Microbial ecosystem constructed in water for successful organic hydroponics

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Conventional hydroponics systems generally use only chemical fertilisers, not organic ones, since there are no microbial ecosystems present in such systems to mineralise organic compounds to inorganic nutrients. Addition of organic compounds to the hydroponic solution generally has phytotoxic effects and causes poor plant growth. We developed a novel hydroponic culture method using organic fertiliser. A microbial ecosystem was constructed in hydroponic solution by regulating the amounts of organic fertiliser and soil, with moderate aeration. The microbial ecosystem mineralised organic nitrogen to nitrate-nitrogen via ammonification and nitrification. A 97.6% efficiency of nitrate-nitrogen generation from the organic nitrogen in the organic fertiliser was achieved. The culture solution containing the microbial ecosystem was usable as a hydroponic solution. Vegetable plants grew well in our organic hydroponics system under continuous addition of organic fertiliser,

1 **and the yield and quality approximated those of vegetables grown by conventional**

2 **hydroponics.**

3 Organic compounds are good fertilisers in soil culture, but they cannot be used as
4 fertilisers in conventional hydroponics because they have phytotoxic effects and inhibit
5 plant growth[1,2,3,4]. In soil, organic nitrogen contained in organic fertilisers is mineralised
6 to nitrate-nitrogen by soil microorganisms via ammonification and nitrification[5].
7 However, there are few microorganisms in the water used for hydroponics, so
8 nitrification barely occurs, whereas ammonification occurs easily because of the
9 presence of saprophytic bacteria contained in organic fertiliser. Many vegetable plants
10 prefer nitrate-nitrogen to ammonia-nitrogen as a nitrogen source, and these plants are
11 easily damaged by excessive ammonium nutrition[6,7,8,9,10,1112,13]. For successful organic
12 hydroponics, it is therefore necessary to establish a method of safely mineralising
13 organic nitrogen to nitrate-nitrogen via ammonification and nitrification in water.

14 Microorganisms are needed to mineralise organic fertiliser. However, in conventional
15 hydroponics, contamination of hydroponic solutions by microorganisms has been
16 regarded as detrimental, because they compete with the plants for oxygen and nutrients
17 in the hydroponic solution and inhibit plant growth[1,14,15,16,17,18,19,20]. In previous studies
18 of organic hydroponics, organic fertiliser has been mineralised by microorganisms to
19 produce an inorganic nutrient solution, but because of their potential phytotoxicity the
20 microorganisms have been eliminated from the culture solution before it was used in the
21 hydroponic system[1,2,4,21]. Therefore, the solution has no ability to mineralise organic
22 compounds; organic fertiliser cannot be incorporated into it during hydroponic
23 cultivation, and mineralisation of the organic fertiliser must be repeated if the solution
24 becomes short on nutrients. For these reasons, in previous studies of conventional and
25 organic hydroponics both organic compounds and microorganisms have been regarded
26 as problematic. In contrast, in the soil, organic compounds and microorganisms are

1 compatible in soil culture and ammonification and nitrification proceed simultaneously.
2 However, in previous studies of organic hydroponics, ammonification and nitrification
3 have been separated in their respective reactors[4,21]. For practical organic hydroponics, it
4 is desirable to construct a microbial ecosystem that can mineralise organic fertiliser via
5 parallel ammonification and nitrification reactions in the hydroponic solution. We
6 therefore examined whether such a microbial ecosystem could be constructed by the
7 addition of a microbial inoculum from natural materials—for example, by the addition
8 of soil.

