The 20th International Meeting on *Frankia* and Actinorhizal Plants

Ken-ichi Kucho[1], Hiroyuki Tobita[2], Shunsuke Utsumi[3], Toshiki Uchiumi[1], and Takashi Yamanaka[4]

[1]Department of Science, Graduate School of Science and Engineering, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890-0065, Japan

[2]Forestry and Forest Products Research Institute, Tsukuba 305-8687, Japan

[3]Field Science Center of Northern Biosphere, Hokkaido University, North 9, West 9, Sapporo, Hokkaido 060-0809, Japan

[4]Tohoku Research Center, Forestry and Forest Products Research Institute, Morioka 020-0123, Japan

A nitrogen-fixing actinobacterial genus *Frankia* establishes root-nodule symbiosis with actinorhizal plants comprising more than 200 species in eight dicotyledonous families (Benson and Silvester 1993)(Huss-Danell 1997)(Kucho et al. 2010). This beneficial symbiosis enables the plants to grow in infertile soils and contributes to revegetation of devastated lands. Since the first description of root nodules in an alder *Alnus glutinosa* (Meyen 1829), a wide variety of research concerning ecology, physiology, cytology, genetics, omics biology, etc. has been conducted on this subject. The 1st International Meeting on *Frankia* and Actinorhizal Plants was held at Petersham, Massachusetts, USA in 1978 (Torrey and Tjepkema 1979). Since then, this international meeting continues to be held almost every two years. The 19th meeting had been organized in Hammamet, Tunisia in March 2018 by Dr. Maher Gtari (Gtari et al. 2019). The memorial 20th meeting was originally planned to be held in Kagoshima, Japan in May 2020. However, due to outbreak of the COVID-19, the conference was postponed to one year later. We had expected a face-to-face meeting but spreading of the disease did not subside. Therefore, it was held online for the first time in the history of the meeting.

The online 20th International Meeting on *Frankia* and Actinorhizal Plants was held on May 29-31, 2021. Fifty-seven scientists attended the meeting while the number of registrations in the originally planned face-to-face meeting had been only 35. This suggests that people who had given up on traveling to Japan was able to join this online event. On the other hand, participants were from 15 countries on every continent except Antarctica, so the time difference was a big problem. The presentation program was organized to fit as many participants as possible, but some presentations were placed at local time which was inconvenient for a part of participants.

There were 19 oral and 8 poster presentations. Oral sessions were conducted using Zoom. Poster sessions were conducted using Zoom and file sharing function of Microsoft OneDrive. Participants viewed PDF files of the posters in a shared folder in OneDrive and had discussions in the Zoom breakout rooms assigned to each poster.

Due to rapid advance in DNA sequencing technology, "Omics" approach was a major topic in this meeting. Majority of researchers used this approach and gained new insights into the actinorhizal symbiosis. Transcriptome analysis of *Alnus glutinosa* (Hocher et al. 2011) identified a lipid transfer protein AgLTP24 was upregulated in roots during establishment of symbiosis, and Gasser et al. (2021) reported this protein was localized at deformed root hairs and *Frankia* vesicles. Several metagenome analyses of cluster-2 *Frankia* strains had been reported (Persson et al. 2015; Nguyen et al. 2016; Nguyen et al. 2019). Berckx et al. (2021a) and Berckx et al. (2021b) presented new metagenome analyses using nodules collected from Philippines, Taiwan and New Zealand

and discussed evolution of the cluster-2 strains. Normand et al. talked about genome analyses of Sp+ *Aluns*-infective strains that sporulate inside nodules and have never been isolated in pure culture (Herrera-Belaroussi et al. 2020; Pozzi et al. 2020). Genomes of the Sp+ strains were significantly reduced in size and lacked more than 1000 genes compared to Sp- strains (Herrera-Belaroussi et al. 2021). Hahlin et al. (2021) reviewed research history of nitrogen fixation, hydrogenase and hydrogen evolution, and reported genome analyses of local source of *Frankia* strains.

