

行政院國家科學委員會專題研究計畫 期中進度報告

陸域熱泉生態系統成因之跨領域研究--總計畫及子計畫
一：生物能量對嗜熱微生物族群繁衍之控制及台灣熱泉多
空間尺度微生物多樣性研究(1/3)
期中進度報告(精簡版)

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執行期間：95年08月01日至96年11月30日
執行單位：國立臺灣大學地質科學系暨研究所

計畫主持人：林立虹
共同主持人：林雨德、于宏燦

報告附件：國外研究心得報告
出席國際會議研究心得報告及發表論文

處理方式：本計畫可公開查詢

中華民國 96 年 12 月 12 日

First year report for the NSC project - Multidisciplinary research on the origins of terrestrial hydrothermal ecosystems

Main contributors: Li-Hung Lin, Pei-Ling Wang, Hon-Tsen Yu, Yu-Teh Lin, Han-Chen Ho and Chun-Yao Chen

The approval of NSC for this three-year multidisciplinary project has provided an essential resource to support our team to systematically characterize the geochemistry and microbial ecosystems of hot springs for the first time in Taiwan. By combining the manpower and instrumentation from four labs in the National Taiwan University and Tzu-Chi University, a total of 75 sets of samples have been collected for geochemical, microscopic, cultivation and molecular analyses through which we would be able to establish bases for better understanding about the distribution, diversity and function of microbial communities persisting in the environments at elevated temperatures. These results also help us to hypothesize the processes for the origin of spring water and the interactive mechanisms among abiotic and biotic factors, which would be further tested in the following years. Below is the summary of the results from our field campaigns and analyses.

The sampling was conducted in three lithological units across Taiwan, including the northern volcanic region, the southwestern sedimentary region and the eastern metamorphic region. Naturally discharged and high-temperature ($\geq 50^{\circ}\text{C}$) springs were chosen as the highest priority for sampling. When these two criteria could not be concomitantly fulfilled, the high-temperature spring water directly drained from the well head is still qualified for our purpose. The major exception occurred at the southwestern region where most springs possessed temperatures below 40°C . Of three lithological units, the northern volcanic region was most intensively investigated primarily because the interaction between volcanic and surface processes rendered the hot springs with a wide range of geochemical and physical properties. Various environmental extremities are apparently coped with by specific physiological strategies inherited within specific microorganisms and regulate the microbial assemblages and their distributions. The other advantage for sampling in the northern region is that the hot springs were densely distributed, thereby facilitating the comparison of microbial communities on various scales.

Multiple tasks, including analyses of aqueous geochemistry, gas geochemistry, isotope geochemistry, community assemblage, cultivation and microcosm experiment, were carried out in order to identify the water origins and establish the regulative mechanisms on microbial physiological expression. Analyses of major anions and

cations yielded consistent results with previous studies. The hot springs in the northern volcanic region were generally acidic with pH values as low as 0.82. The low pH values were primarily generated by the sulfuric acids produced during the oxidation of hydrogen sulfide released from fumaroles. Given sulfate is one of the primary anions, lower pH would be buffered with the transformation between HSO_4^- and SO_4^{2-} . The resultant acidic solution also provided as an effective agent to leach the ferrous iron from the hosted andesitic rocks into the aqueous phase. Ferrous iron at concentrations of tens of ppm was observed in a number of locations. While sulfate was generally at great abundances, chloride concentrations exhibited a bipolar distribution with greater amounts present in either near seashore and the topographic depression (地熱谷) facing the Taipei basin. Lower chloride occurred at the hot springs near the mountainous ridge. To account for the high chloride, sources such as seawater (modern or paleo) or volcanic hydrogen chloride gas would be required. Given the localities of these high chloride samples and significant hydrogen chloride gas has not been detected in the region with intensive fumarolic activity, we therefore hypothesize that modern seawater and formation water may contribute to a fraction of chloride and sulfate observed in the hot springs near seashore and 地熱谷, respectively.

