostatic effect; Serum and pus do not interfere. What still lacks is evidence that 'Patulin' has antibacterial activity *in vivo* and this knowledge is necessary for an understanding of the mechanism of its action and its therapeutic scope.

N.N.D.

Science notes and news

Patulin – A Remedy for Common Cold. – Dr N. K. Basu, Pharmacologist, Scientific and Industrial Research, Delhi, writes: *Current Science* in February 1944 has published a short note on the subject. As it might create a wrong impression in the mind of the lay public regarding the wonderful activity of the substance, the following note appearing in the *Industrial Chemist and Chemical Manufacturer*, December 1943, might be placed before them.

'Considerable publicity has been given of recent weeks to this substance Patulin, which the *British Medical Journal* refers to as the antibacterial derivative of *Penicillin patulum* Bairiar, and the controversy has already started. A sample of the preparation had been sent to the laboratories of the Imperial Cancer Research Fund for therapeutic tests in cancer, and W. E. Gye, suffering at the time from a severe cold, tried a solution of it as a nasal douche. Since then a large-scale clinical trial has been carried out at a naval establishment with satisfactory results. But clinical trials at a primary training wing by officers of the R.A.M.C. led them to conclude that Patulin had no demonstrable effect on the 'course of the series of colds that were treated. Here we have one of those perfect instances where experts disagree'. The *British Medical Journal* sums the situation very neatly by suggesting that serious attempts should be set on foot to elucidate the processes involved in the use of a mould preparation to obtain inhibitory action on the growth of viruses; and there possibly the matter can rest until some more work has been carried out. …