Sen et al. (2021) compared 16S rRNA gene metagenomes between rhizosphere soils of *Alnus nepalensis* and non-rhizosphere soils from Darjeeling hills in India. They found that diversity of bacteria was higher in non-rhizosphere soil, and nitrogen-fixing bacteria such as *Frankia* and cyanobacteria were enriched in rhizosphere soils. Kagiya et al. (2021) analyzed *nifD-K* intergenic spacer sequences of *Frankia* in *Alnus hirsta* nodules and its rhizosphere soils collected from Uryu experimental forest, Hokkaido, Japan. Significantly different *Frankia* operational taxonomic units were detected between the two ecological niches, suggesting that host plants filter infecting partners. Jabberi et al. (2021) evaluated effect of static magnetic field (SMF) on bacterial community in Tunisian phosphogypsum soils by 16S rDNA metagenome analysis. They found that exposure to SMF increased several nitrogen-fixing taxa such as *Frankia*. Sarkar et al. (2021) applied the reverse ecology approach to genomes of *Frankia* and co-inhabiting bacteria in nodules. The results suggest that co-inhabiting bacteria do not compete with *Frankia* but play a supportive role in symbiosis process. Actually, Karthikeyan et al. (2021) reported that co-inoculation of *Micromonospora* with *Frankia* improved growth and biomass of *Casuarina* plants and resistance to a pathogenic bacterium *Ralstonia solanacearum*. Sarkar and Sen (2021) reported comprehensive codon and amino acid usage analyses of transmembrane proteins and proteins involved in metabolic pathways in 44 *Frankia* genomes.

An important topic of this meeting was genetic transformation of *Frankia*. Two laboratories had reported successful transformation of *Frankia* spp. by filter mating with an *Escherichia coli* strain (Pesce et al. 2019) or by electroporation (Gifford et al. 2019). Pesce et al. (2021) presented an application of this mating method to genome editing using CRISPR/CAS9 system (Doudna and Charpentier 2014). On the other hand, however, Kucho (2021) reported that all attempts at the mating transformation made in his laboratory had failed. It was also discussed that cultivation of the *Frankia* transformants has not been successful outside of the two laboratories. Therefore, transformation of *Frankia* is not easily reproducible by universal researchers at present.

Ribeiro-Barros et al. (2021) reviewed salt-stress tolerance of *Casuarina* spp. in aspects of physiology, genomics, transcriptomics, proteomics, metabolomics and discussed potential contribution of the actinorhizal symbiosis to rehabilitation of soil degradation caused by salinization. Djighaly et al. (2021) reported responses of antioxidant enzymes to salt stress, and to inoculation of *Frankia* and arbuscular mycorrhizal fungi in two *Casuarina* species. Studies about heavy metal tolerance were also reported. Rehan et al. (2021) reported *Frankia* strains accumulate cadmium (Cd) and tolerate high concentration of Cd. Worth et al. (2021) reported that co-inoculation of *Alnus glutinosa* with an alder-infecting *Frankia* strain and with an atypical *Frankia* strain enhanced nodulation under copper stress.

Studies about ecology of actinorhizal symbiosis were also reported. Katayama and Tateno reported that even in late autumn *Alnus firma* continued photosynthesis without resorbing nitrogen from leaves and provided photosynthetic products to *Frankia* to support nitrogen fixation (Tateno 2003). They also reported that litter of *Fallopia japonica* enhanced growth of *Alnus inokumae* in volcanic soil by supplying phosphate (Katayama et al. 2021). Tobita et al. (2021) investigated N_2-fixing capacity of three *Alnus* species that were naturally regenerated at the massive landslide area on Mt. Ontake using N stable isotope ratio analysis. They found that contribution of N_2 fixation to N absorption varies not only with elevation but also with vegetation recovery status. Krishnamoorthi et al. (2021) reported nodulation and blister bark disease incidence of *Casuarina junghuhniana* and *C. cunninghamiana* in Tamil Nadu state in southern India. Rajendran (2021) reported exopolysaccharide production by *Casuarina equisetifolia* and its beneficial role for growth of the plants.

Three researchers presented analysis of chemical compounds from actinorhizal plants. Jin et al. (2021) purified compounds from root nodules of *Casuarina equisetifolia* and found that tyramine was a dominant constituent. Banerjee et al. (2021) reported phytochemical profiling of *Elaeagnus* wine and isolated compounds with anti-cancer activity. Nisad et al. (2021) reviewed pharmacological and therapeutic potential of Myricetin, which is a flavonol purified from *Myrica nagi* plant.