Hot springs in the southwestern sedimentary and eastern metamorphic regions possessed neutral to alkaline pH values buffered by high bicarbonate. The major differences among springs from these two regions were the contents of the dissolved organic carbon and whether muddy sediments were discharged along with fluids. Because the host rocks in the eastern region are metamorphic rocks (schists, slates and potentially marble), all the samples collected contain tiny amounts of particulates. Chloride concentrations were also generally less those in the southwestern region. In comparison, unconsolidated sediments originated from marine environments in the southwestern region were discharged into surface. The dissolved organic carbon was at concentrations up to 150 ppmC, thereby facilitating the colonization of organotrophs as well as providing a strong reducing power to maintain the anaerobic condition. Anomalous high chloride (>10000 ppm) was observed in some springs. Whether formation (connate) water contributes substantially to the southwestern spring water would be further investigated. Overall, lithology seems to play an important role on the aqueous geochemistry but could not provide a satisfactory explanation for all geochemical variations and the origins of water. Verification of our speculation and hypothesis will be performed later by using Sr and S isotopic systematics.

Isotopic compositions of spring water indicated that all springs in the northern volcanic and eastern metamorphic regions were meteoric in origin; however, some of which from the northern region were subject to the long-term evaporation processes as revealed by the large deviations of both $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values from global and local meteoric water lines. Such isotopic signatures could be probably explained by the fact that these springs were located at higher elevations where local replenish of groundwater is rather limited. Therefore, the isotopic signatures could be preserved from substantial dilution of precipitation and input of river water. The isotopic compositions of the spring water from the eastern region were the most depleted among all, suggesting that recharge area for these waters were at great elevations.

Of all the measured dissolved gases in the northern volcanic region, carbon dioxide was the most abundant species with concentrations ranging up to $\sim 7400\ \mu\text{M}$. These abundances of dissolved carbon dioxide far exceeded those in equilibrium with atmospheric carbon dioxide at the *in situ* temperatures and pH values, suggesting that the dissolved carbon dioxide discharged from fumaroles has not been completely lost to atmosphere under such low pH conditions. Autotrophic microorganisms might be benefited by such an ample volume of dissolved carbon dioxide. Dissolved oxygen and nitrogen were present at concentrations up to $\sim 100\ \mu\text{M}$ and $\sim 350\ \mu\text{M}$, respectively. These two species were apparently derived from atmospheric counterpart through exchange at the water-air interface. Measurements of dissolved oxygen along the water columns did not yield any substantial decline from surface concentration like those observed in seawater or sedimentary porewater. Abundances of dissolved oxygen were, however, less than those in equilibrium with atmospheric oxygen, suggesting a consumption sink would be required. Potential sources of oxygen consumption include (1) abiotic oxidation of volcanic hydrogen sulfide and (2) biotic oxidation of hydrogen sulfide, elemental sulfur, ferrous iron and hydrogen. Hydrogen and methane abundances varied from below detection limit to tens of μM and were the minor components of dissolved gases. Carbon monoxide was below detection limit. Dissolved gases in the southwestern region were predominantly composed of methane and carbon dioxide (make up $>90\%$ of the total gases). Precise measurement is partially hindered by the extremely high loads of muddy sediments which would easily clog the vacuum streamline used in our sample collection. We are currently developing new sampling protocols.

Gibb's free energies for potential 103 metabolic reactions were estimated on the basis of available aqueous and gas geochemistry. Our calculation indicated that most reactions were energetically favorable. Aerobic metabolisms that used hydrogen

sulfide, ferrous iron, elemental sulfur, hydrogen, methane and ammonium as electron donors particularly possessed great negative free energies. The free energies available for aerobic metabolisms inherit from the fact that oxygen acts as a strong oxidizer (or electron acceptor) due to its high redox potential, thereby making the Gibbs's free energy (ΔG_o) at the reference (or standard) state extremely negative. Therefore even the trace amount of oxygen or electron donor present in spring water could still generate very negative free energy. Although thermodynamics only predicts the fate of a specific reaction, our estimates suggest that aerobic metabolisms could persist in the environment with marginal dissolved oxygen. Anaerobic metabolisms (including nitrogen-, metal-, hydrocarbon-, and sulfur-related metabolisms, and fermentation) yielded negative free energies as well. Few exceptions occurred in sulfate reduction and methanogenesis where the energy yield was less negative than the commonly accepted minimum energy (-20 kJ mol^{-1}) for synthesis of 1/3 mol of ATP molecule. Insufficient energy yields might arise from the extremely low pH values or low substrate concentrations.

