



Association of L-form Bacteria with Plants and their Application in Biological Control of Phytopathogenic Fungi and Bacteria: A Review

P. W. H. K. P. Daulagala^{1*}

¹Department of Botany, Faculty of Natural Sciences, Kandy Regional Centre, The Open University of Sri Lanka, Polgolla, Sri Lanka.

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMB/2021/v21i1030395

Editor(s):

(1) Prof. Hung-Jen Liu, National Chung Hsing University, China.

Reviewers:

(1) Marco Antonio Obregón Mendoza, México.

(2) Annika Durve Gupta, India.

(3) Efrain Orlando Silva Vega, Technical University Of Manabi, Ecuador.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/75031>

Review Article

Received 04 August 2021

Accepted 08 October 2021

Published 16 October 2021

ABSTRACT

L-form bacteria with modified or no cell walls are a special group of bacteria derived or induced from cell walled forms following suppression of their rigid cell wall. They have been used to establish non-pathogenic symbioses with a wide range of plants. These L-form-plant symbioses have been shown to confer resistance against the subsequent challenge of the associated plants by both fungal and bacterial pathogens. As the world population increases, the demand for food also increases and hence control of plant diseases is of paramount importance in producing enough agricultural crops to fulfil the food demand. Plant disease management using chemical fungicides and pesticides etc. is not an ecofriendly approach and hence researchers look for alternative options such as the use of biocontrol agents which are ecofriendly and sustainable. This review paper highlights the published information on the potential of applying L-form bacteria as a biological control agent in management of plant diseases caused by pathogenic microorganisms.

Keywords: *Biological control; L-form bacteria; L-form-plant symbioses; phytopathogens.*

1. INTRODUCTION

L-form bacteria were originally observed and named by Emmy Klieneberger in a pure culture of *Streptobacillus moniliformis* and the name being given out of appreciation for the Lister Institute in London where the work had been carried out. According to Madoff [1], L-forms are defined as a special type of growth derived or induced from a bacterium following suppression of its rigid cell wall. Numerous terms and definitions have been used for this special type of bacteria, such as L-forms, L-variants, L-organisms, cell wall-deficient (CWD) forms and L-phase organisms [2,3,4], although the term 'L-form' has been adopted more recently by many researchers in this field [5,6].

Although observed *in vivo*, L-forms can be induced from cell walled forms of bacteria by agents such as antibiotics (e.g. penicillin) or lytic enzymes (e.g. lysozyme), that interfere with the synthesis of bacterial cell wall. Penicillin, one of the β -lactam antibiotics, is the most commonly used antibiotic which inhibits the final step of bacterial cell wall synthesis and the enzyme lysozyme hydrolyses some linkages between adjacent polysaccharides of the cell wall. During induction of L-form bacteria, classical parental bacterial forms are generally replaced by spherical or pleiomorphic granular cells which are commonly larger than the parental forms from which they are derived [1]. The induction process depends on identifying the appropriate cell wall inhibitors with respect to their type and the suitable concentration. Concomitant with this, newly induced L-forms are prone to reverse to the cell-walled form (i.e. they are unstable), requiring frequent observation and subculture to maintain the L-form state [5]. In an osmotically stabilized appropriate medium, induced L-forms multiply and grow in the presence of inducing agent(s), either singly or in combination.

When the cell walled forms of bacteria are treated with an inducing agent in a hyperosmolar medium, protoplasts or spheroplasts are formed [1]. In spheroplasts, the cell wall is only partially removed and on the other hand, protoplasts are free of any cell wall structure, that is, cell wall-less. But in the presence of an osmotic stabilizer in a proper medium, both protoplasts and spheroplasts may form L-forms [7]. The removal of the cell wall permanently or temporarily results in stable and unstable L-forms respectively. The most conspicuous biochemical characteristics of

stable L-forms are not only the absence of cell wall, but also the permanent loss of ability to re-synthesize their rigid cell wall structure. Accordingly, L-forms can be differentiated into four groups: unstable and stable spheroplast type L-forms and unstable and stable protoplast type L-forms. Unstable L-forms can revert to normal walled parental form when the inducing agent/s is/are omitted from the medium while both stable spheroplast and protoplast type L-forms are not able to revert to the normal walled form. [8]. L-forms can grow and divide indefinitely, but protoplasts can increase their masses only to a certain extent. However, in most cases, protoplasts are unable to divide and grow on normal laboratory media [9].

