

Reply to “*Mycobacterium indicus pranii*” Is a Strain of *Mycobacterium intracellulare*: “*M. indicus pranii*” Is a Distinct Strain, Not Derived from *M. intracellulare*, and Is an Organism at an Evolutionary Transition Point between a Fast Grower and Slow Grower

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Alexander and Turenne in their letter (1) almost completely reiterate their previous comments on our earlier article, which was published in *Nucleic Acids Research* (2), to which we replied; their letter and the rebuttal were subsequently published (18, 19). In our earlier rebuttal, we clearly addressed all these issues and left no scope for revisiting them, and hence we are quite surprised that they did not even cite our original paper, their comments on this paper, or our earlier response in their latest letter. We are once again responding to their comments with a hope that there will be no further room for discussion on these issues.

They state that the name “*Mycobacterium indicus pranii*” does not figure in the “List of Prokaryotic Names with Standing in Nomenclature” and that the designation *M. indicus pranii* does not conform to the binomial naming convention. This opinion is, in our view, irrelevant and unsustainable. While most bacteria follow a binomial naming system, there are several examples of bacteria being named differently. *M. avium paratuberculosis*, from the mycobacterial family itself, is one such example. The fact that the name *M. indicus pranii* has three words does not take away its distinct morphological, biochemical, and genomic identity.

Alexander's and Turenne's claim that they are unaware of any comparison of *M. indicus pranii* with a comprehensive panel of *M. intracellulare* or *M. avium* complex (MAC) reflects their ignorance (deliberate?) of published literature. We state once again that *M. indicus pranii* is very different from all known members of the MAC, including *M. intracellulare*, in various respects that include colony type, growth pattern, biochemical features, chemotaxonomic features, etc. (Table 1). *M. indicus pranii* is a fast grower (6 to 8 days) compared to *M. tuberculosis* (>3 weeks) and members of the MAC complex (including *M. intracellulare*) (>2 weeks). *M. indicus pranii* shares several other biochemical characteristics usually associated with a rapid grower (like *M. smegmatis* and *M. vaccae*), such as the absence of pigmentation and the presence of nitrate reduction, aryl sulfatase (14 days), and catalase, to name a few. Fatty acid methyl ester (FAME) analysis is a highly sensitive indicator of uniqueness of a species and is used to define the precise taxonomic position (3). Our earlier comprehensive FAME analysis (4) and comparison with profiles in the Microbial Identification System (3) showed the presence of a unique metabolic machinery in *M. indicus pranii* very different from that in *M. intracellulare* and the rest of the organisms in the FAME database. Pathway analyses confirmed the presence of unique KEGG pathways in *M. indicus pranii* compared to *M. intracellulare* and *M. avium*, notably the lipopolysaccharide biosyn-

thesis pathway (KEGG identifier [mid00540](#)) and nitrotoluene degradation pathway (KEGG identifier [mid00633](#)). The lipopolysaccharide biosynthesis pathway is involved in the synthesis of lipopolysaccharides (also known as lipoglycans), which elicit strong immune responses. The nitrotoluene degradation pathway is found in soil-dwelling bacteria, is associated with metabolism of 2,4,6-trinitrotoluene under aerobic conditions, and has been reported to be present in *Mycobacterium* sp. strain HL 4-NT-1, isolated from polynitroaromatic compound-rich soil (5).

The name *M. indicus pranii* has been accepted in scientific literature, and this new species has been deposited in the DSMZ, Germany (DSM45239^T), and MTCC, India (MTCC 9506^T), per well-established international guidelines. This saprophytic bacterium, with a much bigger genome than that of *M. intracellulare*, lacks the *mce* operon (required for invasion), is commercially available for therapeutic intervention against leprosy, and is currently undergoing large-scale clinical trials against many dreaded infections and diseases such as cancer, HIV, anal warts, tuberculosis, etc. (reference 6 and references cited therein).

We stand by our key conclusion (2, 7) that *M. indicus pranii* is an ancestor of the *M. avium* complex. The evidence presented by Alexander and Turenne in support of their hypothesis that *M. indicus pranii* is a strain of *M. intracellulare* lack scientific rigor and scrutiny and deserve to be trashed. They assert that phenotypic results can be misleading and hence have suggested genomic sequence comparison for drawing definitive conclusions, primarily based on >99% similarity between *M. indicus pranii* and *M. intracellulare* at the level of DNA sequences of *hsp70*, *gyrA*, *dnaJ*, and 16S rRNA genes. It is surprising that they are unaware of the fact that most members of the mycobacterial family that have biomedical importance display >99.95% similarity (8). Such similarities are often misleading, as they do not represent the full genomic picture, and accordingly, mycobacteria, despite having almost

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TABLE 1 Comparative analyses of *M. indicus pranii* with *M. intracellulare*

