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Biogeosciences Discussions

Interactive comment on "Culturable bacteria in Himalayan ice in response to atmospheric circulation" *by* S. Zhang et al.

Anonymous Referee #3

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This is a short paper that describes the isolation and preliminary identification of several pure bacterial cultures from 4 sub-samples of an ice core from a high mountain Tibetan glacier. The authors characterize the d18O of the ice to determine under what temperature conditions the snow (which is the basis for the ice) was deposited, and attempt to correlate plate counts and isolate diversity with seasonal deposition. They further perform a preliminary identification of isolates by 16S rRNA gene sequencing and attempt to correlate that identity with a potential source–terrestrial or marine. They conclude that culturable bacteria deposited during monsoon season are more diverse, more abundant, and more closely related to marine bacteria than are those deposited during winter seasons.

This paper addresses a topic that is central to microbiology and glaciology right now,

namely, how are microorganisms transported to glacial and other environments, and how do they survive in the ice environment. Unfortunately, I don't think the data are sufficient to support the conclusions in this manuscript, and in some ways, I think the authors missed the point of their own data.

The strength of this paper lies in two areas. The first is the comparison of their results from the East Rongbuk glacier to other Tibetan glaciers, demonstrating significantly lower counts and diversity than in the other glaciers. The second is in the initial description of the diversity observed, although I would have liked to have seen something more than 16S rRNA in the description of these isolates (see below). However, these strengths are not the focus of the paper. Instead, the focus is on the relationship between seasons and microbial deposition–the weakest aspect of the paper.

This study involves samples spanning one season from a single ice core from a single glacier. The counts obtained are just at or below the believable detection limit–0 to 7 CFU per ml is hardly much of a range. Isolate OTU richness is also quite low–the maximum number of different isolates obtained was 15 from sample No. II, and one sample (No. III) had only a single isolate. These values are too low to draw meaningful statistical inference from–is 7 CFU per ml really statistically different from 3 CFU per ml? Is 15 isolates really different from 10? This is particularly hard to assess because no error bars are included in Figure 2. Presumably, they plated multiple times, as the number of different isolates is significantly higher than the number of CFU per ml–so why not include some actual statistics so that we can assess the significance of the results? The authors further use results spanning a single monsoon season to make conclusions about the relationship to monsoon in general–this could easily be a spurious correlation. It is also quite difficult to assess whether these isolates are really from the ice or are contaminants. The authors did an admirable job of decontamination (quite thorough), but no description of any controls for contamination is included. Were any plates left open in the laminar flow hood while sampling or plating was performed? Were any blank plates (sterile water or media) done? Was any sampling done of sur-

BGD

3, S347–S350, 2006

faces that may have contacted the plates? Such controls are not required in cases where high plate counts are expected or obtained, as contamination levels are generally low; however, these samples show exceptionally low plate counts and thus require confirmation of decontamination.

The focus of the paper would be better if it were on the relationship to the other Tibetan glaciers and a more thorough description of the isolates. Even if a relationship to seasonality can't be demonstrated from these samples (which I believe it cannot), it is still of interest to describe bacteria isolated from the ice.

On another note, I am less than enthusiastic about their conclusions regarding the origin of the bacteria based exclusively on 16S rRNA gene sequence. As all practitioners in the field know, implying physiology (even field source) from the nearest cultured relative is problematic. It would be pretty simple to test things like growth under various salt concentrations, temperature ranges, etc. to determine their growth conditions–especially in a sample set of this size. This would go a long way toward confirming the source of the bacteria as well.

Some other, more minor points:

1) This paper could use a bit of English editing. Articles have been dropped, and some sentences are a bit nonsensical. It is not a major issue–the authors are actually quite good for non-primary English speakers, but it could be improved. 2) Why were the web sites for the specific sequences provided instead of the Genbank accession numbers? 3) The d18O data needs to be explained more clearly. Why does 18O enrichment imply winter deposition? This kind of thing is left a bit vague in the paper, and should be clarified.

In summary, this manuscript has some interesting data that is of relevance to the scientific community; however, the analysis of that data left something to be desired. The focus of the manuscript should be shifted away from seasonality in deposition and toward the identity of the isolates and the relationship to other Himalayan glaciers.

BGD
3, S347–S350, 2006

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BGD

3, S347–S350, 2006

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