As the world population increases, an increase in food production is also needed. Therefore, control of plant diseases is of paramount importance in producing agricultural and horticultural crops. A variety of agrochemicals have been introduced to control the diseases in plants and may be applied to seeds, foliage, flowers and fruits or even to the soil. The use of agrochemicals in agriculture is widespread due to their relatively low cost, the ease in application, effectiveness, availability and stability. However, extensive use of chemicals targeting high yield by growers has been a public concern due to the harmful effects on the environment, their undesirable effects on non-target organisms and possible carcinogenicity of some chemicals [10,11]. Considering the drawbacks and the limitations of these chemical agents, the need arose for the development of some other alternative non-chemical methods to control plant diseases. In contrast to the use of chemicals, 'biological control' or its abbreviated synonym 'biocontrol' of plant diseases using microorganisms against the phytopathogens offers a powerful alternative to the use of synthetic chemicals [12]. Biological control is the control of diseases by the application of biological control agents (BCAs) that prevent the development of diseases by pathogens, resulting in minimal impact of the chemicals on the environment [13]. It is well studied and documented that treatment of plants with various BCAs can lead to the induction of resistance to subsequent pathogens which includes cell wall strengthening [14], de nova production of antimicrobial compounds, Pathogenesis Related proteins (PR proteins) and secondary metabolites [15,16,17] and rapid and localized cell death [18].

With regard to plant diseases, the BCAs are usually bacteria or fungi e.g., virulent or avirulent pathogens, non-pathogens and cell wall fragments etc. Biological control may operate via different modes of action including parasitism, antagonism, antibiosis, neutralism, competition and induced resistance of host plants [19]. Out of these modes of action, phytopathologists have promptly begun to characterize especially the pathways of induced resistance in host plants which may result in the protection of plants against the attack by a wide range of pathogens. This effect can be either localized or systemic, spreading far from the attacked organ or inducing defensive responses in the entire plant [20, 21]. Induced resistance can be triggered in plants due to an infection by pathogens or upon root colonization by certain rhizosphere mutualistic microbes. Accordingly, induced resistance can be divided broadly into two categories; systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR develops locally or systemically in response to an infection by a pathogen or treatment with certain chemicals (e.g., 2,6-dichloroisonicotinic acid [INA]). This process is effective against a wide range of pathogens and is mediated by a salicylic acid [SA]-dependent pathway [22]. In contrast, ISR develops as a result of colonization of plant roots by plant-growth-promoting rhizobacteria (PGPR) and is mediated by a jasmonate/ jasmonic acid or ethylene sensitive pathway [23]. SAR is characterized by the activation and expression of a large set of PR genes and by contrast, ISR typically functions without PR gene activation [23,24,25].

2. INDUCTION AND CULTIVATION OF BACTERIAL L-FORMS

L-form bacteria have been induced from many different types of cell walled bacteria. Scientists have induced and obtained L-forms from both Gram positive and Gram negative bacteria including *Agrobacterium*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Streptococcus* and *Streptobacillus* [1]. In addition, L-forms have been successfully obtained from some Gram negative filamentous bacteria such as *Streptomyces hygrosopicus* [26] and *Streptomyces viridifaciens* [27] (Fig. 1).

It is accepted that the induction and subsequent growth and cultivation of L-forms is difficult. L-forms are induced by exposing the cell walled bacteria to an inducing agent in a suitable

medium. The conditions for induction and cultivation are different and need to be adapted to each species and strain. Indeed, although induction is often straightforward, on-going cultivation is frequently challenging. According to Madoff [1], the first step in L-form formation is the enlargement of the bacterial cell. The cells become large, spherical and fragile. This is due to the suppression of cell wall synthesis. In some instances, large bodies fragment releasing typical bacterial forms and alternatively, small L-form colonies develop within or at the periphery of large bodies. On agar medium, they multiply and penetrate the substrate and form colonies resembling mycoplasma often with a typical 'fried-egg' appearance. The center of the colony is dense and embedded in agar. The less dense, foam-like flat periphery is composed of aggregates of large bodies. Compared to mycoplasma, L-form colonies are coarser and larger.

2.1 Association of L-Form Bacteria with Plants

The ability of L-form bacteria to associate with intact plant tissues and cells is considered to be the most significant factor in applying L-form bacteria in biological control measures. There are several ways that L-form bacteria can be introduced into plants. Many L-form-plant associations have been formed with unstable L-forms and it has been reported that the bacteria do not revert to the cell walled form within the plant [28, 29].

