Introducing the probiotics principle: converting organic waste into natural fertilizer in Japan

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Ι

As fears rise about the health risks and water pollution caused by chemical fertilizer, supplies of the base chemicals required to make fertilizer are running out. Phosphorus, nitrogen and potassium are the main chemicals used in fertilizer in Japan and worldwide, yet phosphorus is a resource that is known to be depleting faster than petroleum. Japan is also faced with phosphorus- and nitrate-induced eutrophication problems in fresh and marine water and nitrate contamination in ground water. All these problems necessitate a reduction in use of chemical fertilizers but levels of agricultural production must be maintained to avoid food shortages. Increasingly, the challenge for farmers in Japan and countries around the world is to provide a sustainable food supply using only organic farming methods.

Large-scale monocultures of major crops may offer one way to feed the world but their production depends on the use of massive quantities of chemical fertilizer, pesticide and herbicide. The modern agricultural methods practised in major food-producing countries are unsustainable, causing fundamental problems such as soil deterioration, land and water ecosystem destruction, ground-water contamination and phosphorous depletion. Problems caused by chemical fertilizer have reached record levels worldwide, where the widespread use of nitrogen fertilizer is contaminating the global water supply. Lakes and reservoirs are eutrophying, ecosystem food webs are changing and many precious species are becoming extinct. Efforts to reduce the level of eco-toxicity of agrochemicals or limit the amounts that are used are not enough to halt the ecological deterioration that is being reported. The future preservation of water resources — now an issue of global concern — depends increasingly on radical changes in agricultural practices.

The European Commission (EC) is one of a number of organisations driving the move away from the use of agricultural chemicals toward organic farming methods in the European Union (EU). Defining organic farming as "an agricultural system that seeks to provide you, the consumer, with fresh, tasty and authentic food while

respecting natural life-cycle systems" (EC 2010), EU organic farming policy guidelines call for strict limits on the use of synthetic chemical pesticide and fertilizer and advise farmers to take advantage of on-site resources, such as livestock manure for fertilizer or feed produced on the farm. These and other organic farming principles and objectives underpin a host of EU-recommended common farming practices, designed to minimize the human impact on the environment while ensuring the agricultural system operates as naturally as possible. In Japan, the Ministry of Agriculture enacted the Law on Promotion of Organic Farming in 2006. This law introduced guidelines that accord closely with the standard practices for organic farming endorsed by the EU. Similar guidelines are endorsed or called for worldwide by other organisations and individuals rejecting synthetic chemical insecticides, herbicides and fungicides in favour of natural methods to discourage insect predation and encourage plant growth.

Π

Plants need nitrogen, phosphate and potassium for foliage growth, root development, and flowering to make fruit. Giving plants the right quantities of these nutrients when they are needed most is perhaps the greatest challenge for organic farmers. Applying compost — the rich, humus-like material made when micro-organisms and fungi break down organic matter — allows farmers to supply plants naturally with nitrogen, phosphate, potassium and the nutrients essential for plant growth while also enhancing soil structure. Plants grown on soils with compost are healthier and more resistant to diseases. The composting process also offers a valuable way to recycle nutrients by turning problematic organic wastes into a valuable resource for agriculture (Matsui 2009).

Of the variety of composting processes available, one method currently gaining momentum across Japan uses probiotics to introduce beneficial bacteria during the composting process with the ultimate aim of increasing the supply of nutrients to the soil. The concept of probiotics is derived from human health science, where lactic-acid bacteria species and *Bifidobacterium* species are used to keep the intestinal gut system in a healthy condition and bolster the human natural immune system (Joint Food and Agriculture Organization/World Health Organization Expert Consultation 2001; Hamilton-Miller et al. 2003). Lactic-acid bacteria used to produce yogurt, cheese, pickles, sauerkraut, tsukemono, kimchi and other fermented foods are known to aid the body's natural immune responses and digestion, as well as alleviate lactose intolerance. Bacteria that benefit the human body in these ways are also known to play a similar role in the digestive and immune systems of domesticated mammals, such as cattle, for which lactic-acid-fermented fodder has proved an excellent probiotic food. In each case, "friendly" bacteria help the body — whether human or animal — to protect itself.

