Acta agriculturae Slovenica, 90(november 2007)1, 25–31.

http://aas.bf.uni-lj.si

Agris category codes: L20 COBISS Code 1.01

THE RELATIONSHIP BETWEEN TOTAL AND CULTURABLE BACTERIA IN COLD SOILS

Blaž STRES [a)]

[a)] Univ. of Ljubljana, Biotechnical Fac., Zootechnical Dept., Groblje 3, SI-1230 Domžale, Slovenia, Ph.D., M.Sc., e-mail: blaz.stres@bfro.uni-lj.si.

Received December 20, 2006, accepted January 30, 2007.
Delo je prispelo 20. decembra 2006, sprejeto 30. januarja 2007.

ABSTRACT

Cold ecosystems are essential to global ecology. Microbial activities are predicted to increase in low temperature regions due to global warming thus further affecting the green gas emissions and stored carbon overturn. This all has led to increased awareness how minute is our understanding of bacterial assemblages in the cold soils and also other environments, spurring the idea of standardization of research protocols. This work focused on comparison of recently published data on culturability of bacteria from 23 cold soils in the frame of International polar Year 2007 (IPY 2007). The results show that linear relationship exists between direct counts and numbers of cultured bacteria. 11 environmental parameters were reported in these studies. However, only two categories were present in all, preventing attempt to identify governing environmental factors. As there is such heterogeneity in reporting and performing research in microbial ecology, standardization of approaches and protocols in microbial ecology could improve comparability of results substantially.

Key words: microbiology / bacteria / microbial ecology / cold soil / direct count / cultivation

ODNOS MED SKUPNIM ŠTEVILOM IN ŠTEVILOM GOJENIH BAKTERIJ IZ HLADNIH TAL

IZVLEČEK

Hladni ekosistemi so ključni za globalno ekologijo. Mikrobne aktivnosti naj bi se v teh področjih zaradi globalnega segrevanja povečale in tako dodatno vplivale na povečanje emisij toplogrednih plinov in presnove organskega ogljika v teh tleh. To je povečalo zavedanje o pomankljivem razumevanju bakterijskih združb v hladnih teh in drugih ekosistemih ter sprožilo prizadevanja po standardizaciji raziskovalnih protokolov mikrobne ekologije. V tem delu je prikazana primerjava pred kratkim objavljenih podatkov o stopnji uspešnosti gojenja bakterij iz 23 hladnih tal v okviru mednarodnega polarnega leta 2007 (IPY 2007). Rezultati kažejo linearni odnos med skupnim številom bakterij iz direktnega štetja in iz gojitvenih eksperimentov. Od 11 parametrov okolja objavljenih v teh študijah sta bili le dve kategoriji prisotni v vseh objavah. To je onemogočilo identifikacijo ključnih faktorjev okolja. Zaradi prisotne raznolikosti v poročanju in opravljanju raziskav v mikrobni ekologiji bi standardizacija pristopov in protokolov v veliki meri izboljšala primerljivost raziskovalnih rezultatov.

Ključne besede: mikrobiologija / bakterije / mikrobna ekologija / hladna tla / direktno štetje / gojitvene tehnike