The initial work on cultivation of L-form bacteria and association with plants was carried out by Professor Alan M. Paton, University of Aberdeen, Scotland [30,31]. Later, many researchers were interested on L-form bacteria and the work was further extended using different L-form bacteria and a variety of plants. The associations were basically made by injecting unstable and stable L-form suspensions into different fresh plant parts such as stems/ stolons [32], leaf petioles [33] etc., or by imbibing the germinating seeds in L-form suspensions or by inoculating the germinating seeds using L-form suspensions [34, 35].

As pioneering techniques, Jones and Paton [30] inoculated discs of sterile potato tuber tissue and sterile pieces of cucumber tissue with freshly induced L-forms of *Erwinia carotovora* var. *atroseptica*. L-forms were associated with potato tissues by embedding technique, in which, the

plant tissues were placed in a Petri dish and the surface was saturated with a cloudy suspension of L-form bacteria in molten medium. The same agar was poured over the plant tissues and the plates were incubated. After incubation, tissues were removed from agar and immersed in a pectolyzing solution to separate cells and they were examined to observe the intracellular presence of L-forms.

Many investigations demonstrated that a range of L-form bacteria derived from different cell walled forms could invade various living plant tissues and spread from there to other parts of the plant forming novel viable associations. Attempts were made by researchers to bring either stable or unstable L-form bacteria into intimate contact with plant cells or tissues. In 1984, Aloysius and Paton [31] applied the embedding technique with *Pseudomonas syringae* L-forms using actively growing suspension cultures of soybean cotyledons, derived from callus incubated in a rotary shaker. Further, they carried out some experiments on inoculation of L-form bacteria using sterile potato tubers and stem and root tissues of different plants. A cloudy suspension of L-form bacteria of *Beijerinckia indica* was injected with a fine hypodermic needle to the stems of bean (*Phaseolus*) seedlings growing in pots under greenhouse conditions. Rootlets of sterile germinated seeds of clover (*Trifolium pratense*) and radish (*Raphanus sativus*) growing on an

agar surface were also treated with drops of cloudy suspension of *B. indica* L-forms. An interesting aspect was that whether the used L-forms were in either stable or unstable condition at the time of association, once associated they were maintained in plant tissues without reversion to their respective walled forms. After 48h of incubation at 25 °C, the rootlets were examined by interference microscopy and found that L-form like bodies were present in root hairs and associated epidermal cells and not in control seedlings.

Then with time, research work on L-form bacteria was extended and researchers were able to associate L-form bacteria to form symbioses with a wide range of plants including French dwarf bean [29,36], Chinese cabbage (*Brassica campestris* subsp. *pekinensis*) [37,34,35], strawberry [32] and poplar [33] using different methods. In all these associations, either stable or unstable L-form suspensions were prepared from newly grown exponentially growing liquid cultures and they were either used directly or resuspended in mannitol solution (to provide osmotic protection) before being introduced to plants. Those above mentioned associations were mainly done on fresh tissues by imbibing the germinating seeds (radicle emerged) in L-form suspensions [36,38,34,39,35] or by injecting L-form suspensions to different plant parts by hypodermic inoculations [32,33].

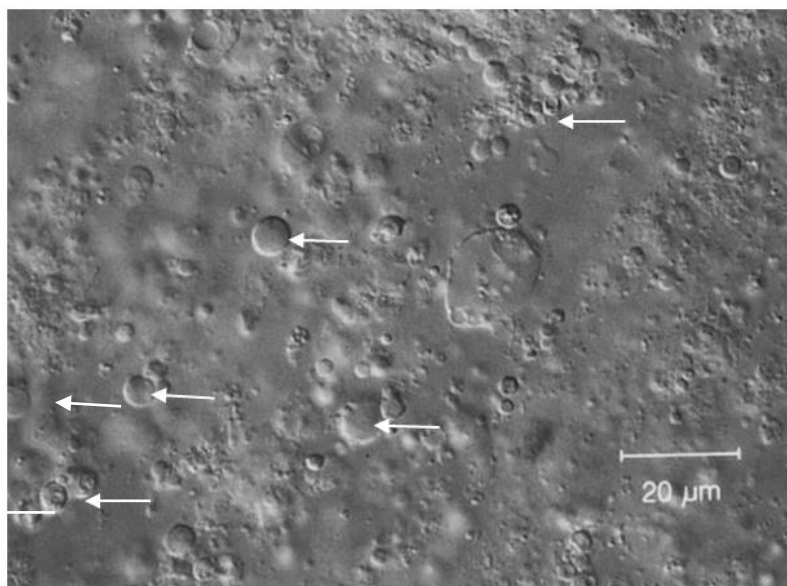


Fig. 1. L-form bacteria of *Streptomyces viridifaciens* NCIMB 8954 under Reichert-Jung Polyvar microscope (indicated by arrows)