Screening of 16S rRNA genes yielded various diversities and assemblages for samples from different regions. For the northern region, archaea were found to outcompete over bacteria in most samples. Microbial communities from the spring waters in the northern region were primarily dominated by aerobic sulfur-, sulfide- or organic- respiring archaea, such as *Sulfolobaceae* and *Thermoproteaceae*. These archaeota are typically found in solfatara with low pH values and high temperatures. Depending on the oxygen and organic content, some of these archaeota could switch between aerobic and anaerobic or between organotrophic and autotrophic metabolisms. Potential metabolisms inferred from 16S rDNA sequences were generally consistent with those from aqueous and gas geochemistry and free energy estimates. The bottom sediments, however, hosted dramatically different microbial assemblages in that crenarchaeota including *Caldisphaeraceae* and *Desulforococcaceae* were the most dominant members. Both these families are capable of reducing elemental sulfur to hydrogen sulfide with either hydrogen or complex organic carbon as an electron donor. *Thermoplasmataceae* which acquired energy from anaerobic fermentation of complex organic matters were present in a minor proportion in some sediment samples. The segregation of anaerobes in bottom sediments from planktonic aerobes suggests that atmospheric oxygen fails to reach the bottom sediments probably because of elevated sulfide contents discharged from fumaroles. The dissolved oxygen was, however, homogenized through diffusion and agitation at concentrations sufficient to maintain the aerobic metabolisms. Despite this overall pattern of microbial distribution, a number of

unclassified crenarchaeota and euryarchaeota were constantly detected. Since no culture was closely affiliated with these environmental sequences, it would be uncertain to draw the inference about their potential functions in the natural setting.

Bacteria were detected in minor samples from the northern volcanic region. When bacteria were present, the communities were almost completely composed of one single family, *Aquificaceae*, the members of which are characterized by acquiring metabolic energy from microaerophilic hydrogen oxidation. The predominance of this family has also been observed in the microbial communities associated with the hot springs in the Yellowstone National Park of USA and was considered to represent the microbial ecosystems at the base of food web fueled by the volcanically generated hydrogen. The physiological characteristics of *Aquificaceae* were, however, not consistent with our geochemical observation. Instead of dissolved hydrogen abundances, the presence or absence of *Aquificaceae* seems to be correlated with the concentrations of dissolved hydrogen sulfide. The *Aquificaceae*-related sequences were detectable when the dissolved hydrogen sulfide concentrations exceeded 5 ppmS. We speculated that that elevated hydrogen sulfide might regulate the dissolved oxygen at concentrations sufficient for abundant metabolic energy but below the threshold that could cause oxidative stress to *Aquificaceae*.

Hot springs in the southwestern region were primarily composed of very complex assemblages of bacteria community. Bacteria dominated over archaea in two samples investigated. Screening of 61 clones from the bacterial library of the Kuan-Chih-Ling hot spring yielded 42 phlotypes at a cutoff threshold of 1% of sequence variance. The phylogenetic affiliation of these sequences in terms of phylum crossed over 11 out of the total 23 phyla, including *Aquificae*, *Thermotoga*, *Nitrospira*, *Chloroflexi*, *Cyanobacteria*, *Bacteroidetes*, *Dictyoglomi*, *Planctomycetes*, *Verrucomicrobia*, *Acidiobacteria* and *Proteobacteria*. The projected overall diversity could range up to above 1000 phlotypes if screening is performed thoroughly. When combining the results from incubation experiments using the sediment slurry from this hot spring as an inoculum, the number of detected phyla would increase to 17. Archaeal assemblages were rather simpler than bacteria and composed of two thermophilic genera (and phlotypes) of euryarchaeota: *Methanotrix* and *Archaeoglobus*. While the former one produces methane from acetate under moderate thermophilic conditions, the latter one is capable of reducing sulfate to sulfide at hyperthermophilic temperatures. The complex assemblages inherited with the sediments in the Kuan-Chih-Ling hot spring suggests multiple interactions among the active members facilitated the enzymatic hydrolysis of organic

carbon into smaller organic acids and hydrogen, which could fuel other terminal electron accepting processes (such as sulfate reduction and methanogenesis) downstream the food chain.

The incubation of the Kuan-Chih-Ling sediment slurries under the confined space, however, changed the community structures substantially. Monitoring of major anions indicated a continuous sulfate reduction over an interval of one month. The lag time increased as the temperature increased. The maximum sulfate reduction rate was, however, the greatest at the highest temperature investigated (80°C). As the temperature was raised from 40°C to 80°C, both bacterial and archaeal communities gradually lost their respective diversities. Correspondingly, community assemblages were also successively replaced by members adapting to the higher temperatures. Sequence analysis revealed consistent results with geochemical assessment. While sulfate reduction and methanogenesis coexisted at 40°C and 50°C, only sulfate reduction persisted at temperatures greater than 60°C.

