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Investigation of the Existence of Gram Negative Endophytic Bacteria Potentially Pathogenic For Man in Vegetables from Organic Cultures

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Abstract

Endophytic bacteria live in vegetable tissues colonizing them actively and exerting beneficial functions for the host. The objective of this study was to investigate the presence of Gram negative endophytic bacteria in vegetables from organic cultures (lettuce, chard, spinach, basil, cauliflower, coriander) purchased at food markets. After disinfecting leaves with a healthy aspect, fragments of approximately 1 cm^2 were placed on LB agar. After the development of bacteria around the fragments, they were isolated in McConkey agar. Several lactose fermenting and non-fermenting colonies were chosen and identified by biochemical assays. Of 46 identified colonies, 27 (58.6%) corresponded to enterobacteria, 14 (30.4%) to the Acinetobacter genus and 5 (10.9%) to the Pseudomonas genus. A relevant number of endophytic pathogen bacteria, including Salmonella, Yersinia and Shigela, among others, was verified. The risk of using animal manure as compost for organic cultures is well known. Generally, the hygiene of vegetables and fruits is given importance in relation with contaminant epiphytic microbiota, but the endophytic microbial population pathogenic for humans is rarely considered, fact which can explain outbreaks where it is not possible to detect the origin of the contamination.

Keywords: *Endophytic Gram negative bacteria; Pathogenic bacteria; Organic cultures*

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Introduction

The importance of the existence of microorganisms is indisputable, without them, higher forms of life could not have arisen, even if maintained. They have fundamental roles in the planet we inhabit, because there are important interactions between microorganisms and animals and microorganisms and plants, and with man, both in the recycling of key nutrients and in the degradation of organic matter, which may be very beneficial or extremely harmful to their hosts [1].

A portion of microorganisms, mainly bacteria and fungi, inhabit the interior of plants. They are the endophytes, colonizing the healthy tissues of the aerial parts of the plant, at some stage of its life cycle, without causing any apparent damage. This concept is also extended to root microorganisms. Endophytes differ from epiphytes that live on the surface of plants, and phytopathogens, which cause disease. Among the endophytic microorganisms, the fungi and bacteria that form the nodules in the roots of the plants to which they are associated, are well studied because of their importance in agriculture, particularly for their participation in the fixation of atmospheric nitrogen (diazotic mycorrhizal rhizosphere). On the contrary, the endophytic of the aerial parts of the plants have only recently aroused the interest of the scientific community, especially for its potentials in the production of biotechnological interest metabolites [2-5].

Although the existence of the endophytic microbiota is well understood, much research has yet to be done on the ecological, genetic and physiological aspects of this interaction. In addition, it is interesting to know the diversity of these organisms, their presence, frequency and functions. There are a number of reasons to deepen the studies of endophytes such as the lack of information to elucidate the biological basis of these interactions and the advantages to the plants already attributed to their presence [6].

Although endophytic-plant interactions are still not well understood, they are known to be symbiotic, neutral, or antagonistic. In symbiotic interactions, microorganisms produce or induce the production of primary and secondary metabolites that may confer several plant advantages such as herbivore and insect attack decrease, abiotic stress tolerance increase, and control of other microorganisms. Examples of metabolites that can be induced by endophytes are phytoalexins, low molecular weight substances with antimicrobial activities produced by plants against the action of microorganisms or stressing agents, mycotoxins and other secondary metabolites produced by fungi that can cause diseases in humans and other animals, and/or, present antimicrobial, antioxidant and antihypertensive properties. New antibiotics, antimycotics such as cryptocardine, immunosuppressants and antineoplastics such as taxol, powerful antitumor, are some examples of secondary metabolites produced by endophytic microorganisms of extreme relevance in the pharmaceutical industry [7-17].

In addition to the importance described for the pharmaceutical industry, endophytic microorganisms are valuable tools as vectors for introducing genes of interest in plants for the production of pest and pathogen inhibitors [18-21].