An exciting and potential commercial application of L-form bacteria is that of using them for associations to protect plants from phytopathogens causing a variety of diseases in plants. This has been achieved in two different ways although more research is required to establish the reliability and safety of the bacterial associations and the methods for large scale treatments. In some associations, L-forms derived from a pathogenic bacterium have been used to protect plants from diseases caused by walled forms of the same or another plant pathogen (including both bacteria and fungi). This type of associations were done by researchers using L-forms derived from the bacterial pathogen *Ps. syringae* pv. *phaseolicola* with different plants, such as French dwarf bean (*Phaseolus vulgaris*) against halo-blight caused by the pathogenic *Ps. syringae* pv. *phaseolicola* [36] and Chinese cabbage against both *Xanthomonas campestris* [37] and grey mould pathogen *Botrytis cinerea* [35]. Further, Walker *et al.* [34] were able to associate L-forms with seeds, for an example, L-forms of *Bacillus subtilis* with radicle emerged Chinese cabbage seeds and tested germination of conidia of *B. cinerea* on cotyledonous leaves obtained from L-form treated seeds.

2.2 Detection of L-Forms in Associated Plant Tissues

The detection of the L-form bacteria in plant tissues continues to be a difficult and challenging aspect of research especially when unstable L-forms are being used. In 1973, Jones and Paton [30], who pioneered the work on L-form-plant associations, examined the discs of sterile potato tuber and cucumber tissues inoculated with L-forms of *E. carotovora* var. *atroseptica* using different techniques; by preparing wet mounts and using phase contrast microscopy, by staining with optical brighteners and by the immunofluorescence technique. Later on, Paton and Innes [29] were also able to locate L-forms in cytoplasm of plant cells by immunofluorescent techniques and light microscopy.

Over the years, with the progression of research with L-form bacteria, researchers were trying to investigate more accurate and developed methods for detection of L-forms in associated plant tissues. Molecular aids such as reporter genes with easily detectable products have been used to study various biological processes. Indeed, work using *lux* reporter genes has been

carried out for research on the L-form-plant symbioses by Waterhouse *et al.* [38] in 1996. In this study, chromosomally *luxAB*-marked L-forms of *Ps. syringae* pv. *phaseolicola* were associated with sterile-germinated Chinese cabbage seeds and their presence in treated seedlings were confirmed by Polymerase Chain Reaction (PCR) of *luxA* gene and by a positive agglutination reaction between plant sap and a conjugated *Staphylococcus* antiserum, which was specific for *Ps. syringae* pv. *phaseolicola*. Strawberry plants which received injections of stable L-form bacteria of *B. subtilis* to stolons and petioles were successfully detected using L-form selective, but not specific Enzyme Linked Immunosorbent Assay (ELISA) [32]. These L-forms injected into mature strawberry plants were maintained for up to 7 days and found that the L-forms had travelled from the site of injection along the entire length of the stolon and, in some plants into the daughter plantlets as well. In another study, histochemical localization of *B. subtilis* P6 *gus* transformant L-form bacteria in treated Chinese cabbage seedlings was determined by a gene system which was successfully applied to detect the distribution of stable L-forms in different parts of the seedlings [39]. The *B. subtilis* P6 *gus* transformant L-form bacteria generated characteristic blue colonies when grown on plates containing L-phase medium and the substrate 5-bromo-4-chloro-3-indolyl-b-D-glucuronide (X-gluc) for β -glucuronidase enzyme. The same blue colour was observed in secondary roots, stems and cotyledons of 1-3 day old L-form treated seedlings with no blue colour development in any part of the control seedlings treated with 5% (w/v) mannitol solution.