Bacteria belonging to the Bacillus, lactic acid and Actinomycetous bacteria groups are proving particularly beneficial to plant life when introduced during the composting process, both as composting agents and in the soil to which the compost is applied (Matsui 2009). The practice of growing certain crops, vegetables and fruits continually, without rotating them, has given rise to hazards from a variety of organisms, including insects, nematodes, fungi, bacteria and viruses, which attack crops. Although plants, like humans, have a natural immune system to defend them when under attack from various organisms, they are not always strong enough to fend off attack. They may, therefore, benefit from the provision of friendly bacteria that strengthen their immune systems¹ through processes such as commensal or mycotrophic symbiosis. Recent studies of endophytic microorganisms — organisms that reside in the living tissues of virtually all plants — show that their relationships with the host plants range from beneficial and symbiotic to pathogenic (Subhash et al. 2010). Utilizing the endophytic function of Lactobacillus and Bacillus bacteria to stimulate plant growth, while ecologically suppressing the activity of pathogenic endophytic microorganisms in the host plant, helps to develop the natural immune system of the host plant.

This, I believe, is the essence of "probiotic agriculture". While the scientific understanding is new, using bacteria groups to compost organic waste is not. The use of human excrement or night-soil on crops has a long history in Japan. Cattle farming is not widespread in Japan so the supply of animal dung is limited. The practice of using human excrement as fertilizer went into decline following the promotion of sewage works in Japan after the second world war. However, its use has been revived in recent years, with organic farmers adding human excreta as well as animal dung — both rich sources of nitrogen, phosphate and potassium — to the organic waste (rice stalks, etc) gathered during the autumn harvest to make compost.² Over the past 20 years, I have observed the practices of many prominent Japanese organic farmers who are skilled in probiotic approaches to farming: whether by adding rotten leaves — a prime source of Bacillus bacteria — into their organic waste when composting, or by adding Lactobacillus spp. in either powder or solution form, directly on to their crops for use as a natural pesticide. Their efforts show that Gram-positive bacteria species in general can be used to protect plants and crops from the hazards of modern continuous cropping, which include the nematodes that can decimate potato crops and a number of insect types that attack soybeans and maize. They are also effective to control fungal diseases such as powdery mildew, downy mildew and phythium blight, which attack many plants; club root, a common disease caused by Plasmodiophora brasscae that affects cabbages, turnips and other plants belonging to the Cruciferae (mustard) family; and Fusarium wilt, which can afflict solanaceous crop plants (tomato, potato, pepper and eggplant) and banana plants (Matsui 2009).

III

Let's look in some detail at how the three types of bacteria that have been identified are used to compost organic wastes. Typically, one bacteria group will be chosen over another according to the type of waste that is being composted. Organic waste must, therefore, be sorted carefully to ensure the bacteria chosen will work effectively. Efforts to control the oxygen supply, temperature and level of moisture of the compost come next: such efforts are vital, if optimum conditions for bacterial activity are to be maintained throughout the composting process. Bacteria can be added in a wide variety of ways: introduced "naturally", in the case of rotten leaves carrying the *Bacillus* species in the simplest case, or applied more scientifically, in a powder or solution administered through carefully measured inoculations, as pure cultures, or in a mixed culture containing all three bacteria groups.

Bacillus species such as Bacillus subtilis, Bacillus subtilis var. natto and Bacillus thuringiensis can rapidly decompose any type of organic waste, including animal dung, raw garbage and sewage sludge. As these aerobic bacteria require the presence of oxygen to live and grow, strict aerobic conditions must be observed throughout the composting process to ensure an optimum air supply. Good ventilation is also a priority: composting organic waste that contains protein produces high levels of ammonia, which occur when amino acids are broken down. The resulting odour problems can only be dealt with by keeping composting mounds or composting yards well ventilated. Rain protection must also be provided above the mound.