9 Field soil, nursery soil, bark compost and seawater were chosen as appropriate
10 inoculum sources of the microorganisms needed to mineralise organic nitrogen to
11 nitrate-nitrogen in water (Fig. 1) in the presence of a fish-based soluble fertiliser.
12 Distilled water without inoculum but with added fish-based fertiliser generated
13 ammonia-nitrogen (data not shown) but no nitrate-nitrogen. In the absence of the
14 fertiliser, neither nitrate-nitrogen nor ammonia-nitrogen was detected in any inoculated
15 flasks containing field soil, nursery soil, bark compost or seawater (data not shown).
16 Without aeration by shaking, ammonia-nitrogen, but no nitrate-nitrogen, was generated
17 in the inoculated flasks (data not shown). These results indicated that the saprophytic
18 bacteria contained in the distilled water could generate only ammonia-nitrogen from the
19 (organic) fish-based fertiliser, but microorganisms added to the water from the field soil,
20 nursery soil, bark compost or seawater could mineralise the organic nitrogen to nitrate-
21 nitrogen; aeration was necessary for this process.

22 Inoculum requirements were examined with bark compost and a one-off addition of
23 2.5 g/L corn steep liquor (CSL) (Fig. 2). Nitrate-nitrogen was generated in flasks by the
24 addition of bark compost at 5 g/L but was not detected in flasks when 0.5 g/L or no
25 compost was added. Nitrate-nitrogen content increased as the ammonia-nitrogen content
26 decreased in the solution with bark compost at 5 g/L. These results suggest that bark

1 compost inoculum at about 5 g/L was needed to mineralise organic compounds to
2 nitrate-nitrogen, and that addition of too little inoculum would damage the nitrifying
3 bacteria, because nitrification by these bacteria is especially susceptible to inhibition by
4 organic compounds[22].

5 Various kinds of organic fertilisers were able to be mineralised to nitrate-nitrogen.
6 When added at 1 g/L, corn oilcake, soybean curd refuse, milk, tomato plant residue
7 (leaves and stems), fish powder, dry beer yeast, yeast extract, dry fermented chicken
8 manure, dry fermented beef manure, or digested slurry from methane fermentation was
9 mineralised to nitrate-nitrogen in water with an inoculum of nursery soil at 5 g/L (data
10 not shown). However, the carbon-to-nitrogen ratio (C/N) of organic fertiliser was
11 important for generating nitrate-nitrogen from organic fertiliser. Organic fertilisers with
12 a C/N ratio of less than 11, such as soybean curd refuse (10.4) and milk (10.8), were
13 able to be mineralised to nitrate-nitrogen. However, high-C/N organic fertilisers, such
14 as rice bran (18.1) and low-grade spirits distilled from sake lees (11.8), were not
15 appropriate for generating nitrate-nitrogen (data not shown). These results suggest that
16 addition of organic fertiliser with a C/N ratio of 11 or more causes nitrogen starvation of
17 the microorganisms[23]. We therefore considered that organic fertilisers with a C/N ratio
18 below 11 should be used to generate nitrate-nitrogen.

19 We then examined the optimum dose of organic fertiliser (Fig. 3). Fish-based soluble
20 fertiliser was added daily for 7 days from the start of incubation to water containing an

1 inoculum of bark compost at 5 g/L. Nitrate-nitrogen was generated only in those
2 containers to which we had added the fertiliser at 0.5 g/L daily for 7 days. The
3 efficiency of nitrate-nitrogen generation from the organic nitrogen in the fertiliser was
4 97.6%. Nitrification was inhibited in containers that received fertiliser at 2.5 g/L daily
5 for 7 days. This result indicated that the nitrifying bacteria were susceptible to inhibition
6 by exposure to organic compounds present at high concentrations in the water.
7 Nitrification of ammonia by nitrifying bacteria, such as the obligate
8 chemolithoautotrophs *Nitrosomonas* and *Nitrospira*, is particularly inhibited by the
9 presence of organic compounds[5,22,24,25,26,27], except for several ones[28]. To mineralise
10 organic fertiliser to nitrate-nitrogen in water, we therefore considered that only a small
11 amount of organic fertiliser could be added to the water.

12 Addition of organic fertiliser after the generation of nitrate-nitrogen resulted in a
13 decrease in nitrate-nitrogen concentration (data not shown). This suggested that
14 denitrification was inducible in the presence of both nitrate-nitrogen as an oxygen
15 source and organic compounds as an energy source[29,30]. Therefore, to suppress
16 denitrification, it was necessary to stop adding the organic fertiliser to the water before
17 nitrate-nitrogen generation.