Organic farming or organic farming is the term often used to designate the production of vegetables and other plant products without the use of synthetic chemicals such as inorganic fertilizers (nitrates, phosphates, etc.) and pesticides, or genetically modified organisms. This production system, which excludes the use of fertilizers, agrochemicals and growth regulators, is based on the use of animal manures, composting and biological control of pests and diseases. But it is precisely in fertilization with manure that if not properly treated before employment (and it is known that even pig feces and human faeces are used in soil fertilization) which results in a high source of microbial contamination.

The objective of this work was to investigate the presence of potentially pathogenic Gram negative bacteria in the feet of vegetables grown from organic crops (lettuce and curd, chard, basil, spinach, coriander and cabbage) purchased from supermarkets in the Higienópolis region of the city of São Paulo.

Material and Methods

Sterilization of the collected material: The collected material was processed within 24 hours, after collection, washing it abundantly with running water and neutral detergent. Then, in an aseptic chamber, the material was immersed in 70% (v / v) ethyl alcohol for 1 minute, then 3% (v / v) sodium hypochlorite for 4 minutes and again in 70% ethyl alcohol per 30 seconds to remove excess hypochlorite, and finally rinsed with sterile distilled water 3 times to remove any residue.

Endophytic isolation: After several colonies isolated from bacteria developed around the fragments of leaves and stems were randomly selected and transferred (culture by depletion) to plates containing McConkey selective medium. After incubation for 24-48 hours, isolated fermentative colonies of lactose (red) and non-fermenting colonies of lactose (whitish colonies) were transferred for biochemical tests (reactions provided by the Enterokit ™ Probac® Brazil) and further testing complementary, as proof of the presence of the enzyme cytochrome oxidase for those bacteria suspected to be Pseudomonas and/or Acinetobacter.

Results

Different vegetables derived from organic crops purchased from a supermarket in the Higienópolis region, São Paulo city: Lettuce (Lactuca sativa, family Astaraceae) were qualitatively analyzed; basil (Ocimum gratissimum L., family Lamiaceae); Chard (Beta vulgaris var. cicla (L) K, Koch, family Amaranthaceae); spinach (Spinacia oleraceae, Amaranthaceae family); cabbage (Brassica oleraceae, Brassicaceae family) and coriander (Coriandrum sativum L., family Apiaceae).

From the lettuce, two feet of crisp lettuce, a flat lettuce foot and a crisp purple lettuce foot were analyzed. Of the other vegetables, only one foot. Of all, three leaves were taken at random to make the analyses. A total of 46 Gram negative bacterial colonies were identified, of which 27 (58.7%) were enterobacteria, 14 (30.4%) to the genus Acinetobacter and 5 (10.9%) to the genus Pseudomonas (Table 1).

Lineages found	Classification
A1	Acinetobacter sp.
A2	Acinetobacter sp.
A3	Acinetobacter sp.
A4	Acinetobacter sp.
A5	Pseudomonas aeruginosa
A6	Escherichia coli
A7	Acinetobacter sp.
A8	Acinetobacter sp.
A9	Acinetobacter sp.
A10	Acinetobacter sp.
A11	Acinetobacter sp.
A12	Klebsiella pneumoniae
A13	Serratia marcescens
A14	Hafnia alvei
A15	Yersinia enterocolitica
A16	Shigella spp. or Yersinia pestis
A17	Pseudomonas sp.
A18	Klebsiella pneumoniae
M1	Proteus vulgaris
M2	Escherichia coli
M3	Acinetobacter sp.
M4	Acinetobacter sp.
M5	Pseudomonas aeruginosa
M6	Acinetobacter sp.
C1	Salmonella sp.
C2	Proteus mirabilis
C3	Proteus vulgaris
C4	Salmonella sp.
C5	Pseudomonas putrefasciens
C6	Klebsiella oxytoca

ARC1	Acinetobacter sp.
ARC2	Proteus vulgaris
ARC3	Edwarsiella parda
ARC4	Salmonella typhi
AC1	Klebsiella oxytoca
AC2	Proteus mirabilis
AC3	Klebsiella pneumoniae
AC4	Serratia marcescens
E1	Serratia marcescens
E2	Escherichia coli
E3	Acinetobacter sp.
Co1	Escherichia coli
Co2	Pseudomonas putrefasciens
Co3	Proteus vulgaris
Co4	Salmonella spp.
Co5	Salmonella typhi