Figure 1 shows *Bacillus* spp. aerobic composting carried out on an industrial scale in one of several sewage works in Japan currently using this method to compost dewatered sewage sludge for use as natural fertilizer. Aerobic conditions are secured in concrete-floored composting yards, such as the one shown in the figure, by channelling air into the compost through vinyl or cast-iron pipes placed in shallow trenches underneath the mound. This ensures that an optimum air supply reaches the bottom layer of compost, as well as the top layer, which is exposed to the natural circulation of air. Bacteria give off heat when they begin work decomposing organic matter. When the mound is big enough, the temperature within it rises to between 85 °C and 100°C. Vapour will start to rise off the mound when the temperature reaches higher than the atmospheric temperature, as can be seen in the second and third compartments from the left in the figure. The preparation of seven compartments allows industrial-scale composters to increase and lower the temperature of the compost mound by mixing and moving it from one compartment to the next, starting new batches of compost as older ones reach maturity. The temperature of the compost increases up until the third or fourth compartment, where it peaks, before starting to

fall, lowering to between 30 $^{\circ}$ C and 40 $^{\circ}$ C by the time it reaches maturity in the seventh compartment. In industrial-scale composting, the seven compartments correspond to the number of weeks required to stabilize compost in countries where temperatures drop sharply in winter, for example, in Europe, north America, China or Japan. Small-scale composters using hyperthermophilic composting in aerobic conditions do not need to use compartments but they must keep the compost well mixed, well aired and within an acceptable temperature range at all times for composting to be successful.

It is important to note that hyperthermophilic composting at these high temperatures is enough to kill viruses, the eggs of worms and insects and all bacteria in the Gram-negative group, which contains most of the bacterial pathogens to humans and animals. Anaerobic bacteria cannot grow because of the aerobic conditions observed in the composting process. Some Gram-positive, spore-forming bacteria may survive but these are, in general, not harmful to humans or to crops. Indeed, many of the spore-forming *Bacillus* species, which remain in the compost where they germinate, long after the compost has been added to soil by farmers, function as microbial pesticides, herbicides and fungicides, making them beneficial to the crops on which the compost is applied. One notable exception is the dangerous bacteria, *Bacillus anthracis*, which must be treated immediately by medical vaccination if contracted. Other than this extremely rare exception, compost produced using this method can be pronounced safe (Figure 2).

Lactic-acid bacteria, including Lactobacillus spp., Enterococcus spp., Lactococcus spp., Pediococcus spp. and Leuconostoc spp., can work rapidly to decompose plant material and any type of organic waste in facultively anaerobic condition. Bacterial species in this group prefer a more anaerobic condition to decompose organic waste that is rich in carbohydrate and sometimes work together with yeast species such as Saccharomyces spp. and Schizosaccharomyces spp. Rainwater protection over the mound of compost is a strict requirement, as is good drainage from the bottom of the mound. When the mound is big enough, the temperature within it rises to exceed 60° C to 70 °C and activates thermophilic composting. Although Gram-negative bacteria species and some of the spore-producing Gram-positive bacteria may survive at these temperatures, lactic acid produced by bacteria breaking down carbohydrate, fat and oil brings the pH of the composting mound down below 5, creating acidic conditions in which most Gram-negative bacteria and pathogenic viruses cannot survive. In principle, only a number of worm and insect eggs and some of the spore-producing Gram-positive bacteria may survive in these acidic conditions — none of them posing much of a problem. More problematic, perhaps, is the pathogenic yeast Candida pneumocystis jiroveci, which is an important cause of opportunistic respiratory-tract infections in patients with compromised immune systems, and pathogenic lactic acid Clostridium perfringens, which can produce food-poisoning toxins that trigger

abdominal cramps and diarrhea. Both of these pathogens may survive the composting process but it is rare for them to do so.

It takes three to four months for compost to mature using this method, depending on the climate. Compost made in winter will take longer to mature in countries in the north temperate zone, for example. Lactic-acid bacteria produce lactic acid when composting organic waste that contains carbohydrate, fat and oil and amino acids when composting waste that contains protein: this drops the pH of compost and creates a low-pH and amino-rich compost that is particularly good for plant growth. Another benefit of this composting method is that it produces much less ammonia than composting using *Bacillus* species.

Actinomycetous species — including Actinoplanes spp., Ampullariella spp., Dactylosporangium spp. and Streptomyces spp. — play an important role in the decomposition of any type of organic waste, particularly oil, fat and plant cell walls containing lignin, latex and chitin. Aerobic conditions should be observed throughout the composting process and both rainwater protection and good drainage must be given to the compost mound. When the mound is big enough, relatively high temperatures of 35 °C to 65 °C are reached within it. Mesophilic composting of this kind does not create high enough temperatures to kill all Gram-negative bacteria. Pathogenic viruses cannot survive a prolonged composting period, however, and they will die away — together with some Gram-negative bacteria — in the long period of starvation that results when the availability of organic food depletes over the course of composting. The eggs of worms and insects may survive, along with some species of spore-producing, Gram-positive bacteria that "sleep" (the surviving spores wake up in the soil later on when conditions become favourable). Fortunately, most of these are not harmful to crops.