18 We examined the growth of komatsuna (*Brassica rapa* var. *peruviridis*) in a
19 hydroponic nutrient solution in which we had constructed a microbial ecosystem that
20 optimally mineralised organic nitrogen to nitrate-nitrogen. We started cultivation when
21 the ammonium ion concentration in the hydroponic solution had declined to less than 10
22 mg/L. This value was chosen because in preliminary cultivation tests growth was
23 inhibited when more than 10 mg/L of ammonium ions was added (data not shown).
24 Sixty komatsuna plants were cultivated in a hydroponic system by a deep-flow
25 technique[31], with CSL directly added to the hydroponic solution as an organic fertiliser.
26 The average komatsuna head fresh weight in this system was 39.9 g/plant, close to the

1 40.1 g/plant achieved with chemical fertiliser. The average dry root weights in the two
2 systems were 0.85 g/plant and 0.41 g/plant, respectively. The average ascorbic acid
3 concentrations in the leaves were 2442 mg/L and 2308 mg/L, respectively. The average
4 nitrate ion concentrations in the leaves were 987 and 5108 mg/L, respectively. It is
5 interesting that root weight in the organic system was twice that in the inorganic
6 fertiliser system: root development was likely promoted by the presence of the
7 microorganisms in the hydroponic solution. It is also interesting that the leaf nitrate ion
8 content of plants in the organic system was less than one-fifth that in the plants
9 cultivated with the chemical fertiliser. This suggests that nitrate-nitrogen is generated
10 gradually by the microorganisms, whereas in conventional hydroponics the inorganic
11 nutrients are likely to be absorbed immediately by the plant and accumulate in the
12 leaves. Nitrate ion present at high concentrations in crops is toxic to humans and other
13 animals[32]. Organic hydroponics therefore offers the advantage of low concentrations of
14 nitrate ion in crops.

15 We also performed a growth study on tomato, *Solanum lycopersicum* (Fig. 4). The
16 respective average yields of fruits grown with conventional, CSL, and fish-based
17 soluble fertiliser were 383.8, 518.4, and 512.5 g per fruit cluster. The average Brix
18 values (a measurement of the mass ratio of dissolved sugar to water) of the respective
19 fruits were 5.8, 5.5 and 5.4, respectively. The average ascorbic acid contents of the
20 fruits were 26.2, 24.8, and 22.8 mg/100 g fruit, and the average glutamic acid contents
21 were 126.9, 85.2, and 103.3 mg/100 g fruit, respectively. These results suggest that the
22 yield and quality of tomato fruits from the organic fertiliser system were not inferior to
23 those from the inorganic chemical system. Root hairs and biofilm developed on the
24 roots submerged in the solutions to which CSL or the fish-based fertiliser had been
25 added, whereas they were not observed in the conventional hydroponics system (Fig. 4).
26 They also developed on the roots of the komatsuna cultivated with the CSL (see above).
27 These results suggest that the presence of biofilm on the submerged roots in the

1 hydroponic solution induces the development of root hairs. Little is known about the
2 mechanism of root hair development[33]. The influence of biofilm on the development of
3 root hairs is an important topic for future study.

4 Biofilm collected from the root surface was able to mineralise organic fertiliser to
5 nitrate-nitrogen in a flask (data not shown). Interestingly, nitrate ions were not detected
6 in the hydroponic solution of organic hydroponics about 2 weeks after the start of plant
7 cultivation, even if large amounts of organic fertiliser had been added (data not shown).
8 These results suggest that the biofilm on the surfaces of roots submerged in the
9 hydroponic solution degrades the organic fertiliser to nitrate-nitrogen, which is absorbed
10 immediately by the roots without diffusing into the hydroponic solution.

