Qeios

Peer Review

Review of: "Isolation of a Novel Plant Growth-Promoting Dyella sp. from Danish Natural Soil"

A. Keith Cowan[1]

1. Rhodes University, South Africa

This is a long-winded and convoluted account of the isolation of a bacterium (from precisely where is not clear) that may, based on phylogenetic relations and an apparent impact on Arabidopsis and tomato tissue fresh mass, be considered a novel plant growth-promoting microbe. The outcome of measures of hormone production, as presented in the S1 file for auxin (not shown is nutrient mineralisation efficiency for N, P, K) and upon integration into the manuscript, may have provided more substantial support than a mere mention. Nevertheless, a manuscript with the same title, “Isolation of a novel plant growth-promoting Dyella sp. from a Danish natural soil” but with additional authors (Laura Dethier, J. Rasmus P. Jespersen, Jemma Lloyd, Elena Pupi, Wanru Zhou, Fang Liu, Yang Bai, Barbara Ann Halkier, Deyang Xu) and doi:10.1101/2025.01.24.634660 can be found at BioRxiv and this raises the question: why BioRxiv and Qeios? Why not Qeios only? And why are only three of the listed authors on the frontice of this submission to Qeios? Is this the same article? And for the Supplementary Information file (S1), the authors listed are Laura Dethier, J. Rasmus P. Jespersen, Jemma Lloyd, Elena Pupi, Wanru Zhou, Fang Liu, Yang Bai, Barbara Ann Halkier, Deyang Xu. Is this correct?

Abstract & Introduction: language is contrived and contains redundant expressions/terms that need to be tempered or omitted such as ‘environmental soils’. Why not simply, ‘soils’? And ‘natural soil derived from an alder” – why not ‘soil obtained from an alder swamp’? In the Abstract, what is meant by “promoted Arabidopsis seedling weight”? Mass surely? And then it should be dry (dry matter accumulation)? This is particularly true if as it is stated “we identified a new species from the Dyella genus which increased biomass of tomato and Arabidopsis seedlings in agar as well as the shoot biomass of Arabidopsis grown in both the alder swamp soil and potting soil.” And since the candidate PGP bacterium “promoted the elongation of lateral roots without affecting lateral root number and primary root elongation” it can be

 doi.org/10.32388/35TBAW

assumed that nutrient load must play a role since Arabidopsis plants purposefully suppress root growth when nutrients are in excess (New Phytologist (2022) 235: 1426–1441 doi:10.1111/nph.18255).

In the Introduction, the reason behind this study is not well explained. It is moot whether prior "research has focused on isolating microbes from nutrient-poor and/or arid soils". It is more likely that a range of habitats, both rich and poor in resources, are targeted as potential havens for PGP bacteria. And species related to the "rhizobacterium Bacillus megaterium" classified as the genus Prestia in 2020, based on comprehensive phylogenomic and comparative genomic analyses that revealed unique conserved signature indels (Int J Syst Evol Microbiol, 2020. 70(1):406-438; 2020. 70(11):5753-5798; 2020. 70 (12):6531-6533).

While the text in the second paragraph of the Introduction may provide some background, it is insufficient for hypothesis formulation. That is, the underpinning information is scant and somewhat superficial. Consequently, there is no precise aim, and the objectives are either absent or poorly defined. For example, why was Arabidopsis grown in an alder swamp soil? Why were PGP bacteria not isolated directly from the swamp soil? I think the reason is lacking.

In the Materials & Methods: Why was alder swamp soil targeted? Why were other soil types not explored? Why was the soil used not classified, or at best, why were proximate and ultimate (including microbiology) analyses not carried out? And, if the collected soil was placed in "hermetically sealed (plastic bags) before sterilisation by gamma irradiation (18 kGy on each side of the soil bag; Sterigenics, Espergaerde, Denmark) to eliminate microbes", from where did the novel 75 isolated strains arise? Were these Arabidopsis endophytes or?

In the section ***Plant materials and growth conditions***, it is stated that "Arabidopsis Col-0 seeds were laboratory stocks, while DR5rev::GFP (CS9361) seeds were obtained from the Arabidopsis Biological Resource Center (ABRC)." From what year of production, how stored, and what viability, or GI? The same information for "Tomato seeds (Solanum lycopersicum cv. Moneymaker) obtained from ....". Please provide complete and accurate information.

