Is *Yersinia pestis* a distinct species?

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Comparison of characters from similar living forms had been the basis of evolutionary studies since centuries. With the limitations posed by morphological characters and the advances in techniques generating enormous molecular data, such comparisons have now become more meaningful and informative. Over the past decade, comparison of one or more genes and more recently, of whole genome sequences of pathogenic bacteria and their relatives have not only helped decipher ways in which human diseases caused by these pathogenic bacteria might have developed but also provides information about the evolutionary descent of the bacteria under study. A recent study by Chain *et al.*[1], proves the latter application of such comparisons beyond doubt. The authors completely sequenced the fully virulent human isolate *Yersinia pseudotuberculosis*, strain IP32953 and compared the same with available sequences of two strains of *Yersinia pestis* (strain O, CO92 and M, KIM10+)[2,3], and provided an interesting insight as to how the latter pathogen could have evolved from the former just a few hundred years ago by acquiring a few genes and inactivating some of the existing ones.

Y. pestis, the causative agent of plague, and the enteric food- and water-borne pathogens *Y. pseudotuberculosis* and *Y. enterocolitica* comprise the three species of the genus *Yersinia*. Amongst the three species, *Y. pestis* is primarily a disease-causing pathogen of rodents or other wild mammals that usually is transmitted by fleas and is often fatal. This species has been further subdivided into three biovars (Antiqua, Medievalis, and Orientalis) based on minor phenotypic differences. Even among *Y. pestis* strains isolated from a single country, some degree of restriction fragment length polymorphism according to ribotyping and pulsed-field gel electrophoresis has been recorded. However, proposals for the reclassification of these two species as two related subspecies that were based on highly identical serotyping, phage typing, DNA–DNA hybridization and 16S rRNA gene sequence analysis, have been rejected due to the different transmission routes of the two and because of the historical importance of *Y. pestis* for human history. The higher virulence of *Y. pestis* compared to *Y. pseudotuberculosis* may be an outcome of the presence of two *pestis*-specific plasmids, one of which codes for murine toxin, a phospholipase-D homolog facilitating flea midgut colonization, thus enhancing host-to-host transmission.

Achtman *et al.*[4], used a technique called Multi Locus Sequence Typing (MLST), for studying the population genetic structure of *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. They sequenced fragments of five housekeeping genes and a gene involved in the synthesis of lipopolysaccharide from 36 strains of *Y. pestis* and from 12 to 13 strains of each of the other species representing the global diversity of these species. It was found that all the *Y. pestis* strains possessed identical alleles for all the six gene fragments. Moreover, these gene fragments were highly similar to those found in *Y. pseudotuberculosis* and variations, if any between them, were within the range as noted for the *Y. pseudotuberculosis* strains. Thus it was concluded that *Y. pestis* is a highly conserved clone of *Y. pseudotuberculosis,* so much so that but for tradition, it might as well be named as *Y. pseudotuberculosis*.

The MLST data was further extrapolated to determine the approximate time at which the two species separated in the evolutionary history. The time since the last bottleneck from which all strains of *Y. pestis* descended was calculated to be around 1000 years at 50% confidence limits. This is just prior to the first recorded pandemic of plague (Justinian's plague) which dates back to AD 541–767.

Whole genome comparisons of two strains of *Y. pestis* (KIM10+ and CO92) with *Y. pseudotuberculosis* IP32953 confirmed the close association of the two species[1]. The IP32953 chromosome encodes 3974 predicted genes of which 2976 (75%) share greater than or equal to 97% identity to their homologues in *Y. pestis*. However, there are some major differences that are probably responsible for the differences in pathogenicity of these two species. Amongst these are the 317 genes unique to IP32953 that are absent in either of the *Y. pestis* strains. Similarly, 112 genes were found only in *Y. pestis* strains that were absent in *Y. pseudotuberculosis*.

Chain *et al.*[1] further confirmed the presence or absence of these gene fragments in the other *Y. pestis* and *Y. pseudotuberculosis* strains available with them using PCR. Of the 85 IP32953-specific genes tested, 11 genes were specific for the *Y. pseudotuberculosis* species (i.e. present in all *Y. pseudotuberculosis* and absent from all *Y. pestis* isolates). Of the 112 genes uniquely associated with the two *Y. pestis* genomes, 105 were tested for, of which only 32 genes, located in six clusters, were present in all *Y. pestis* strains and were absent from all *Y. pseudotuberculosis* strains that were examined. Almost all these genes code for hypothetical proteins. In addition, a total of 204 genes (5% of the gene complement) have functional analogues in *Y. pseudotuberculosis* but are inactivated in both strains of *Y. pestis*. These include several genes involved in metabolism and thus may account for observed physiological differences between the two species.