11 Biofilms in organic hydroponics are suitable media for observation of the interaction
12 between rhizobacteria and plant roots. This interaction occurs in soil culture but is
13 difficult to observe without disruption of the rhizosphere structure. In conventional
14 hydroponics it is easy to observe plant roots, but there is no interaction between
15 microorganisms and the roots because there are few microorganisms in the hydroponic
16 solution. In contrast, our organic hydroponics method allows observation of the
17 interaction whenever necessary (Fig. 4). Biofilm is an important subject for studies of
18 bacterial communication and interaction; the possibility that the bacterial community in
19 the biofilm interacts with the plant roots is very interesting[34]. In organic hydroponics, it
20 is possible to easily observe the interaction between the roots of general crops, such as
21 tomato, and the biofilm on them. This is an important advantage in biofilm research.

22 Tests for susceptibility to bacterial wilt disease were performed with tomato plants
23 and the phytopathogenic bacterium *Ralstonia solanacearum*, which causes bacterial wilt
24 disease of tomato. *Ralstonia solanacearum* caused wilt in more than half the plants in
25 the control experiment, in which chemical fertiliser was added. In contrast, no wilted
26 tomato plants were observed in containers in which CSL was used as an organic

1 fertiliser (Fig. 5). *Ralstonia solanacearum* was detected in the conventional hydroponic
2 solution at an estimated density of 6×10^4 cells/mL. It was not detected in the CSL
3 hydroponic solution. It is not clear why *R. solanacearum* disappeared from the
4 hydroponic solution. In conventional hydroponics, microbial contamination of
5 hydroponic solutions often results in root disease[15,16]; this is why microorganisms are
6 generally eliminated from the solution. Conversely, for some reason, the microbial
7 ecosystem in the organic hydroponic solution inhibited root disease.

8 Our results demonstrated that our novel technique, in which we used a microbial
9 ecosystem that mineralised organic fertiliser to inorganic nutrients in the hydroponic
10 solution, was a feasible alternative to conventional hydroponics. The ability to add
11 organic fertiliser directly to the hydroponic solution is very convenient. In contrast, in
12 previous studies of organic hydroponics, inconvenient procedures have been regarded as
13 an integral part of the use of organic fertiliser, and the two reactions of ammonification
14 and nitrification have been conducted in separate tanks and then organic compounds and
15 microorganisms have been eliminated from the mineralised solution before its
16 use[3,4,35,36,37,38].

17 Our method of organic hydroponics had two phases: construction of the microbial
18 ecosystem in water, followed by hydroponic cultivation. Construction of the microbial
19 ecosystem is particularly important. If nitrification fails during system construction, the
20 solution will be inadequate for hydroponic nutrition, because many vegetable plants are
21 easily damaged by excessive ammonium nutrition[6,7,8,9,10,11,12]. If denitrification fails to
22 be suppressed during system construction, the solution will not be suitable for supplying
23 nutrients, because the nitrate-nitrogen will immediately be lost and plant growth will
24 therefore be poor. It is important during construction of a microbial ecosystem in water
25 to activate nitrification gradually by the addition of small amounts of organic fertiliser

1 and to suppress denitrification by stopping the addition of organic fertiliser before
2 nitrate-nitrogen generation.

3 Hydroponic cultivation enables the addition of large amounts of organic fertiliser to
4 accompany increasing plant nutrient demand. During summer cultivation of tomato we
5 were able to add CSL at 5 g/L daily (data not shown), whereas daily addition of fish-
6 based soluble fertiliser at 2.5 g/L inhibited nitrification during the construction of our
7 microbial ecosystem (see Fig. 3). This result indicates that mineralisation of organic
8 fertiliser is likely to be promoted, and denitrification is unlikely to occur, if plants
9 rapidly absorb the nitrate-nitrogen produced by mineralisation.

10 This method is suitable for use in a closed recirculating hydroponic system. In
11 conventional hydroponics, the use of recirculating nutrient systems can result in
12 accumulation of phytotoxic organic compounds in the reused nutrient solution. These
13 compounds can inhibit plant growth[39,40], and the solution must occasionally be
14 discarded. In contrast, in our method the organic compounds are degraded to inorganic
15 nutrients by the microbial ecosystem.