INTRODUCTION

Arctic, alpine and other cold ecosystems are critical to global ecology as they represent almost a third of Earth's terrestrial surface. These soils store a third of Earth’s soil carbon, are large sources of atmospheric methane, are very sensitive to climate change, and are already changing rapidly. The intention of International Polar Year 2007 (http://www.ipy.org/) is to strengthen the awareness of scientific community about the importance of cold regions of the world and to increase our understanding of microbiological components of these vast ecosystems. So far, the basic responses of microorganisms to environmental factors in cold ecosystems have been identified. To survive at low temperature, microbes can reduce their cell size and their capsular polysaccharide coat thickness, as well as they can change their fatty acid and phospholipid composition. Moreover, they can decrease the fractional volume of cellular water, increase the fraction of ordered cellular water and extract energy by catalyzing redox reactions of ions in aqueous veins in ice or in thin aqueous films covering solid particle surface (Price and Sowers, 2004). The unfrozen microsites are surrounded by ice and therefore have limited gas exchange. According to Christensen and Tiedje (1990) and Christiensen and Cristiensen (1991), the availability of labile carbon in this water film is high as a consequence of microorganisms being killed by freezing or hygroscopic effects, and from organic matter from broken aggregates. Further, nutrient concentrations in the liquid water film also increase due to ion exclusion from the growing ice grid (Edwards and Cresser, 1992; Stahli and Stadler, 1997). The process of ion exclusion therefore contributes significantly to the establishment of nutrient enriched microsites covered with liquid water at temperatures below zero. As the cold soils are dominated by strong gradients in environment structure and extreme variation within the course of a single year these ecosystems are one of the most challenging on earth for terrestrial life. While a number of groups around the world are contemplating complex research questions, each study is focused on a single local area, usually on a specific subset of the microbial community, and usually uses a suite of analytical techniques. The aim of present study was to gain insight into basic relationship between total and culturable bacteria in various cold soils from recent publications and to correlate the findings to reported environmental parameters.

MATERIALS AND METHODS

Data selection

Literature on enumeration and cultivation of bacteria in cold soils was explored using available public databases: Medline (http://www.ncbi.nlm.nih.gov/PubMed), ScienceDirect (http://www.sciencdirect.com) and ASM (http://aem.asm.org/searchall). The following criteria for literature exploration and selection were adopted: **(i)** publication should be less than ten years old, **(ii)** it should report on more than two cold soil samples, **(iii)** the methodological approaches to direct enumeration of bacteria should be comparable, **(iv)** the methodological approaches to cultivation should be comparable, **(v)** the data on environmental parameters should be provided. The following publications reporting on 23 cold soil samples were selected, focusing on various soils from Antarctica (Zdanowski *et al.*, 2001; Aislabie *et al.*, 2006) and young glacial forefield soils from Switzerland (Sigler *et al.*, 2002).

Data analysis

The environmental data on 23 cold soils were explored. The data categories on various soil properties shared among studies were selected. Two relationships were explored: first, the relationship between environmental data categories, and second, the relationship between environmental parameters and biological variables. The analyses were performed using simple linear regression using MS Excel.

The data on cold soil total and culturable bacteria was compiled and the values were log10 transformations of the original values obtained from the literature. The relationship between total and culturable bacteria in cold soils was analyzed by linear regression of the abundance data according to Lemke and Leff (2006).

RESULTS AND DISCUSSION

In the present study elucidation of basic relationship between environmental parameters and total and culturable bacteria counts was attempted based on recently published data on 23 cold soils.

The relationship between the only two environmental parameters shared among studies, total carbon and total nitrogen, appears to be linear, $R^2 = 0.8321$ (Fig. 1). In case the soil samples with higher carbon content (> 3%) are removed, the goodness of fit remains basically unaffected ($R^2 = 0.824$). This correlation increases substantially ($R^2 = 0.9875$) if one single data point of soil 1 from Antarctica is omitted from analysis. This tight correlation of carbon and nitrogen is in accordance with previously published literature, however, the fractions of various forms of carbon and nitrogen play more crucial role in survival strategies of microbial communities than total abundance per se (Stenstrom *et al.*, 2001). In addition, the majority of the cold environments analyzed in this study are not rich in carbon as 82% (19 of 23) have less than 0.7% of carbon, indicating the oligotrophic nature of cold soils.