Community structures in the eastern region were much simpler than those in the northern and southwestern regions. Only two phylotypes were observed in the Hung-Yeh hot spring with one affiliated with *Aquificaceae* and the other with *Korarchaeota*. Again the former one is capable of oxidizing hydrogen under the microaerophilic condition. The physiological characteristics of the latter one have not been validated yet due to the lack of any culture representative. Such a coexistence of *Aquificaceae* and *Korarchaeota* has been observed in the Calcite Spring of the Yellowstone National Park of USA and Iceland, both of which were characterized by high bicarbonate contents and stream flows. Given the geochemical context of this hot spring, it is likely that the observed *Korarchaeota* possess similar metabolisms with those of *Aquificaceae*. We are currently developing a new strategy to cultivate the observed *Korarchaeum*.

Enrichment experiments were performed to target on iron reducers and oxidizers, organotrophic and autotrophic sulfate reducers, methanogens, aerobic and anaerobic organotrophs, elemental sulfur oxidizers and reducers, and hydrogen oxidizers at acidic and neutral pH values. Positive enrichments of sulfate reduction, iron oxidation and reduction, fermentation, and elemental sulfur oxidation and reduction were obtained for samples from the northern region. The results of enrichments were generally consistent with the 16S rDNA sequences and geochemical assessment. Although the enrichment of iron reduction was positive, the analysis of 16S rRNA genes failed to detect any sequence affiliated with the known thermophilic or

hyperthermophilic iron reducers in the corresponding samples. It is likely that either the proportion of iron reducers is too low to be detected or unclassified sequences represent the population capable of reducing iron. Enriched elemental sulfur reducers exhibited faster growth at 90°C than at 80°C and preferred sub-neutral pH over acidic pH values even though the *in situ* pH value was at ~3. Sequence analysis for the enrichment after a 10⁸-fold dilution indicated that one strain was affiliated with crearchaeal *Desulforococcus* sp. at 93% similarity. Further isolation and physiological tests are currently undergoing.

Sulfate reduction and anaerobic fermentation also appeared to be positively enriched for samples from the southwestern region. A co-culture composed of *Thermotoga* sp. and *Archaeoglobus* sp. was obtained through several serial dilutions. Most *Archaeoglobus* strains and related environmental sequences are recovered primarily from deep sea hydrothermal vents. Only a limited number of environmental sequences affiliated with *Archaeoglobus* have been reported for terrestrial environments. The strategy adapted by *Archaeoglobus* in terrestrial environments would provide further insights into how microorganisms can cope with the fluctuated temperature, oxygen intrusion, and salinity, and the restriction of substrate availability.

The samples from the eastern region yielded positive growth of hydrogen oxidizers and iron reducers. One strain was isolated from the former enrichment and its 16S rDNA sequence was closely affiliated (>99%) with the *Aquificales* detected from the environmental DNA of the corresponding sample.

In conclusion, the lithology apparently plays an essential role on regulating the geochemistry of the spring water, which in turn constrains the diversity, assemblage, and distribution of thermophilic communities. Variations in geochemistry and community assemblages, however, can not be satisfactorily accounted for by only this factor. Proxies for the other local processes are required to unravel the operating mechanisms hidden behind. Our analyses also revealed that the turnover of the microbial communities is rapid with regard to the environmental changes. A small perturbation would shift the dynamic equilibrium optimized for the transient stability. Our one-year survey and analyses already allowed us to establish the hypotheses which will be tested in the coming year. Two manuscripts that summarize the available results are currently under development and will be wrapped up for submission at the beginning of the next year.

赴國外研究心得報告

計畫編號	95-2627-M-002-004-
計畫名稱	陸域熱泉生態系統成因之跨領域研究-總計畫及子計畫一：生物能量對嗜熱微生物族群繁衍之控制及台灣熱泉多空間尺度微生物多樣性研究
出國人員姓名 服務機關及職稱	林立虹，台灣大學地質學系，助理教授
出國時間地點	日本 Yokosuka 12/26~12/28, 2006
國外研究機構	Japanese Agency of Marine Science and Technology