16 The inhibition of bacterial wilt disease of tomato is also very valuable, because it is
17 difficult to suppress this disease in conventional hydroponics with chemical inorganic
18 fertilisers[20,41]. All microorganisms have been regarded as deleterious in conventional
19 hydroponics on the assumption that some could be pathogenic. In our preliminary
20 cultivation experiment, microbial contamination of the solution by soil dust resulted in a
21 serious decrease in crop yield in our conventional hydroponics system (data not shown),
22 whereas the soil appears to have been a key component of our organic hydroponics
23 system for construction of microbial ecosystem. Susceptibility to contamination by
24 microorganisms in conventional hydroponics may be due to the enrichment of
25 microorganisms in the inorganic nutrient solution, using the plant root as the sole
26 carbon source. The tolerance of our organic system to microbial contamination might be

1 due to the fact that carbon is freely supplied by the continuous addition of organic
2 fertiliser to the hydroponic solution.

3 When we began to establish this method, we were aware of the concepts used in
4 Japanese sake fermentation. Japanese sake is made by simultaneous multiple parallel
5 fermentation, saccharification by *Aspergillus oryzae,* and ethanol production by yeast,
6 suppressing the fermentation of acetic acid. The relationship among these reactions is
7 similar to that of activation of ammonification and nitrification and suppression of
8 denitrification. If we had not studied the fermentation of sake, we could not have
9 devised our method of 'multiple parallel mineralisation'.

10 **Methods Summary**

11 To optimise conditions for construction of a microbial ecosystem capable of
12 mineralising organic fertiliser in water, we examined what kinds of inoculum (field soil,
13 nursery soil, bark compost, or seawater) were appropriate. We then determined the
14 optimum dose of inoculum (0 to 5 g/L of bark compost was added as inoculum with 2.5
15 g/L CSL) and the optimum dose of organic fertiliser (0.5 to 5 g/L of fish-based soluble
16 fertiliser was added with 5 g/L bark compost as inoculum). The criterion for successful
17 construction of a microbial ecosystem was nitrate-nitrogen generation from organic
18 nitrogen contained in the organic fertiliser. For plant cultivation and analysis, the
19 culture solution in which the microbial ecosystem was constructed was made by
20 mineralising CSL or fish-based soluble fertiliser with inoculum of bark compost and
21 then used as the hydroponic solution. During cultivation of komatsuna or tomato,
22 organic fertiliser was daily added directly to the hydroponic solution; oyster shell lime
23 was also added to supplement minor nutrients such as Ca, Mg, Mn, B, Zn, Fe, Cu, and
24 Mo. The plants were cultivated in a closed, recirculating nutrient hydroponic system.
25 For the control experiment, conventional hydroponics was conducted with addition of
26 chemical fertiliser solution; nitrogen addition was regulated to the same rate as in the

organic solution. For inoculation of bacteria phytopathogenic to tomato, *Ralstonia solanacearum* was inoculated into the hydroponic solution. Wilted plants were counted during cultivation. The hydroponic solution was collected 18 days after pathogen inoculation and examined for the presence of *R. solanacearum* by plate culture with selective tetrazolium-chloride medium plates.

Methods

Construction of a microbial ecosystem

To find an appropriate source of microorganisms to mineralise organic fertiliser, we added 5 g/L of field soil collected from a field of garden pea, *Pisum sativum*, at our institute; 'Nae-ichiban' nursery soil (Sumirin-Nousankougyo Co., Ltd., Kaifu, Aichi, Japan); or 'Golden bark' bark compost (Shimizu-kou Mokuzai-Sangyou Kyoudou Kumiai, Shizuoka, Shizuoka, Japan) as an inoculum source to a 100-mL flask of distilled water containing 1 g/L fish-based soluble fertiliser (Makurazaki Gyokyo-Kumiai, Makurazaki, Kagoshima, Japan). As a control, distilled water containing fish-based soluble fertiliser without inoculum was used. The fertiliser contained 6.3% N, 0.14% P_2O_5, and 1.1% K_2O. We also tested 100 mL of seawater collected from Kinu-ura Harbour in Aichi Prefecture instead of distilled water without inoculum of soils or bark compost. The flasks were shaken (120 strokes/min) for 20 days at 25°C and the nitrate-nitrogen concentrations determined.