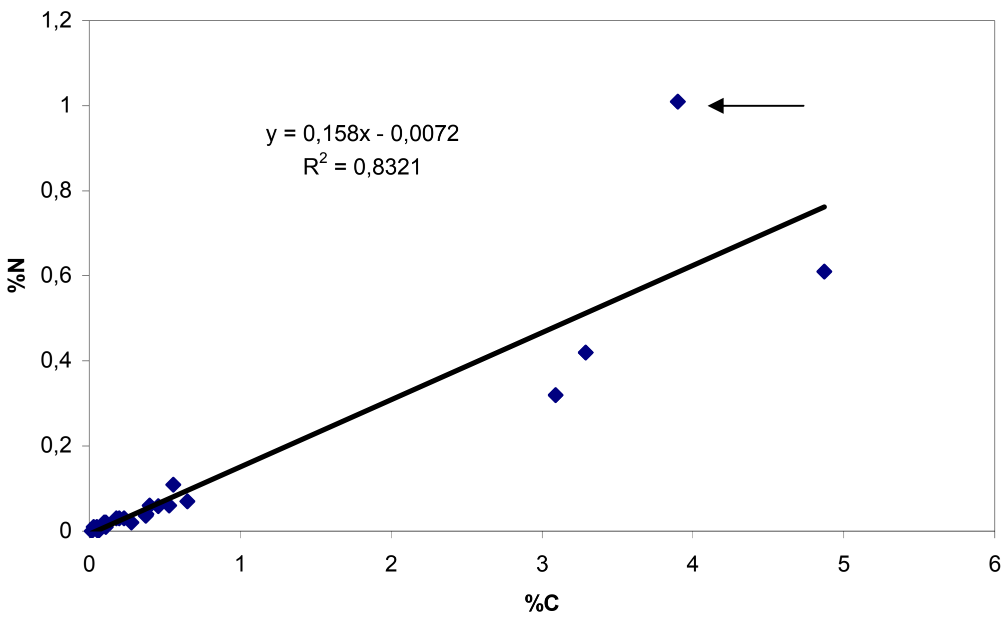

Figure 1. The linear relationship between total carbon (% C) and total nitrogen (% N) present in the 23 cold soils. The data were adopted from Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006). When the data of soil 1 from Antarctica (marked by arrow) is omitted the correlation increases to R2 = 0.9875.

Slika 2. Linearni odnos med deležem skupnega ogljika (% C) in dušika (% N) v 23 hladnih tleh. Podatki so iz naslednjih virov: Zdanowski in sod. (2001), Sigler in sod. (2002) in Aislabie in sod. (2006). Pri analizi brez podatkov za tla 1 Antarktike (označeno s puščico) je korelacija $R^2 = 0{,}9875$.

The studies of Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006) reported altogether on 11 soil properties: soil particle size distribution, pH, C:H:N:S ratio, total C, total N, C/N ratio, soil moisture, soil temperatures, total phosphorus, electrical conductivity, nitrate concentration. The fact that only two categories (total C, total N) were present in all three studies is alarming. This illustrates the diversity of research approaches in microbial ecology. All the published data presented here are based on single measurements in time. The analysis of only one sampling point is not terribly informative from ecological perspective aiming at resolving questions as: What organisms are found in cold soils? Are there local endemic species and communities? How widely distributed are those species and communities? How do they vary across the gradients? Do communities actually vary in their composition and size seasonally or does the growing-season-active community just get frozen in at the onset of winter period? In this respect, there is a true need for standardization of methodological approaches. In addition, reporting on standard set of environmental parameters measured using rigorous approaches of soil chemistry should be adopted in everyday microbial ecology practice.

Out of 11 categories only two of them could be used for analyses. Soil total carbon and total nitrogen are linearly correlated and are thus inefficient in explaining the differences observed in bacterial abundances (figure 2). Various functions could be fitted to the data, however, with very little or no ecological relevance (data not shown). This highlights the importance of reporting environmental attributes thus facilitating identification of governing environmental factors shaping microbial communities. On the other hand, modeling of environmental gradients in controlled laboratory experiments could give better clues on environmental factors shaping microbial communities at hand.