Finally, we thank all the participants and presenters for your corporation to make this unusual meeting a success. We sincerely hope the next meeting will be face-to-face and be able to have more intimate communication each other.

References

Banerjee S, Sarkar I, Sen A, Kar P. 2021. Phytochemical profiling of *Elaeagnus* wine with special reference to its anti-stress and anti-cancer properties. Poster session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May

29-31 (online).

Benson DR, Silvester WB. 1993. Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. Microbiol Rev. 57:293–319.

Berckx F, Nguyen T Van, Bandong CM, et al. 2021a. Evolution and distribution of the basal *Frankia* clade, *Frankia* cluster-2. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Berckx F, Wibberg D, Brachmann A, et al. 2021b. *Frankia* associated with *Coriaria arborea* and *Coriaria sarmentosa* in New Zealand. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Djighaly PI, Diagne N, Ngom D, et al. 2021. Effect of symbiotic associations with *Frankia* and arbuscular mycorrhizal fungi on antioxidant activity and cell ultrastructure in *Casuarina equisetifolia* (L.) and *C. obesa* (Miq.) under salt stress. Poster session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Doudna JA, Charpentier E. 2014. The new frontier of genome engineering with CRISPR-Cas9. Science (80-). 346:1258096. https://doi.org/10.1126/science.1258096.

Gasser M, Alloisio N, Fournier P, et al. 2021. Role of antimicrobial peptides of the "lipid transfer proteins" family in actinorhizal symbiosis. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Gifford I, Vance S, Nguyen G, Berry AM. 2019. A stable genetic transformation system and implications of the type IV restriction system in the nitrogen-fixing plant endosymbiont *Frankia alni* ACN14a. Front Microbiol. 10: https://doi.org/10.3389/fmicb.2019.02230.

Gtari M, Benson DR, Nouioui I, et al. 2019. 19th International Meeting on *Frankia* and Actinorhizal Plants. Antonie Van Leeuwenhoek. 112:1–4. https://doi.org/10.1007/s10482-018-1202-x.

Hahlin A-S, Pawlowski K, Normand P, et al. 2021. A *Frankia* strain infective on *Alnus* sp. the so-called local source – past and present. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Herrera-Belaroussi A, Normand P, Pawlowski K, et al. 2020. *Candidatus* Frankia nodulisporulans sp. nov., an *Alnus glutinosa*-infective *Frankia* species unable to grow in pure culture and able to sporulate *in-planta*. Syst Appl Microbiol. 43:126134. https://doi.org/10.1016/j.syapm.2020.126134.

Herrera-Belaroussi A, Sellstedt A, Pawlowski K, et al. 2021. Comparative genomics to study *Frankia* Sp+ lineages. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Hocher V, Alloisio N, Auguy F, et al. 2011. Transcriptomics of actinorhizal symbioses reveals homologs of the whole common symbiotic signaling cascade. Plant Physiol. 156:700–711.

Huss-Danell K. 1997. Actinorhizal symbioses and their N_2 fixation. New Phytol. 136:375–405.

Jabberi M, Martinez M, Najjari A, et al. 2021. Effect of a static magnetic field on putative nitrogen-fixing Terrabacteria of Tunisian phosphogypsum decipherided through metataxonomic analyses. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Jin Y, Xu Y-T, Huang Z-W, et al. 2021. Metabolite pattern in root nodules of the actinorhizal plant *Casuarina equisetifolia*. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Kagiya S, Kucho K, Utsumi S. 2021. Filtering in a plant–bacterium symbiotic network in a natural environment: Does host genetic variation matter? Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Karthikeyan A, Nicodemus A, Kanchanadevi K. 2021. Effect of *Frankia* and *Micromonospora* on growth and health improvement in *Casuarina* clones. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Katayama S, Omori T, Tateno M. 2021. Fresh litter acts as a substantial phosphorus source of plant species appearing in primary succession on volcanic ash soil. Sci Rep. 11:11497. https://doi.org/10.1038/s41598-021-91078-6.