Table 1: *Classification of Gram-negative endophytic bacteria found in vegetables from organic crops based on biochemical tests.*

Note: The genera Pseudomonas do not belong to Enterobacteriaceae and Acinetobacter. Subtitles: M1 to M6 lineages isolated from basil. Sections C1 to C6 isolated from cabbage. ARC1 to ARC4 strains isolated from purple curly lettuce. Lines AC1 to AC4 isolated from Swiss chard. E1 to E3 lineages isolated from spinach. Co1 to Co5 strains isolated from coriander.

Discussion

Endophytic microorganisms (bacteria and fungi) are those that live in at least one period of their life cycle. Healthy plant tissues can remain dormant or actively colonize tissues in a localized or systemic way, exerting several beneficial activities for the host plant as well as nitrogen fixation, production of biologically active metabolites such as antibacterial and antifungals, growth factors, etc. However, many endophytic bacterial species are pathogenic or potentially pathogenic to humans [2].

Organic agriculture or organic farming excludes the use of synthetic chemicals such as inorganic fertilizers (nitrates, phosphates, etc.) pesticides, and genetically modified organisms, but is based on the use of animal manures, composting and biological control of pests and diseases. But, it is precisely in fertilizing with manures that if not properly treated before employment that results in a high source of microbial contamination.

The use of excreta is motivated by the recognition of its valuable nutrient content for plants. It is known that pig manures and human faeces are used as vegetable fertilizers. Human excreta may also contain pathogenic microorganisms, which directly threaten human health. Diarrhea and parasitic diseases are important factors contributing to the Global Burden of Disease, where the major causes are the environmental transmission through contaminated water and food crops, or through direct contact with fecal contaminated sources. Excreta usually does not contain industrialized chemical contaminants, but must be treated to reduce levels of pathogens at safety levels. Human metabolites such as hormones may also exist, but reuse on farmland would decrease the negative impact as well as on water sources [24].

A system for the control and management of microbial exposure with respect to wastewater and excreta use was drafted and published by the World Health Organization (WHO) in the 1980s [25] and revised thereafter [26]. This report focused on the management and management of feces and urine, taking into account current information on risk management and limiting a strategy of separation at source.

The presence of disease-causing organisms in human excreta is the result of infection of the individuals. This type of infection does not necessarily manifest itself with clinical symptoms (asymptomatic carriers) but may lead to excretion of the pathogens in question. For organisms that infect the gastrointestinal tract, this contamination occurs primarily through feces. In this work, several bacterial genera of clinical relevance such as Klebsiella, Serratia, Hafnia, Yersinia, Shigella, Salmonella, Edwarsiella, Proteus, Escherichia, and the non-fermenters Acinetobacter and Pseudomonas were identified.

Conclusion

All the microorganisms identified in the present study occupy the same ecological niche, such as water, soil, plants, intestinal tract of warm-blooded animals, etc. and can trigger diseases such as urinary tract infections and gastroenteritis, and if not treated quickly and effectively can lead to severe conditions and even death.

Even after undergoing a thorough disinfection process, the organic vegetables analyzed presented pathogenic potential for consumers, since if the individual has the impaired immune system, when eating this type of vegetable, it can develop some of the diseases mentioned above.

People generally buy organic products because they are labelled healthier because they do not have pesticides and pesticides. But the use of natural manure, composed of animal waste and animal waste is susceptible to bacterial contamination. All plants have endophytic microorganisms that may be symbiotic or commensal; however, few studies have been conducted regarding the interaction of these endophytic organisms with the direct consumers of host plants. Therefore, it is necessary to carry out studies to establish the relationship between consumption of this type of food (organic vegetables) and outbreaks, once which demonstrated in this work the existence of bacteria potentially pathogenic to humans.

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