As rapid means to indicate the L-form association with plants, ELISA and agglutination techniques were successfully employed to detect L-forms of unstable *Ps. syringae* pv. *phaseolicola* associated with Chinese cabbage seedlings [35]. Agglutination test showed the presence of *Ps. syringae* pv. *phaseolicola* antigens in Chinese cabbage seedlings treated with L-forms of *Ps. syringae* pv. *phaseolicola* and no agglutination was detected in any control seedling treated with 5% (w/v) mannitol solution. These results were strongly supported by ELISA. Interestingly, the ELISA results confirmed the agglutination test results with the highest ELISA absorbance being found in the same seedlings that gave the most intense agglutination. Roots showed the highest absorbance indicating the presence of more

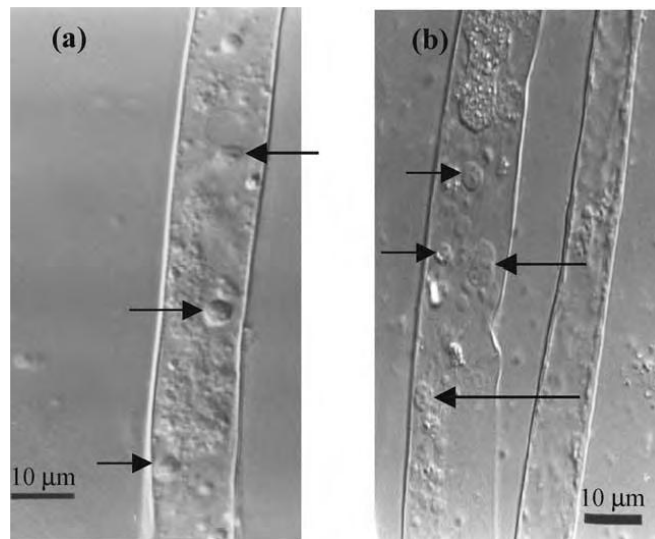


Fig. 2. Typical pleomorphic L-form-like cells of *Ps. syringae* pv. *phaseolicola* with granules, as indicated by arrows, in root hairs of Chinese cabbage seedlings (5 d old) under phase contrast (a) and interference (b) microscopy [35]

Pseudomonas antigens in root extracts. The leaves and cotyledons of the L-form treated plants also contained the bacterial antigens but to a significantly lower extent than the roots. Re-isolation experiments indicated a systemic distribution of L-form bacteria in seedlings with the highest population in roots. Further, microscopic observations clearly demonstrated the presence of intracellular L-form-like cells in the root hairs of 3 and 5 day old L-form associated seedlings (Fig. 2).

3. L-FORMS AS A POTENTIAL BIOCONTROL AGENT AGAINST PHYTOPATHOGENIC FUNGI AND BACTERIA

Plant pathogens, pests and weeds are the key factors that cause major losses and damages to agricultural crops. Plants are attacked by these different groups of pathogens individually or sometimes by more than one pathogen, causing more severe disease development. Among the plant pathogens, fungi are the most destructive group of pathogens that causes enormous losses in yield and quality of field crops, fruits and other edible plant materials. Some important examples for phytopathogenic fungi include *Rhizoctonia solani*, *Pythium* spp., *Alternaria solani*, *Fusarium oxysporum* and *Botrytis cinerea*. The recognition that bacteria cause diseases in plants is relatively slow. Important examples for pathogenic bacteria include pathovars of *Ps. syringae*, *Ralstonia*

solanacearum, *Agrobacterium tumefaciens*, *Xanthomonas oryzae* pv. *oryzae*, pathovars of *Xanthomonas campestris* and *Xanthomonas axonopodis* and *Erwinia amylovora* [40].

Protection in plants against phytopathogens has been previously reported with L-form bacteria [36, 41, 38, 32, 34, 35, 33] in particular with *Ps. syringae* pv. *phaseolicola* [36, 38, 35] and the crucifer specific pathogen *Xanthomonas campestris* pv. *campestris* [41, 38]. Initially late Professor Alan Paton and coworkers, University of Aberdeen [30, 31] used L-form bacteria in plant associations and since then several investigations on L-form-plant associations were undertaken by many L-form experts revealing the potential of these novel symbioses in biological control of plant diseases caused by both fungi and bacteria. Unstable L-form bacteria of *Ps. syringae* pv. *phaseolicola* have been successfully used in biological control of diseases in bean and Chinese cabbage plants [36, 38]. An interesting observation found by Amijee *et al.* [36] was that, unstable L-forms of pathogenic *Ps. syringae* pv. *phaseolicola* did not cause any disease when associated with French dwarf bean (*Phaseolus vulgaris*), like their walled form. Most interestingly, it was found that plants associated with this L-forms of *Ps. syringae* pv. *phaseolicola* and subsequently challenged by its own pathogenic walled form which causes halo blight in bean, were protected with lower disease incidence compared to the non-associated control plants. That means although the cell