It takes three to five months, depending on the composting temperature, air supply and climate, to get matured compost using this method. In Japan, where winters are cold, it will take five months rather than three if composting outside using this method (Figure 3). When composting organic waste that contains carbohydrate, fat, oil and protein, *Actinomycetous* bacteria produce low-molecule organic acids and amino acids that reduce the pH of the compost and may help the growth of plants in the fields. The matured compost has a uniquely rich smell of earth or fungus, which is created when the bacteria forms whitish, lace-like filaments that cover the surface of the compost mound. Bark compost produced by *Actinomycetous* species composting is already commercially available. In addition, many types of antibiotics produced by the fermentation of the *Streptomyces* and other *Actinomycetous* species are known to be effective in compost to control some Gram-negative pathogens that affect crops and vegetables. Important Gram-positive pathogenic *Actinomycetous* species including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Corynebacterium diphtheriae* and *Mycobacterium bovis* — all well known and easily diagnosable diseases that affect



Figure 1. Hyperthermophilic composting of dewatered sewage sludge in aerobic conditions using *Bacillus* species

Source: author



Figure 2. Composted sewage sludge produced by the south Kagoshima city sewage works and certified for use as quality fertilizer

Source: author







Figure 3. Winter composting (left) using *Bacillus* and *Actinomycetous* spp. bacteria in a quiet corner of a frozen vegetable field at Mugi no ie (right), a working organic farm in Shiga prefecture, Japan Source: Kenji Yamamoto Photo Office

humans and domesticated animals — can be controlled by antibiotics produced by other *Actinomycetous* species.

IV

Selecting the correct species of bacteria as the catalytic function for composting organic waste is the key to successful probiotic agriculture.³ This is because probiotic composting depends on choosing a catalytic bacterium or mixture of catalytic bacteria that can foster the presence of friendly bacteria in the compost that is the end product. When this compost is applied to soil on which plants and crops are being grown, the bacteria can start to proliferate and work as microbial pesticides against bad microbes. For example, the application of bark compost made using *Actinomycetous* bacteria suppresses problems for strawberry plants and watermelon caused by continuous cropping, while *Bacillus* species composting has proved effective in preventing the nematodes that feed on the roots and sieve tubes of potatoes (Matsui 2009). In each case, the bacteria also actively improve the taste of the crops: bark compost greatly improves the sugar content of strawberries, for instance, and *Bacillus* species compost enhances the sweetness of potatoes.

The improvement in the taste of crops that have been grown with probiotically made compost is caused by the plant hormones produced by the bacteria used in the composting process. Bochow (2001) has shown that Bacillus species produce auxin, a key plant hormone that regulates the direction of plant growth toward sunlight. Others have reported that Actinomycetous species produce cytokinin, another key plant hormone for regulating plant growth and development (Nishimura 2003). Cytokinin promotes cell division in plant roots and shoots and regulates cell enlargement, chloroplast development, plant senescence or aging and cell differentiation — all processes that relate to plants' ability to extend leaves and bear blossom and fruit (Kirinuki et al. 1976; Nishimura 2003). My own studies have shown that Lactobacillus species produce both auxin and cytokinin (Matsui and Matsuda 2011). When probiotically produced compost is introduced to the soil, the beneficial and effective bacteria in the compost can work in either endophytic or ectophytic ways. Ectophytic bacteria stimulate plants around the surface of roots and leaves, while endophytic bacteria live in sieve tubes where a symbiotic relationship develops. Endophytic bacteria also excrete auxin and/or cytokinin, which stimulate plant growth and enhance the plant's ability to bear blossom and fruit. The sweetness of fruit comes from its sugar content. Lactobacillus species stimulate the growth of any plant and hasten the bearing of blossom and fruit. Many farmers in Japan and other countries, who use friendly or beneficial bacteria in their farming practices, state that the taste of leaf vegetables is recognizably better and the sweetness of all fruits is enhanced. Their

opinions would appear to be endorsed by the increasing numbers of consumers in Japan and elsewhere, who appear willing to pay a higher price for organic farm products on account of their flavour as well as their perceived safety.