工作記要：

1. 時間：12/26~12/28, 2006。
2. 地點：JAMSTEC HQ, Yokosuka, Japan。
3. 參與人員：
台灣：林曉武、劉家瑄、楊燦堯、黃奇瑜、林立虹。
日本：Hideaki Machiyama, Wataru Azuma, Ken Takai, Katsuhori Fujikura, Samito Morita, Tomohiro Toki, T. Nunoura。
4. 目的：討論 2007 年 3 月 JAMSTEC 研究船 Natsushima 來台合作研究：(1) 測站選擇；(2) 參觀研究船與 ROV Hyper Dolphin；(3) 台灣科學家需要之採樣儀器；(4) 合作原則與研究內容。
5. 時程：
 1. 12/26 下午：台北出發，至東京 Narita 機場後轉乘多班火車於晚上 9 時抵達，適逢當地多年少見之大雨，在火車站旁麵館略進晚餐後，轉搭計程車至 JAMSTEC HQ 夜宿。
 2. 12/27 上午：9 點至 JAMSTEC HQ 5 樓日式之會場集合，此為日我雙方人員會議駐足之處。Dr. Machiyama 與 Dr. Fujikura 帶領我方人員赴 HQ 旁停泊之 R/V Natsushima 參觀研究船與 ROV，瞭解此研究船研究室與住宿狀況、ROV 之採樣能力與我方之配合需求。R/V Natsushima 於參觀後即出港。
 3. 12/27 下午：日我雙方科學家自我介紹。12 名科學家闡述其在本航次之研究目的與需求後已快超過餐廳休息時間，急忙赴餐廳晚餐。待晚餐後雙方人員又回返會議室繼續討論，直到夜深才停。我方科學家於返回住宿處後又繼續討論。
 4. 12/28 上午：討論樣品與合作事宜至 11 時，因需轉搭多次火車至東京 Narita 機場，故雖然仍有討論事項，但必需停止。急忙搭車赴車站，經過多次轉車至 Narita 機場時，只剩下不多時間。此次航前會議討論多項重要事項，時間雖然非常緊迫，但與會學者都顯現非常強烈的興趣，故能在非常短暫的時間達成共識。

5. 12/28 晚：抵達台北。

6. 會議結論：

A. 科學研究主旨：

1. 生物與微生物研究：Nunoura、林立虹、Fujikura、林曉武、黃奇瑜
2. 海床：Azuma、劉家瑄、古佳艷
3. Carbonate 與 gas hydrate：楊燦堯、Toki、Morita、Machiyama、林曉武、黃奇瑜
4. 泥火山來源與發育：Morita、Azuma、林曉武、楊燦堯、劉家瑄
5. 強烈地震與泥火山之相關性：Azuma、劉家瑄

B. 方法：

1. 放射性元素使用：Natsushima 將攜帶放射元素專用貨櫃：林曉武
2. Fluid & gas sample equipment：Azuma
3. Magnetometer：劉家瑄

C. Dive sites：

1. Passive sites：F(主要) 或 G(次要)。
2. Active sites：B(主要) 或 C1+C2(次要)。
3. Co-seismic rupture：2006 年 12 月恆春地震產生之破裂面應是重要研究項目，希望 NARL 可協助爭取同意進入該研究區域，而 Azuma 將爭取更多時間來進行此種難得且是本研究最主要之目的。

D. 需求事項：

1. 確定 submarine cable 位置避免造成 ROV 潛航危險：林曉武。

E. 科學分享：

1. 樣品分享：
 - a. 原則上依據 IODP 樣品分配原則分取，各科學家將研究需求向台日二方之 Co-Chief Scientists (即林曉武與 Machiyama) 提出後，由二位 Co-Chief Scientists 本著科學重要性與需求於討論後決定。
 - b. 所有科學家需於 3 星期內將科學目的與需求提出，並在 1 月底作出決定。
2. Co-Chief Scientists 負責與同意日後研究成果之發表，並參與發表。

F. Post-Cruise Meeting：林曉武，Machiyama。

原則上將在台灣舉行，時間訂在 9 月底附近；Co-Chief Scientists 將負責與主持會議之召開。

8. 綜合結論：

雙方科學家對 2007/3 月之台日雙方第一次海洋研究航次破冰合作都有非常高之期盼，科學家對未來合作有充分的討論與瞭解合作的重要性。另外在會議當天台灣西南海域研究區域發生強烈地震，此地震與本研究主題非常切合，即斷層、泥火山活動與台灣西南板塊地震帶之相關性。由於此種海床變動非常容易造成海嘯，故不僅可能對台灣產生嚴重危害，且可能對鄰近國家產生嚴重後果，更加上此強烈地震吻合本研究主題之理論，故而台、日本科學家都希望我方能向國科會申請核准進入該海域進行研究，希望能夠瞭解台灣西南海域斷層與地震活動之相關性。