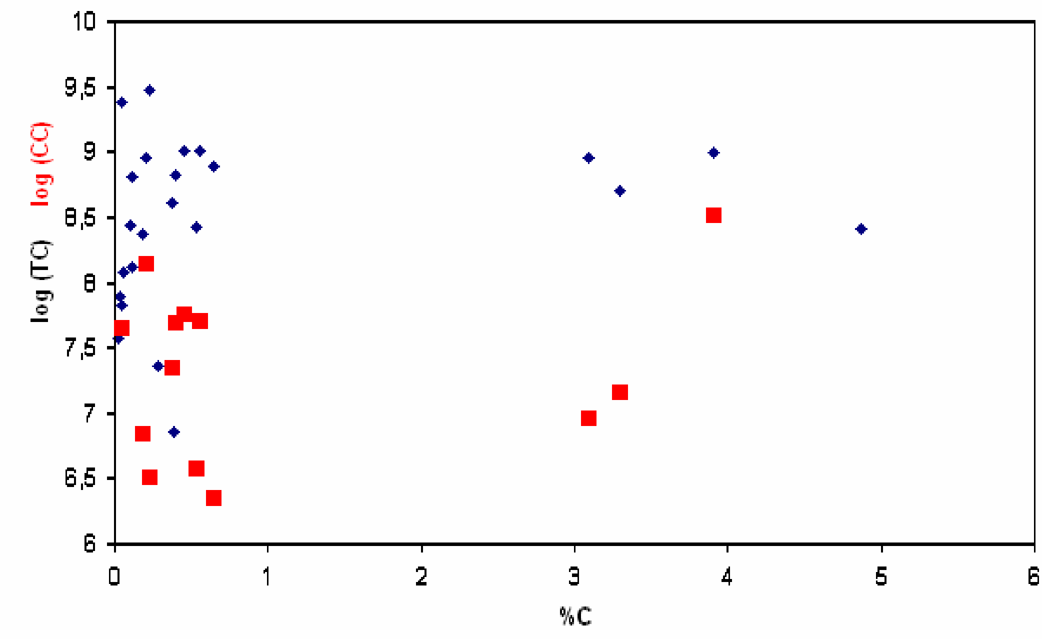

Figure 2. Total (TC) (♦) and culturable (CC) (■) bacteria as a function of total organic carbon (%C) present in 23 cold soils. Values of abundance data were log 10 transformations of values obtained from Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006).

Slika 2. Skupno število (TC) (♦) in število vzgojenih celic (CC) (■) kot funkcija skupnega organskega ogljika (%C) v 23 hladnih tleh. Prikazane so logaritmirane vrednosti podatkov o skupnem številu in številu vzgojenih celic iz literature: Zdanowski in sod. (2001), Sigler in sod. (2002) in Aislabie in sod. (2006).

The relationship between total and culturable bacteria in 23 cold soils is presented in figure 3. Two interesting features can be observed. First, total abundance of bacteria spans from 10^7 to 10^9 per g of cold soil, thus giving rise to comparable abundance of bacteria as in other temperate soils (Curtis *et al.* 2002; Torsvik *et al.* 2002). Second, a linear relationship exists between total bacterial number in cold soils and the number of culturable bacteria, in spite of differences in counting and cultivation approaches used to collect the data. The linear correlation coefficient (R^2 = 0.64) between total and culturable bacteria is comparable to recent study conducted on sediment and stream water (R^2 = 0.76) that included data on nine previously published samples (Lemke and Leff, (2006)). This demonstrates that the percentage of cells that are culturable is consistent across different cold soil environments.

Some of the variation in the number of culturable bacteria may be in general accounted for by a wide range of culturing factors like buffer used in preparation of soil suspension, media type, temperature of incubation, duration of incubation, humidity, gas composition and oxygen saturation level, used by different investigators. However, differences among soils are also likely to play part, especially as media composition were very similar in the studies of Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006). All investigators used media derivatives of nutrient broth as a carbon source solidified with agar.

From regression results, one would predict that average percent of culturable bacteria from a cold soil would be 0.1% that is approximately six times less than predicted for oligotrophic water streams and sediments (Lemke and Leff, 2006).