V

Many Japanese farmers, who apply synthetic chemical pesticide and herbicide to fields that they cultivate for commercial products, say bluntly that they would not dream of applying those same chemicals to fields that are cultivated for their own consumption. It is perhaps no wonder, then, that in the past 20 years, I have met increasing numbers of small-scale growers eager to pioneer, practise and promote organic farming in Japan (Figure 4).

These changes in farming practices are welcomed by increasing numbers of citizens and consumers, predominantly Japanese women, who have been active agents of change in lobbying for agricultural practices that do not threaten the natural environment. In my home city of Kyoto, the Kyoto Kankyo [Environment] Action Network, which my wife founded, runs a neighbourhood initiative that has 200 households (out of the 3,000 households in Omuro school ward) recycling household organic waste for composting. The compost is subsequently used by organic farmers living in Miyama-cho, north of Kyoto city. They then sell their organic produce from my garage twice a month, every second Tuesday (Figure 5). At the national level, women in the organic farming movement have lobbied against genetically modified soya products and demanded safer food products to feed their families. The food industry is slowly but surely responding to calls such as these: in recent years, signs advertising "gen-nouyaku" (reduced-chemical) and "mu-nouyaku" (no-chemical, i.e. organically grown) products have appeared in high-street supermarkets and stores run by farmers' cooperatives all over Japan as increasing numbers of outlets sell organic products catering to consumers' changing tastes.

How does the composting method I have described fit in with the trend towards a more sustainable way of farming? Clearly, the application of friendly bacteria to agriculture offers great potential to reduce the use of chemical fertilizer and agrochemicals, including antibiotics used for domesticated animals. The positive role it can play in organic farming is at least four fold: it recycles nutrients by finding a use for organic waste; it produces compost that provides a rich humus to enhance the soil; it reduces the need for synthetic chemical pesticides and it provides us with tasty and authentic food. These four merits, made possible by applying a probiotic principle to compost production, must be taken advantage of by greatly expanding the current scale of probiotic composting. Non-governmental organizations, such as the Japan Organic Agriculture Association (JOAA), Organic Farming Promotion Association, Organic







Figure 4. Mr Takashi Yamazaki (top left) talks to assembled guests at the Bansei Kyokai — a sustainability study group held at Mugi no ie about local organic farming's importance to the global environment; Dr Mariko Nakagiri (top right and below) enthrals the same audience with a lecture about the economic life and ethics of Ninomiya Kinjiro, an agricultural reformer of the Edo period Source: Kenji Yamamoto Photo Office











Figure 5. Mrs Megumi Matsui (top left) explains organic waste recycling to fellow residents in the Kyoto Kankyo Action Network; an organic waste composting box (bottom left), newly installed outside the Matsui home in 2004; farmers (top right) sell organic produce grown in Miyama-cho, northern Kyoto prefecture, at the bi-monthly Miyama ichiba (market) held outside the Matsui home; an elderly resident (center right) in Omuro school ward takes advantage of the market on her doorstep; Omuro school ward residents (bottom right) on a Kyoto Kankyo Action Network field trip to Miyama-cho see how their compost is being used to make organic produce

Source: Kyoto Kankyo Action Network

Farming Research Council and the Center of Japan Organic Farmers Group, are currently leading the way. In the future, city governments could tackle their organic waste problems by producing compost that is cheaper for farmers to use than chemical fertilizers and brings significant environmental benefits.