walled form of a pathogenic bacterium may cause disease symptoms in a particular host plant, the same plant seems to be able to tolerate L-forms of that particular walled form with producing lower disease symptoms. Afterward, it was found that L-forms derived from lux- marked *Ps. syringae* pv. *phaseolicola* could be associated with Chinese cabbage providing protection against a heterologous pathogen, *Xanthomonas campestris* [38]. They found that leaves of control plants treated with 5 % (w/v) mannitol and heat killed walled form of lux-marked *Ps. syringae* pv. *phaseolicola* were killed and wilted showing advancement of disease symptoms. But the test plants treated with viable L-forms of lux-marked *Ps. syringae* pv. *phaseolicola*, prior to the challenge by *X. campestris* remained healthy and no sign of disease development, after 6 days of treatment. This was also an evidence to demonstrate that L-forms could induce resistance in host plants and involve in plant defence system. Another research carried out by Walker *et al.* [34], found that radicle emerged Chinese cabbage seeds associated with L-forms of *B. subtilis* showed a significant reduction of conidial germination of *B. cinerea* compared to the control plants treated with 5 % mannitol solution. It was stated that this biocontrol mechanism was unclear, but L-forms of *B. subtilis* produced antibiotics in pure cultures and suggested that they could be active against the conidia and mycelia of *B. cinerea*, *in vitro* [42].

Chitinases (EC 3.2.1.14), a major group of PR proteins have been reported to play a major role in defence responses of plants against pathogens, especially fungi whose cell walls are made up of chitin. Chitinases are present constitutively in many plant species and these are mostly endochitinases that are known to inhibit fungal growth [43]. Chitinases, in combination with β -1.3 glucanases lyse mycelial tips of fungi or are involved in releasing elicitors that can activate plant defense mechanisms [44].

Plant protection has been previously reported with L-form bacteria by many researchers against both fungal and bacterial pathogens, but the mechanisms of protection were not clearly studied. Although systemic acquired resistance was inferred [36], an enhanced resistance in L-form associated Chinese cabbage seedlings against the Grey mould pathogen *B. cinerea* due to the induction of chitinases in L-form

associated plants was observed [35]. A significant induction of chitinolytic enzymes was detected in Chinese cabbage seedlings treated with L-forms of *Ps. syringae* pv. *phaseolicola* at 31 days after treatment, compared to the mannitol treated control plants. The whole plant pathogenicity bioassay showed that the symbiotic L-form association had provided protection to the plants against the Grey mould pathogen *B. cinerea* in a manner similar to systemic acquired resistance. This work showed that L-form bacteria had induced PR proteins in treated plants and the plants were protected through the induction of systemic resistance.

Interestingly, as it has been reported by researchers working on L-form bacteria, they can be easily associated without having any detrimental effects on plants and it is possible to utilize induced or genetically engineered L-form bacteria as a successful biocontrol agent against a range of phytopathogenic fungi and bacteria.

4. CONCLUSION

Harmful pests and pathogens are the most serious biotic agents causing drastic losses and damages to the agricultural crops. A number of strategies are being employed by the growers to increase the yield by minimizing the losses and they often rely on the use of different agrochemicals. Due to the serious health and environmental problems of these agrochemicals, a need arose for the development of some other alternative non-chemical methods to control plant diseases, especially eco-friendly biological control systems. This review shows that L-form bacteria can be used as an alternative biocontrol strategy against phytopathogenic bacteria and fungi. Further work is needed to investigate the nature of plant - L-form symbioses in detail and the mechanisms and long life of protection against plant pathogens.