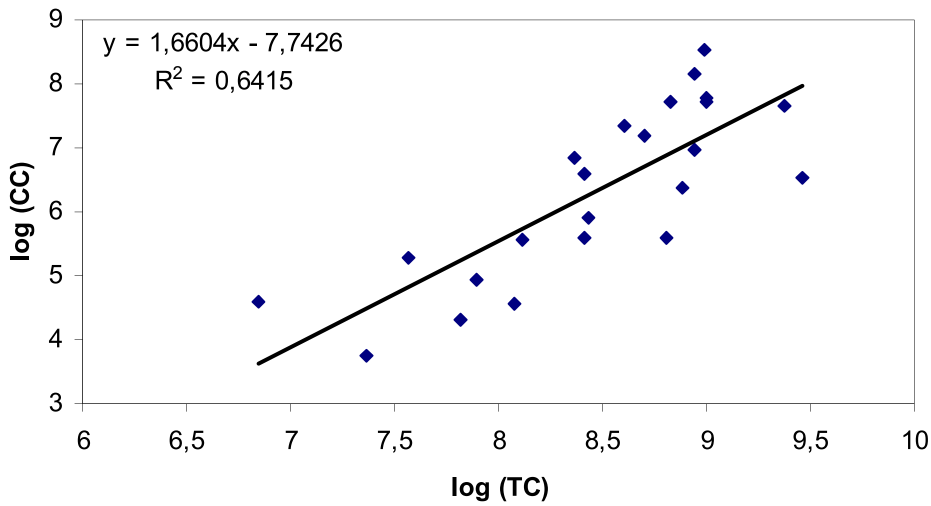

Figure 3. Relationship between total (TC) and culturable (CC) bacteria in 23 cold soils. Values were log 10 transformations of values obtained from literature: Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006).

Slika 3. Odnos med skupnim številom (TC) in številom kultiviranih bakterij (CC) v 23 hladnih tleh. Prikazane so logaritmirane vrednosti podatkov iz literature: Zdanowski in sod. (2001), Sigler in sod. (2002) in Aislabie in sod. (2006).

Based on relatively low success of cultivation approaches, bacterial communities appear to be numerically dominated by bacteria in viable but not culturable (VBNC) state. This suggests that the pool of nonculturable cells includes representatives of species that are under some circumstances culturable, as well as other types of bacteria that resist cultivation under the current conditions of cultivation. There is lack of data on how a wider variety of species divide, grow, reproduce, mature and die off, as prokaryotic life cycles have been described for a few species only (Colwell and Grimes, 2000). In general, it appears that few free-living populations possess the energy and resources to reproduce constantly, and therefore, in the case of bacteria, will all surely not always be culturable.

Because a substantial number of "nonculturable" cells retain measurable metabolic activities (Creach *et al.*, 2003; Kell *et al.*, 1998) a reproductive phase seems to represent only a brief part of the life cycle carried out by a small portion of the population, usually not exceeding 1% of population. However, Stenström *et al.* (2001) reported on reversible transition between active and dormant microbial states in soil upon glucose addition and consumption, where the respiration rate of growing microorganisms represented 5–20% of overall microbial community respiration.

CONCLUSIONS

The results of this study showed that percentage of culturable cells is consistent across 23 different cold soil environments despite differences in techniques used. Using culturing approaches adopted by Zdanowski *et al.* (2001), Sigler *et al.* (2002) and Aislabie *et al.* (2006) it appears to be possible to cultivate only 0.1% of total microbial community. However, more elaborated and imaginative approaches to culturing were already shown to be well suited to yield much higher numbers of cultivated bacteria from temperate soils. Bakken and Olsen (1984) and Lindahl and Bakken (1995) have shown that using appropriate techniques it is possible to retrieve up to 40% of total bacterial counts from soil. Unfortunately, none of these approaches have been tested on cold soils so far.