Feature (reference[s])	Result for:	
	<i>M. indicus pranii</i>	<i>M. intracellulare</i>
Growth pattern ^a (4, 16, 17)		
Growth rate	Rapid (6–8 days)	Slow (>2 weeks)
Growth at 45°C	Yes	No
Pigmentation	No pigment	Scotochromogenic
Colony type	Smooth	Variable
Intracellular growth	No	Yes
Biochemical features ^a (4, 16, 17)		
Nitrate reduction	+	–
Aryl sulfatase (14 days)	+	–
Catalase semiquantitative	+	–
Chemotaxonomic features ^b		
Fatty acid fraction	Higher	Lower
18:1 ω 7c	Present	Absent
20:0 fatty acid	Absent	Present
18:1 ω 9c	Lower	Higher
16:1 ω 10c	Absent	Present
Unique metabolic genes and pathways (KEGG)		
Unique genes	Present	Absent
UniProt: J9WDV5	Present	Absent
UniProt: J9WLX6	Present	Absent
UniProt: J9WHE4	Present	Absent
Unique nitrotoluene degradation pathway (KEGG identifier eco00633)	Present	Absent

^a Saini et al. (4), Saxena et al. (16), Katoch (17).

^b Saini et al. (4).

100% similarity in these marker genes, have been assigned different species status (9–11). For example, with respect to the 16S rRNA gene, *M. kansasii* and *M. gastri* share 100% identity and *M. malmoense* and *M. szulgai* share 99.9% identity, as do *M. microti* and *M. bovis*. Similarly for other markers, despite identity to the extent of 100%, distinct species status has been given by taking into account the difference in ecological niches, host preferences, etc. (12). Therefore, drawing evolutionary evidence based on such assumptions of marker gene identities is erroneous (10) since candidate genes do not represent the entire genome complexity. Alexander and Turenne mention a complex genetic event as an illustration to support their claim that *M. indicus pranii* is derived from *M. intracellulare*. We respond that this single genetic event could not form the basis of an argument that *M. indicus pranii* is not a different species. The inversion, gain in transposons, is highly unlikely during evolution of a strain under environmental conditions. Furthermore, mycobacterium evolution involves genome reduction (13); hence, *M. indicus pranii* with a much bigger genome is very unlikely to be derived from *M. intracellulare* just because of the acquisitions of the transposons and the inversion events. On the contrary, it can be argued that the predecessor of *M. indicus pranii* witnessed selective massive gene acquisitions as a prelude to a soil-water interface habitat that evolved into a parasitic lifestyle (2). Genomic analysis (2) of *M. indicus pranii* demonstrated the presence of genetic features required for its unique lifestyle. They include 24 sigma factors (a very large number); the abundance of cytochrome P450 genes, genes involved in aerobic metabolism of phenol during degradation of plant substrates; machinery for biodegradation of cyanide and for thiocyanate degradation (absent in pathogenic mycobacteria); the presence of a complete hydrogenases enzyme complex, etc.

In summary, the array of features, including growth, biochemical and chemotaxonomic characteristics, and metabolic genes and pathways, and finally the complete genomic analyses of *M. indicus pranii* constitute the comprehensive basis of our assertion that *M. indicus pranii* occupies a unique phylogenetic place, making it the immediate predecessor of opportunistic mycobacterial species represented by the *M. avium* complex. We believe that the progenitor status of *M. indicus pranii* lends support to the idea of a shared aquatic past between saprophytic and pathogenic mycobacteria (14, 15) and provides new insights on “evolutionary habitat diversification and advent of pathogenic attributes in mycobacterium” (2).

REFERENCES

- Alexander DC, Turenne CY. 2015. “*Mycobacterium indicus pranii*” is a strain of *Mycobacterium intracellulare*. *mBio* 6(2):e00013-15. <http://dx.doi.org/10.1128/mBio.00013-15>.
- Saini V, Raghuvanshi S, Khurana JP, Ahmed N, Hasnain SE, Tyagi AK, Tyagi AK. 2012. Massive gene acquisitions in *Mycobacterium indicus pranii* provide a perspective on mycobacterial evolution. *Nucleic Acids Res* 40:10832–10850. <http://dx.doi.org/10.1093/nar/gks793>.
- Haack SK, Garchow H, Odelson DA, Forney LJ, Klug MJ. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. *Appl Environ Microbiol* 60:2483–2493.
- Saini V, Raghuvanshi S, Talwar GP, Ahmed N, Khurana JP, Hasnain SE, Tyagi AK, Tyagi AK. 2009. Polyphasic taxonomic analysis establishes *Mycobacterium indicus pranii* as a distinct species. *PLoS One* 4:e6263. <http://dx.doi.org/10.1371/journal.pone.0006263>.
- Vorbeck C, Lenke H, Fischer P, Knackmuss HJ. 1994. Identification of a hydride-Meisenheimer complex as a metabolite of 2,4,6-trinitrotoluene by a mycobacterium strain. *J Bacteriol* 176:932–934.
- Singh Y, Kohli S, Sowpati DT, Rahman SA, Tyagi AK, Hasnain SE. 2014. Gene cooption in mycobacteria and search for virulence attributes: comparative proteomic analyses of *Mycobacterium tuberculosis*, *Mycobacterium indicus pranii*, and *Mycobacterium goodii*.