Krishnamoorthi S, Mohan V, Suraj PG, et al. 2021. Status of *Frankia* nodulation in *Casuarina* plantations of Tamil Nadu and Andhra Pradesh in southern India. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Kucho K. 2021. Genetic transformation of *Frankia* CcI3: in our laboratory's case. Poster session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Kucho K, Hay AE, Normand P. 2010. The determinants of the actinorhizal symbiosis. Microbes Environ. 25:241–252. https://doi.org/10.1264/jsme2.ME10143.

Meyen J. 1829. Über das Hervorwachsen parasitischer Gebilde aus den Wurzeln anderer Pflanzen. Flora (Jena). 12:49-64.

Nguyen T Van, Wibberg D, Battenberg K, et al. 2016. An assemblage of *Frankia* Cluster II strains from California contains the canonical *nod* genes and also the sulfotransferase gene *nodH*. BMC Genomics. 17:796. https://doi.org/10.1186/s12864-016-3140-1.

Nguyen T Van, Wibberg D, Vigil-Stenman T, et al. 2019. *Frankia*-enriched metagenomes from the earliest diverging symbiotic *Frankia* cluster: they come in teams. Genome Biol Evol. 11:2273–2291. https://doi.org/10.1093/gbe/evz153.

Nisad K, Kumar HPP, Ramesh N, et al. 2021. Myricetin- A lead from the forest. Poster session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Persson T, Battenberg K, Demina IV, et al. 2015. *Candidatus* Frankia datiscae Dg1, the actinobacterial microsymbiont of *Datisca glomerata*, expresses the canonical *nod* genes *nodABC* in symbiosis with its host plant. PLoS One. 10:e0127630. https://doi.org/10.1371/journal.pone.0127630.

Pesce C, Kleiner V, Friedman L, et al. 2021. Genetic tools in *Frankia*: Yes we can, from complementation to site-specific mutagenesis. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Pesce C, Oshone R, Hurst SG, et al. 2019. Stable transformation of the actinobacteria

Frankia spp. Appl Environ Microbiol. 85:e00957-19. https://doi.org/10.1128/AEM.00957-19.

Pozzi ACM, Herrera-Belaroussi A, Schwob G, et al. 2020. Proposal of "*Candidatus* Frankia alpina", the uncultured symbiont of *Alnus alnobetula* and *A. incana* that forms spore-containing nitrogen-fixing root nodules. Int J Syst Evol Microbiol. 70:5453–5459. https://doi.org/10.1099/ijsem.0.004433.

Rajendran K. 2021. Exopolysaccharide substances (EPS) production by *Frankia* and microbial interaction for the sustainable nutrient management of *Casuarina equisetifolia* in farm forestry. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Rehan M, Hesis A Al, Farid M, et al. 2021. Cadmium resistance mechanism in *Frankia alni* ACN14a. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Ribeiro-Barros AI, Ramalho JC, Pawlowski K. 2021. Salt stress tolerance in *Casuarina*: an overview under the context of saline agriculture. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Sarkar I, Chettri S, Sen G, Sen A. 2021. Reverse Ecology analysis of *Frankia* and their co-inhabiting microbial populations indicate a strong complementary association among them. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Sarkar I, Sen A. 2021. Comprehensive metabolomic profiling of *Frankia* reveals differential amino acid usage based on their biosynthetic energy cost. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Sen G, Sarkar I, Chetrri S, et al. 2021. Rhizosperic metagenomic analysis of *Alnus nepalensis* reveals the abundance of nitrogen fixing symbiotic microbes. Oral session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Tateno M. 2003. Benefit to N_2 -fixing alder of extending growth period at the cost of leaf nitrogen loss without resorption. Oecologia. 137:338–343. https://doi.org/10.1007/s00442-003-1357-6.

Tobita H, Shibata M, Nakashizuka T, et al. 2021. Evaluation of N_2 fixation in *Alnus* species 35 years after a massive landslide on Mt. Ontake in central Japan. Poster session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).

Torrey JG, Tjepkema JD. 1979. Symbiotic nitrogen fixation in actinomycete-nodulated plants. Preface. Bot Gaz. 140:i–ii. https://doi.org/10.1086/337026.

Worth M, Swanson E, Tisa L. 2021. Co-inoculation of *A. glutinosa* with *Frankia* strains QA3 and *Frankia inefficax* EuI1c under copper stress. Poster session presented at 20th International Meeting on *Frankia* and Actinorhizal Plants; May 29-31 (online).