While microbial ecology is well poised to make a major leap in understanding microbial life, it is constrained by the lack of coordinated study (Morris *et al.* 2002). Thus, a key to this would be to start with coordinating the development of the protocols from sampling up to molecular analyses as this would give strong grounds for intercomparison of results. In addition, this would enable researchers to perform new analyses from different perspectives using collections of already published data to gain additional insights. However, this would constitute the first really coordinated study in microbial ecology in the world and International Polar Year 2007 (http://www.ipy.org/) constitutes a remarkable opportunity to accomplish this task.

ACKNOWLEDGMENTS

I acknowledge the support of late Ivan Mahne, who directed my attention to this topic.

REFERENCES

Aislabie, J.M./ Chhour, K.M./ Saul, D.J./ Miyauchi, S./ Ayton, J./ Paetzold, R.F./ Balks, M.R. Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. Soil Biol. Biochem., 38(2006), 3041–3056.

Bakken, L.R. Separation and purification of bacteria from soil. Appl. Environ. Microbiol., 49(1985), 1482–1487.

Price, B.P./ Sowers, T. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc. Nat. Acad. Sci., 101(2004), 4631–4636.

Christiensen, S./ Tiedje, J.M. Brief and vigorous N_2O production by soil at spring thawing. J. Soil Sci., 41(1990), 1–4.

Christensen, S./ Christensen, B.T. Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. J. Soil Sci., 42(1991), 637–647.

Colwell, R.R./ Grimes, D.J. Nonculturable microorganisms in the environment. Washington, America Society for Microbiology, ASM Press, 2000.

Creach, V./ Baudoux, A.C./ Bertu, G./ Rouzic, L. Direct estimate of active bacteria:CTC use and limitations. J. Microbiol. Meth., 52(2003), 19–28.

Curtis, T.P./ Sloan, W.T./ Scanell, J.W. From the cover: estimating prokaryotic diversity and its limits. PNAS, 99(2002), 10494–10499.

Edwards, A.E./ Cresser, M.S. Freezing and its effects on chemical and biological properties of soil. In: Advances in soil science (Ed.: Steward, B.A.). New York, Springer-Verlag Inc., 18(1992), 59–79.

Kell, D.B./ Kaprelyants, A.S./ Weichart, D.H./ Harwood, C.R./ Barer, M.R. Viability and activity of readily culturable bacteria: a review and discussion of the practical issues. Antonie van Leeuwenhoek, 73(1998), 169–187.

Lemke, M.J./ Leff, L.G. Culturability of stream bacteria assessed at the assemblage and population levels. Microb. Ecol., 51(2006), 365–374.

Lindhal, V./ Bakken, L.R. Evaluation of methods for extraction of bacteria from soil. FEMS Microb. Ecol. 16(1995):135–142.

Morris, C.E./ Bardin, M./ Berge, O./ Frey-Klett, P./ Fromin, N./ Girardin, H./ Guinebretière, H.H./ Lebaron, P./ Thiéry, J.M./ Troussellier, M. Microbial biodiversity: approaches to experimental design and hypothesis testing in primary scientific literature from 1975 to 1999. Microbiol. Mol. Biol. Rev., 66(2002), 592–616.

Sigler, W.V./ Crivii, S./ Zeyer, J. Bacterial succession in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microb. Ecol., 44(2002), 306–316.

Stähli, M./ Stadler, D. Measurement of water and solute dynamics in freezing soil columns with time domain reflectometry. J. Hydrol., 195(1997), 352–369.

Stenstöm, J./ Swenson, K./ Johansson, M. Reversible transition between active and dormant microbial states in soil. FEMS Microb. Ecol., 36(2001), 93–104.

Torsvik, V./ Øvreas, L./ Thingstad, T.F. Prokaryotic diversity – magnitude, dynamics and controlling factors. Science, 296(2002), 1064–1066.

Zdanowski, M.K./ Weglenski, P. Ecophysiology of soil bacteria in vicinity of Henryk Arctowski Station, King George Islan, Antarctica. Soil Biol. Biochem., 33(2001), 819–829.