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The 125th anniversary of the first postulation of the soil origin of endophytic bacteria – a tribute to M.L.V. Galippe

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In both managed and natural ecosystems, a wide range of various non-nodulating bacteria can thrive as endophytes in the plant interior, and some can be beneficial to their hosts (Hallmann and Berg 2007; Reinhold-Hurek and Hurek 2011). Colonization mechanisms, the ecology and functioning of these endophytic bacteria as well as their interactions with plants have been investigated (Hardoim et al. 2008; Compant et al. 2010). Although the source of colonization can also be the spermosphere, anthosphere, caulosphere, and the phyllosphere, most endophytic bacteria are derived from the soil environment (Hallmann and Berg 2007; Compant et al. 2010).

Endophytes were neglected for a long time, the term being lost and then rescued after re-definition of the one originally coined by De Bary (1866) for pathogenic fungi entering inside leaves (and subsequently

extended to include all organisms entering plant tissues). However, already 125 years ago there was a report of the presence of some microsymbionts living inside different plant tissues and a hypothesis about their soil origins. In 1887, M.L.V. Galippe (Fig. 1) was the first scientist to report that various vegetable plants could host microbes within their interior, and he believed that these microbes were derived from the soil environment. This has followed by the works of Jorissen, Marciano, and others, who began to describe the occurrence of healthy plants that were not free of microorganisms in their interior (described in Laurent 1889).

Galippe isolated different microorganisms from the roots and from the center of the stems of various vegetable plants grown in the municipal experimental gardens of Gennevilliers, near Paris, France. Here, the soil was irrigated with sewage water in order to determine if plants grown in soil enriched with microorganisms would be invaded by them. He also isolated microbes from vegetables obtained from a market in order to demonstrate that all plants can potentially host microorganisms (Galippe 1887a). This was done on such plants as carrot (*Daucus carota* L.), onion (*Allium cepa* L.), potato (*Solanum tuberosum* L.), celery (*Apium graveolens* L.), turnip (*Brassica rapa* L.), sugar beet (*Beta vulgaris* L.), lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleacea* L.), salsify (*Tragopogon porrifolius* L.), and radish (*Raphanus sativus* L.).

No microbes were definitively identified upon their morphology in the work of Galippe apart from his

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Fig. 1 M.L.V. Galippe, a pioneer on microbes colonizing plants and their soil origin

descriptions of bacteria as ‘*Micrococcus*’ and “colored cells”, and so the presence of other bacteria (as well as fungi), cannot be excluded. Nevertheless, Galippe was still the first scientist to put forward a hypothesis about the soil being the origin of microbes isolated from plants. Indeed, 125 years later, this ground-breaking hypothesis continues to be used by the community of scientists working on endophytes.

Although Galippe had proposed as early as 1887 that plants hosted microbes and that the microorganisms isolated from the plant tissues were derived from the soil environment, the rhizosphere concept was later more formally proposed, in 1904 by L. Hiltner, who also called the bacteria derived from the rhizosphere and entering endorhizal tissues as ‘bacteriorrhiza’ (Hiltner 1904; Hartmann et al. 2008). Galippe further claimed that the mechanisms of colonization of those microbes from the soil to the plant interior needed to be investigated, and he proposed (but did not demonstrate) that some of them could possibly play a beneficial role in the life of their host plants (Galippe 1887a). It was difficult to establish this concept among well-known scientists such as Pasteur, Chamberland, Fernbach, Laurent, and others at that time. Indeed, they demonstrated contradictory results and claimed that plants are normally free of microbes

(Compant et al. 2010). This claim was believed for a long time, although some other scientists had also demonstrated that microbes could not only live within healthy plants but also that they could live in particular parts of them at the same time, such as seeds (for instance, Marcano, Hiltner), or stems (Marcano, Jorissen; discussed in Laurent 1889).

Although M.L.V. Galippe was highly criticized in the nineteenth century for his study, he undoubtedly still made the first postulation of soil-derived microbes internally colonizing plants, and that all vegetables could host microbes. However, this demonstration and postulation were forgotten for a long time after he first made them.

Although he had been criticized, Galippe again discussed, in another manuscript in 1887, the presence of microorganisms inside plant hosts, as well as the colonization of plants by microbes from the soil. Galippe maintained his hypothesis that these microbes were derived from the soil, even including those isolated from stems (Galippe 1887b). A year later, Fernbach (1888) did the same experiments as Galippe and took vegetables from the market, assuming that if any soil adapted to growing vegetables was sufficiently rich in microorganisms, then they would inevitably enter the vegetables if it was physiologically possible to do so. He duly found bacteria and fungi inside healthy plants, such as tomato (*Solanum lycopersicum* L.), carrot, turnip, and sugar beet, but then Fernbach (probably influenced by Pasteur) claimed that these microbes were contaminants obtained during experimentation and that plants are sterile, and, therefore, that no microbes from the soil could enter them. However, at about the same time, Hellriegel and Wilfarth (1888) published their famous studies on the exceptional mineral N-independence of leguminous plants, as well as the importance of the endophytic bacteria within the root nodules.

It is 125 years since Galippe’s first report/hypothesis of the soil origin of endophytic microorganisms, and his claim that all plants could host microbes in their interior, and it is now well accepted that plants generally host a wide range of phylogenetically distinct endophytic bacteria and fungi in various organs (Bacon and White 2000), and that almost all of these microbes are derived from the soil environment (Ryan et al. 2008; Rosenblueth and Martínez-Romero 2006; Hardoim et al. 2008; Compant et al. 2010). Given that Galippe was the first to make these postulations

concerning endophytes and soil, and especially in consideration that his work was criticized at the time he made them, and that his work was subsequently forgotten, his name is long overdue for rehabilitation, and hence 2012 marks a special 125th anniversary for research on soil-derived endophytes.

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