It is stated in the section ***Culture-independent analysis of the alder swamp soil and root-associated microbiota*** that the authors profiled bacterial communities from unplanted bulk soil and plant roots (endosphere and rhizoplane) of plants grown in alder swamp soil. But has this soil been sterilised using gamma irradiation? So, no microbes are present - correct? If it was the half collected and not sterilised, why was soil sterilised and what was it used for?

It is also stated that “Arabidopsis seeds were sown in alder swamp soil mixed with sand (1:1 v/v) and grown for 5 weeks.” Was this the sterilised or unsterilised soil? Was the sand also of ‘natural’ origin? Was it sterilised? It is then stated that “Roots were washed in phosphate-buffered saline (PBS), placed in Lysing Matrix E tubes (MP Biomedicals), and stored at -80°C with unplanted soil aliquots. The experiment was conducted twice with 7 root and 3 soil replicates.” Roots of what? Arabidopsis? Why store at -80°C with unplanted soil aliquots? And which experiment was “conducted twice with 7 root and 3 soil replicates”. Throughout this manuscript, the text is either ambiguous or confusing, which misleads the reader. In short, the work is premised on a very weak rationale, has no aim or hypothesis, and there are no clearly defined and elaborated experiments whether conducted once, twice or thrice.

The section Isolation of bacteria from the root endosphere states, “Seeds were sown in pots containing alder swamp soil mixed with sand (1:3 v/v) ..... Bacteria were isolated from eight-week-old plants using a modified limiting dilution method ..” Was this the sterilized or unsterilised soil? Was the sand ‘natural’, and if so, from where? Was the sand sterile? It is stated that roots were the source of endophytes (i.e., ***an endosymbiont, often a bacterium or fungus***, that lives within a plant for at least part of its life cycle without causing apparent disease ...) but from where did these arise if the substrate was microbe free due to gamma irradiation? A much better and more explicit description of the work is needed.

A final comment on the Material and Methods section relates to the following statements “Seedlings were grown for an additional 10 days before measuring fresh weight.” and ‘Shoot fresh weight was measured after 24 days, and ....”, and “Arabidopsis seeds were sown the next day and shoot fresh weight was measured after 6 weeks of growth”. Why was it fresh weight (you mean mass, surely) that was determined? Throughout this manuscript fresh weight is regarded as equivalent to biomass (Introduction, Figure 1 caption, and in the Results). Biomass is defined as the organic material derived from living or recently living organisms, like plants and animals, and can be used as a renewable energy source or feedstock for various products. So, and as a consequence, the logical measure should be tissue dry mass which will reflect an accumulation of fixed carbon. Note, there are well-defined methods for the determination of tissue (shoot, root, leaf, etc.) dry mass. Very surprisingly, no method for determination of plant tissue biomass is recounted in the Material and Methods section. This is surprising as a change in mass is the only measured criterion used to describe any of the 75 isolates as plant growth promoting. This is a serious oversight that must be addressed.

To further confuse the reader, in the Results section, data in supplementary files is referred to from the outset and seems to be key support data for the ‘cartoons’ that form part of Figure 1. For example, it is

stated that “The root-associated microbiota of Arabidopsis plants grown in soil collected from an alder swamp in North Zealand, Denmark (Figure 1A) was analyzed and compared with the microbiota from bulk soil.” What is meant by “bulk soil”? This aspect is not clearly elaborated in the Methods section. Indeed, the Materials and Methods are very poorly presented and a complete re-write of this section is needed and according to accepted convention for scientific publications. For example, in the Results the reader is introduced to new methods including “Unconstrained Principal Coordinate Analysis (PCoA) of Bray-Curtis distances”, “Permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis distances” and “.. bacterial alpha-diversity based on the Shannon index ...” none of which is elaborated in the Materials and Methods. Data in S2 and S3 must be integrated correctly into the Results section of the manuscript. And consideration should be given to the other data sets. To squash all results into a single plate is also contrived. Rather show the actual result, its interpretation and lead to the next experiment.

In view of the above, the convoluted, ambiguous, and sometime confounding description of the methods, the lack of experimental design, set-up and execution, and the poor overall quality of presentation, and the improper integration of data into the manuscript, the Results and Discussion must be considered moot and await a considerable revision of this manuscript.

Declarations

Potential competing interests: No potential competing interests to declare.