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Notes

- ¹ The concept of using friendly bacteria to promote plant growth was first developed in Japan in the 1970s by eminent Japanese horticulturalist Dr Teruo Higa, a retired professor of the University of the Ryukyus, Okinawa. Higa's proposals for an "earth-saving revolution" based on the use of "effective microorganisms" (as he called them) in agricultural practice was distributed in Japanese and English by Sunmark Publishing in the 1990s (see Higa 1993; 1994).
- Starting after the autumn harvest, farmers dig a large hole in a corner of their field and dump their farm waste into it, together with fermented human excreta. Natural composting starts with native *Bacillus* spp. and other aerobic bacteria active in the upper part of the hole and native anaerobic bacteria, such as lactic acid and yeast active in the bottom part. The compost matures in five months just in time for spring, when it is added to new crops and paddy fields. Farmers using this method may not necessarily be aware what types of aerobic and anaerobic bacteria and yeast are at work but they are very sure of the quality compost that is its result!
- This is probably the main difference between the probiotic approach outlined in this paper and the aforementioned effective-microorganisms (EM) method proposed by Dr Teruo Higa, who endorses a combination of several species of bacteria, yeast and fungi in a commercial solution called EM Bokashi for use in organic farming. EM Bokashi requires fermentation by users to amplify the proliferation of EM before it can be used. This particular fermentation method only supports the growth of anaerobic lactic-acid bacteria and yeast so that other species of bacteria may not function in EM Bokashi. There is also no clear indication of the amount of bacteria that needs to be applied for composting. By selecting a single catalytic bacterium or combination of bacteria in the correct amount for a single application, the probiotics approach endorsed in this paper offers a more simple composting method that is easier to put into practice. Other than match the right catalytic bacterium or bacteria to the kind of waste that is to be composted, composters need do little more than guarantee the correct aerobic or anaerobic conditions, watch the temperature in and around the compost mound and mix and move the mound when required (or leave it as it is for as long as is necessary). Nature should take care of the rest.

References

Bochow, H., El-Sayed, S. F., Junge, H., Stavropoulu, A. and Schmiedeknecht, G. (2001) 'Use of *Bacillus subtilis* as biocontrol agent. IV. Salt-stress tolerance induction by *Bacillus subtilis* FZB24 seed treatment in tropical vegetable field crops, and its mode of action', *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* [Journal of Plant Diseases and Protection], 108 (1), pp. 21–30.

European Commission (2010) 'Organic farming: good for nature, good for you', \(\http://ec.europa.eu/agriculture/organic/organic-farming/what-organic_en\), accessed December 3, 2010.

Food and Agriculture Organization/World Health Organization Expert Consultation Report (2001) 'Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria', (http://www.who.int/

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- foodsafety/publications/fs_management/en/probiotics.pdf\, accessed November 4, 2009.
- Hamilton-Miller, J. M. T., Gibson, G. R. and Bruck, W. (2003) "Some insights into the derivation and early uses of the word 'probiotic'", British Journal of Nutrition 90: p. 845.
- Higa, T. (1993) An Earth-Saving Revolution: Solutions to Problems in Agriculture, the Environment and Medicine. Sunmark Publishing Inc.
- Higa, T. (1994) An Earth-saving Revolution II: the Proven Effects of EM Technology. Sunmark Publishing Inc.
- Kirinuki, T., Ofuji, H., and Suzuki, N. (1976) 'Chitin-Lytic and β-1, 3-Glucanase activity of Actinomycetes and their effects for preventing fusarium-wilt of cucumber' [in Japanese], The science reports of the Faculty of Agriculture, Kobe University, 12 (1), pp. 41–48.
- Matsui, S. (2009) 'Baiomas junkan katsuyo wo kanou ni suru purobaiotikusu nougyou genri' (Probiotics principle that can help organic farming), Journal of Environmental and Sanitary Engineering Research, 23(3). The Association of Environmental and Sanitary Engineering Research, Kyoto University, pp. 81–87.
- Matsui, S., and Matsuda, T. (2011). An analysis of the production of auxin and cytokinin by *Lactobacillus* fermentum (forthcoming publication, title provisional).
- Nishimura, T. (2003) 'Housenkin wo riyo shita kofukakachi karumia sosikibaiyokin karano kogatahachimono sakushutsu gijutsu ni kansuru kenkyu' (a study of a new technology for making high-price potted dwarf trees by a tissue culture using *Actinomycetes* species), PhD thesis (Graduate School of Bio-resources, Mie University).
- Subash, J., Bhore and Sathisha, G. (2010) 'Screening of endophytic colonizing bacteria for cytokinin-like compounds: crude cell-free broth of endophytic colonizing bacteria is unsuitable in cucumber cotyledon bioassay', World Journal of Agricultural Sciences, 6(4): pp. 345–352.

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