9. 會議感想：

本次會議非常緊湊，而且有具體結果。不僅雙方都瞭解研究需求與重要性，而且都有強烈之合作研究興趣。日方同意負責所有研究船需求，我方可攜帶額外設備，更加上強烈地震之發生皆有助於提升瞭解台灣之地震與泥火山相關成因之研究外，更希望借助此一合作研究，奠定未來合作契機，相信對提升我國科研與日本雙方關係必有相當之助益。

另外借由參觀日本 JAMSTEC HQ 亦瞭解日本對海洋研究之巨大投入，儀器設備都是國際一流，科學家在有了充足之經費與研究儀器、船隻，自信心非常充足，更有邁向國際一流海洋研究之企圖心。希望台灣海洋研究亦有類似之一天。

出席國際學術會議心得報告

計畫編號	95-2627-M-002-004-
計畫名稱	陸域熱泉生態系統成因之跨領域研究-總計畫及子計畫一：生物能量對嗜熱微生物族群繁衍之控制及台灣熱泉多空間尺度微生物多樣性研究
出國人員姓名 服務機關及職稱	林立虹，台灣大學地質學系，助理教授
會議時間地點	Taupo, New Zealand, 11/10-11/16, 2007
會議名稱	International Symposium on Environmental Biogeochemistry, ISEB 18
發表論文題目	Temperature dependent succession of microbial communities associated with a muddy hot spring in southwestern Taiwan

一、參加會議經過

11/09 – 11/10 Taipei - Taupo

11/10 – 11/16 Taupo; attending meeting

11/16 – 11/19 Auckland

11/19 – 11/20 Auckland - Taipei

二、與會心得

The ISEB 18 was held at Taupo, New Zealand from 11/10 to 11/16 of 2007. The major theme of this meeting was the “Biogeochemistry at the extremes” by taking the advantages that Taupo is located at the one of the most geothermally active areas in New Zealand, which resulted from magmatism associated with the subduction of the Pacific plate underneath the Australia-Antarctic plate. Volcanic gases were discharged through the localized fractures to surface environments at which interaction between hydrogen sulfide and dissolved oxygen generated extremely acidic solutions that would further leach transition metals (such as arsenic) out of rocks. The high temperature steam also raised the groundwater temperatures up to the boiling point. Such an interaction creates habitats with various physical and chemical characteristics for specialized microorganisms thriving at the chemical disequilibrium. This phenomenon is also similar with what we constantly observed in the northern Taiwan. The major differences are that the spatial scale of these geothermal features is much widespread and less interfered by man-made activity in New Zealand than in Taiwan. The advantage in Taiwan, however, lies in the fact that we have geothermal features not only related with volcanic activity but also with sedimentary and metamorphic rocks. The diverse environments facilitate to design sampling and experiment targeting on the specific groups of metabolisms or assemblages that could not be easily achieved elsewhere.

Three posters related with this project were presented in the meeting and grasped the attention of people working in the microbial ecosystems of the geothermal and hydrothermal

environments. We have established the contact with Dr. Kirk Nordstrom of USGS who is an aqueous chemist specialized on hydrothermal geochemistry and has paid huge efforts in characterizing the chemistry of the hot springs in the Yellowstone National Park of USA. He will perhaps visit Taiwan next year to discuss more about the arsenic contamination in our southwestern groundwater and arsenic release in the hot springs of the northern Taiwan.

We also have a very good discussion with Dr. Hugh Morgan of the University of Waikato, New Zealand about the screening and distribution of nanoarchaea in both our and their hot spring samples. The sample exchange is currently undergoing and hopefully collaborative research will be initiated in the near future. Several discussions with other scholars provided very helpful comments to our current project.

Overall, abundant geothermal features in New Zealand open another gate for studying the microbial ecosystems at extremely acidic and hot conditions. While our research project already gained recognition in this field, we are hoping to develop an international collaborative project that would need to acquire the samples from both Taiwan and New Zealand in five years. The ultimate goal is to unravel whether biogeography plays a role in regulating the diversity and distribution of specific groups of microorganisms in hot springs.