- bacterium indicus *pranii* and other mycobacteria. *Int J Med Microbiol* 304:742–748. <http://dx.doi.org/10.1016/j.ijmm.2014.05.006>.
7. Rahman SA, Singh Y, Kohli S, Ahmad J, Ehtesham NZ, Tyagi AK, Hasnain SE. 2014. Comparative analyses of nonpathogenic, opportunistic, and totally pathogenic mycobacteria reveal genomic and biochemical variabilities and highlight the survival attributes of *Mycobacterium tuberculosis*. *mBio* 5(6):e02020. <http://dx.doi.org/10.1128/mBio.02020-14>.
 8. Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H, Pryor M, Duthoy S, Grondin S, Lacroix C, Monsempe C, Simon S, Harris B, Atkin R, Doggett J, Mayes R, Keating L, Wheeler PR, Parkhill J, Barrell BG, Cole ST, Gordon SV, Hewinson RG. 2003. The complete genome sequence of *Mycobacterium bovis*. *Proc Natl Acad Sci U S A* 100:7877–7882. <http://dx.doi.org/10.1073/pnas.1130426100>.
 9. Fox GE, Wisotzkey JD, Jurtshuk P, Jr. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int J Syst Bacteriol* 42:166–170. <http://dx.doi.org/10.1099/00207713-42-1-166>.
 10. Janda JM, Abbott SL. 2002. Bacterial identification for publication: when is enough enough? *J Clin Microbiol* 40:1887–1891. <http://dx.doi.org/10.1128/JCM.40.6.1887-1891.2002>.
 11. Tonjum T, Welty DB, Jantzen E, Small PL. 1998. Differentiation of *Mycobacterium ulcerans*, *M. marinum*, and *M. haemophilum*: mapping of their relationships to *M. tuberculosis* by fatty acid profile analysis, DNA-DNA hybridization, and 16S rRNA gene sequence analysis. *J Clin Microbiol* 36:918–925.
 12. Koeppel A, Perry EB, Sikorski J, Krizanc D, Warner A, Ward DM, Rooney AP, Brambilla E, Connor N, Ratcliff RM, Nevo E, Cohan FM. 2008. Identifying the fundamental units of bacterial diversity: a paradigm shift to incorporate ecology into bacterial systematics. *Proc Natl Acad Sci U S A* 105:2504–2509. <http://dx.doi.org/10.1073/pnas.0712205105>.
 13. Ahmed N, Dobrindt U, Hacker J, Hasnain SE. 2008. Genomic fluidity and pathogenic bacteria: applications in diagnostics, epidemiology and intervention. *Nat Rev Microbiol* 6:387–394. <http://dx.doi.org/10.1038/nrmicro1889>.
 14. Ahmed N, Saini V, Raghuvanshi S, Khurana JP, Tyagi AK, Tyagi AK, Hasnain SE. 2007. Molecular analysis of a leprosy immunotherapeutic bacillus provides insights into mycobacterium evolution. *PLoS One* 2:e968. <http://dx.doi.org/10.1371/journal.pone.0000968>.
 15. Djelouadji Z, Raoult D, Drancourt M. 2011. Palaeogenomics of *Mycobacterium tuberculosis*: epidemic bursts with a degrading genome. *Lancet Infect Dis* 11:641–650. [http://dx.doi.org/10.1016/S1473-3099\(11\)70093-7](http://dx.doi.org/10.1016/S1473-3099(11)70093-7).
 16. Saxena VK, Singh US, Singh AK. 1978. Bacteriological study of a rapidly growing strain of mycobacterium. *Lepr India* 50:588–596.
 17. Katoch VM. 1981. A report on the biochemical analysis of mycobacterium W. *Lepr India* 53:385–389.
 18. Alexander DC. 2012. *Mycobacterium indicus pranii* (MIP) is a strain of *Mycobacterium intracellulare*. *Nucleic Acids Res* 40:10832. http://nar.oxfordjournals.org/content/40/21/10832/reply#nar_el_48.
 19. Tyagi AK, Saini V, Hasnain SE. 2013. On behalf of all the authors, the response to Dr. Alexander's letter is as follows. *Nucleic Acids Res*. 40:10832. http://nar.oxfordjournals.org/content/40/21/10832/reply#nar_el_83.