ACKNOWLEDGEMENTS

The author is grateful to Dr. E.J. Allan Atkins for the valuable comments on the early version of this manuscript.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Madoff S. Introduction to bacterial L-forms. In: S. Madoff (Ed) Bacterial L-forms, Marcel Dekker Inc., New York. 1986; 1-20.
2. Gumpert J. Taubeneck U. Characteristic properties and biological significance of stable protoplast type L forms. In: I. Potrykus, C.T. Harms, A. Hinnen, R. Hutter, King PJ, Shillito (Eds) RD, *Experienta Supplement* 46, 6th International Protoplast Symposium, Birkhäuser Verlag, Basel. 1983; 227-241.
3. Madoff S. Lawson JW. The L-forms of bacteria. In: *Balows, A. (ed): The prokaryotes*. Springer, New York 1992; 4068.
4. Mattman LH. Cell wall deficient forms. *Stealth Pathogens*. (3rd ed.) Boca Raton, FL, USA, CRC Press Inc; 2001.
5. Allan EJ. Hoischen C. Gumpert J. Bacterial L-forms. *Advances in Applied Microbiology*. 2009; 68:1-39.
6. Studer P. Borisova M. Schneider A. Ayala JA. Mayer C. Schuppler M. Schuppler M. Loessner M. Briens Y. The absence of a mature cell wall sacculus in stable *Listeria monocytogenes* L-form cells is independent of peptidoglycan synthesis. *PLoS ONE*. 2016;11(5): e0154925.
7. McGee Z. Witter R. Gooder H. Charache P. Wall-Defective Microbial Variants: Terminology and Experimental Design. *The Journal of Infectious Diseases*. 1971;123(4):433-438.
8. Maxted WR. Specific procedures and requirements for the isolation, growth and maintenance of the L-phase of some microbial groups. In: J.R. Norris and G.W. Ribbons (Eds.), *Methods in Microbiology* vol.7A, Academic Press, London & New York. 1972; 423-449.
9. Weibull C. Structure of bacterial L-forms and their parental bacteria. *Journal of Bacteriology*. 1965; 90: 1467-1480.
10. Agrios GN. *Plant Pathology*. (3rd ed.), Academic Press, Inc., New York; 1988.
11. Heydari A. Misaghi IJ. Balestra GM. Pre-emergence herbicides influence the efficacy of fungicides in controlling cotton seedling damping-off in the field. *International Journal of Agricultural Research*. 2007;2: 1049-1053.
12. Heydari A. Pessarakli M. A Review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biological Sciences*. 2010;10: 273-290.
13. Vinale F. Ghisalberti EL. Sivasithamparam K. Marra R. Ritieni A. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. *Letters in Applied Microbiology*. 2009;48:705-711.
14. Bradley CA. Black WE. Kearns R. Wood P. Role of production technology in mycoinsecticide development. In "Frontiers in Industrial Mycology" G. F. Leatham, Ed Chapman & Hall, New York. 1992; 160-173.
15. Hammerschmidt R. Phytoalexins: What have we learned after 60 years? *Annual Review of Phytopathology*. 1999; 37: 285-306.
16. Misra AK. Gupta VK. *Trichoderma: Biology, biodiversity and biotechnology*. *Journal of Eco-Friendly Agriculture*. 2009; 4: 99-117.
17. Gupta VK. Misra AK. Gaur RK. Jain PK. Gaur D. Sharma S. Current Status of Fusarium Wilt Disease of Guava (*Psidium guajava* L.) in India. *Biotechnology*. 2010;9:176-195.
18. Alvarez ME. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. *Plant Molecular Biology*. 2000;44:429-442.
19. Chisholm ST. Coaker G. Day B. Staskawicz BJ. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell*. 2006; 124: 803-814.
20. Pieterse CMJ. Zamioudis C. Berendsen RL. Weller DM. Van Wees SCM. Bakker P. A. H. M. Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*. 2014;52: 347-375.
21. Verbon EH. Trapet PL. Stringlis IA. Kruijs S. Bakker PAHM. Pieterse CMJ. Iron and immunity. *Annual Review of Phytopathology*. 2017; 55: 355-375.
22. Walters DR. Newton AC. Lyon GD. Induced resistance: Helping plants to help themselves. *Biologist*. 2005; 52: 28-33.
23. Pieterse CMJ. Van Wees SCM. Van Pelt JA. Knoester M. Laan R. Gerrits H.