Genomic differences that may play a role in the unique pathogenic characteristics of *Y. pestis* include absence of the lipid A acyltransferase gene *htrB* (YPTB2490), absence of a cluster of nine coding sequences (YPTB3450-YPTB3459) encoding several hemolysin homologues in IP32953, presence of insecticidal toxin homologues either in complete or inactivated form and the presence of in-frame indels in two loci (*srfA* and *srfB*) encoding putative virulence factors[1]. Furthermore, nine regulatory genes are inactivated in *Y. pestis* and these may have effects on its phenotype, including virulence. These include genes involved in sorbose utilization, synthesis of capsular polysaccharide colanic acid, motility, raffinose utilization, constitutive glyoxylate bypass, hexose phosphate utilization and finally sigma 54-dependent genes. Although the precise role of these genes in *Yersinia* virulence cannot be determined solely on the basis of genome sequence, their possible functions need to be investigated.

Thus, overall these studies provide an insight into how *Yersinia pestis* and *Yersinia pseudotuberculosis* could have diverged from a common ancestor just a thousand years ago, the approach has certain limitations. The absence of a reliable molecular clock to measure the rates of genome rearrangement, IS transposition, and gene inactivation limits the direct comparison of the evolutionary distances between these two *Yersinia* species calculated by Achtman

et al.[4] and Chain *et al.*[1]. It is also difficult to predict how the new genes could have been acquired by *Y. pestis* and by what mechanism. One can only have some idea about the overall picture rather than exact molecular mechanisms that took place during evolution. In this scenario, gene inactivation or IS-mediated rearrangements might have led to changes that increased virulence and that facilitated flea-borne transmission. The selective pressure for the same might have been provided by the concomitant and dramatic change in lifestyle undergone by *Y. pestis*, ensuing from its continuous association with the host and dependency on the flea vector for survival, which resulted in wholesale inactivation of as much as 13% of its genome that we observe today. These results may represent an intermediate stage in genome compaction, as proposed in the evolution of other pathogens. Moreover, the uncertain role played by horizontal gene transfer into the chromosome of *Y. pestis* cannot be ruled out. To account for this, Chain *et al.*[1] rightly hypothesized that the acquisition of at least some of the six chromosomal regions uniquely conserved in *Y. pestis* strains, in conjunction with the high degree of gene inactivation may be responsible for the increased pathogenicity of this species. Whole-genome comparisons of pathogen near neighbours of distinct characteristics lay the foundation for future mutational, functional, and animal studies that will ultimately help elucidate the mechanisms underlying the emergence of new pathogens.

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2. Parkhill, J. *et al.*, *Nature*, 2001, **413**, 523–527.
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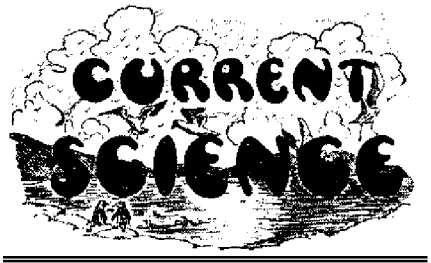

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Industrialization of India Essential for World Progress

'Perhaps the most important factor which will have world-wide implications will be our attempt to raise the standard of living in India. Politics does play an important part in all events. it is obvious that the best and quickest way of bringing about national development is for India to have a National Government representative of the people. The present absence, however, of such a government does not justify that the thinking men and women of India should not devise ways and means of bettering the lot of their fellow-beings to the best of their ability under present circumstances and in view of the future. I am not convinced that the rich and the wise in the land have done all they can for agricultural and industrial development of India.

'It has been urged by some that the problem of India is largely biological: that health, food and population are our real bottlenecks. Those who know India intimately are fully aware of the facts that attention to agriculture alone cannot solve the problem of India's poverty. Biology must be helped by physics, chemistry and engineering, even by mathematics. India cannot be healthy, prosperous and self-respecting, and education, medicine, and agriculture cannot play their important role unless a good bit of India's population is devoted to pursuits other than agricultural . . . .

. . . If I would not be misunderstood, I would make a suggestion to those European and Indian friends who are interested in the industrialisation of India not to fight for less or more to either side, but to come to terms honourable for both and do something to help Indian industry. It is obvious that European friends in India will have to yield to the natural aspirations of India, namely, that industry in India should be largely managed by Indians themselves. Indian businessmen should see that co-operation with the allied powers is the quickest method of developing India. The energy spent in fighting may be better spent in co-operative development. If the bye-product industries of coal-distillation, the petroleum industry, the textile industry, the woollen, cotton, sugar and jute industries and the metallurgical and chemical industries are developed, the country will have a different complexion altogether and a co-ordinated programme of development in all directions will become a possibility. This plea I am entitled to make as President of the Indian Science Congress, as I am convinced that science has no future in India unless our agriculture and our industries are fully developed; more food and more health are dependent upon these factors. Scientific and industrial research thrives best when it is applied to material benefit to human kind and to existing industries and existing agricultural enterprises'.