- Weisbeck PJ. Van Loon LC. A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell*. 1998; 10:1571-1580.
24. Van Loon LC. Bakker PAHM. Pieterse CMJ. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology*. 1998; 36: 453–483.
 25. Choudhary DK. Prakash A. Johri BN. Induced systemic resistance (ISR) in plants: mechanism of action. *Indian Journal of Microbiology*. 2007; 47: 289–297.
 26. Hoischen C. Gura K. Luge C. Gumpert J. Lipid and Fatty Acid Composition of Cytoplasmic Membranes from *Streptomyces Hygroscopicus* and Its Stable Protoplast-Type L Form. *Journal of Bacteriology*. 1997; 179 (11): 3430-3436.
 27. Innes CMJ. Allan EJ. Induction, growth and antibiotic production of *Streptomyces viridifaciens* L-form bacteria. *Journal of Applied Microbiology*. 2001; 90: 301–308.
 28. Paton AM. L-forms: evolution or revolution? *Journal of Applied Bacteriology*. 1987;63:365 – 371.
 29. Paton AM. Innes CMJ. Methods for the establishment of intracellular associations of L-forms with higher plants. *Journal of Applied Bacteriology*. 1991; 71: 59-64.
 30. Jones SM. Paton AM. The L-phase of *Erwinia carotovora* var. *atroseptica* and its possible association with plant tissue. *Journal of Applied Bacteriology*. 1973;36: 729-737.
 31. Aloysius SKD. Paton AM. Artificially induced symbiotic associations of L-form bacteria and plants. *Journal of Applied Bacteriology*. 1984; 56: 465–477.
 32. Ferguson CMJ. Booth NA. Allan EJ. An ELISA for the detection of *Bacillus subtilis* L-form bacteria confirm their symbiosis in strawberry. *Letters in Applied Microbiology*. 2000; 31: 390-394.
 33. Daulagala PWHKP. Induction of Resistance in Poplar to *Melampsora larici-populina* using L-form Bacteria. *Asian Journal of Biology*. 2018; 6 (3): 1-9.
 34. Walker R. Ferguson CMJ. Booth NA. Allan EJ. The symbiosis of *Bacillus subtilis* L-forms with Chinese cabbage seedlings inhibit conidial germination of *Botrytis cinerea*. *Letters in Applied Microbiology*. 2002;34:42–45.
 35. Daulagala PWHKP. Allan EJ. L-form bacteria of *Pseudomonas syringae* pv. *phaseolicola* induce chitinases and enhance resistance to *Botrytis cinerea* infection in Chinese cabbage. *Physiological and Molecular Plant Pathology*. 2003;62:253-263.
 36. Amijee F. Allan EJ. Waterhouse RN. Glover LA. Paton AM. Nonpathogenic association of L-form bacteria (*Pseudomonas syringae* pv. *phaseolicola*) with bean plants (*Phaseolus vulgaris* L.) and its potential for biocontrol of halo blight disease. *Biocontrol Science and Technology*. 1992; 2:203-214.
 37. Waterhouse RN. Strang JA. Amijee F. Tyson RH. Allan EJ. Glover LA. Molecular detection of *Pseudomonas syringae* pv. *phaseolicola* L-forms associated with Chinese cabbage. *Microbial Releases*. 1994; 2: 273-279.
 38. Waterhouse RN. Buhariwalla H. Bourn D. Rattray EJ. Glover LA. CCD detection of *lux*-marked *Pseudomonas syringae* pv. *phaseolicola* L-forms associated with Chinese cabbage and the resulting disease protection against *Xanthomonas campestris*. *Letters in Applied Microbiology*. 1996;22: 262–266.
 39. Tsomlexoglou E. Daulagala PWHKP. Gooday GW. Glover LA. Seddon B. Allan EJ. Molecular detection and beta-glucuronidase expression of *gus*-marked *Bacillus subtilis* L-form bacteria in developing Chinese cabbage seedlings. *Journal of Applied Microbiology*. 2003; 95(2):218-224.
 40. Mansfield J. Genin S. Magori S. Citovsky V. Sriariyanum M. Ronald P. Dow M. Verdier V. Beer SV. Machado MA. Toth I. Salmond G. Foster GD. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*. 2012; 13: 614– 629.
 41. Buhariwalla HK. Bacterial L-form associations with plants. Ph.D.Thesis. University of Aberdeen. 1993.
 42. Allan EJ. Amijee F. Tyson RH. Strang JA. Innes CMJ. Paton AM. Growth and physiological characteristics of *Bacillus subtilis* L-forms. *Journal of Applied Bacteriology*. 1993;74: 588–594.

43. Roberts WK. Selitrennikoff CP. Plant and bacterial chitinases differ in antifungal activity. *Journal of General Microbiology*. 1988; 134:169 – 179.
44. Ryan CA. Oligosaccharides as recognition signals for the expression of defensive genes in the plants. *Biochemistry*. 1988;27:8879- 8883.

© 2021 Daulagala; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<https://www.sdiarticle4.com/review-history/75031>