To determine the optimum dose of inoculum, bark compost at 0, 0.5, or 5 g/L was added to a flask of 100 mL distilled water containing 0.25 g CSL (Sakata, Yokohama, Kanagawa, Japan), 3.3% N, 3.4% P_2O_5, and 3.2% K_2O. The flask was shaken (120 strokes/min) for 13 days at 25°C and the nitrate-nitrogen concentrations determined.

To determine the optimum dose of organic fertiliser, fish-based soluble fertiliser was added at 0.5, 2.5, or 5 g/L daily for 7 days from the experiment start to 2 L of water

1 containing 5 g/L bark compost as a microbial inoculum. The experiment was performed
2 at an ambient temperature of 25°C in a bucket; the water was aerated (19.6 kPa) with a
3 NISSO α-4000 aeration pump (Marukan Co. Ltd., Kasukabe, Saitama, Japan) for 15
4 days. An RQ-Flex Plus Analyser (Merck, Frankfurt, Germany) was used to quantify
5 nitrate and ammonium ions.

6 **Plant cultivation**

7 From 7 November to 11 December 2007, 60 komatsuna seedlings (Sakata,
8 Yokohama, Kanagawa, Japan) were cultivated in a closed, recirculating nutrient system
9 (M-shiki Suikou Co., Ltd., Yatomi, Aichi, Japan), length 465 × width 60 × height 18 cm,
10 by a deep-flow technique in a glasshouse at Tsu, in Mie Prefecture. The microbial
11 ecosystem in the hydroponic solution was constructed before we began cultivation. CSL
12 was added at 0.5 g/L daily for 3 days to 200 L of water containing 2.4 g/L bark compost
13 as a microbial inoculum in a tank; the water was aerated (19.6 kPa) for a total of 22
14 days by two aeration pumps (NISSO α-4000). The solution contained 177 mg/L nitrate
15 ions and 5 mg/L ammonium ions when it was used as the hydroponic solution. Four
16 grams of CSL (equivalent to 120 mg nitrogen) was added directly to the hydroponic
17 solution daily during cultivation. Two kilograms of oyster shell lime (Urabe Industry
18 Co., Ltd., Fukuyama, Hiroshima, Japan), containing 0.34% N, 0.29% P_2O_5, 0.7% K_2O,
19 0.8% MgO, 0.9% Fe, 390 ppm boron, 1400 ppm Mn, 84 ppm Zn, 12 ppm Cu, and 8
20 ppm Mo, as a minor-nutrient supplement, was suspended in 3 L of solution collected
21 from the hydroponic tank and kept overnight. The next day, the suspension supernatant
22 was added to the hydroponic solution. This procedure was repeated daily during
23 cultivation.

24 From 7 June to 13 October 2006 we used a nutrient film technique[42] to cultivate 24
25 'House-Momotaro' tomato plants (Takii & Co., Ltd., Kyoto, Japan) in a greenhouse in
26 the same type of closed recirculating hydroponic system as used above (M-shiki Suikou

1 Co., Ltd.). The microbial ecosystems in the hydroponic solutions were constructed
2 before we began cultivation. CSL was added at 0.2 g/L daily or fish-based soluble
3 fertiliser at 0.1 g/L daily for 5 days to 200 L of water in respective tanks containing 5
4 g/L of bark compost as a microbial inoculum; the hydroponic system was aerated by
5 recirculation of the nutrient solution with an MD-15R-N water pump for 12 days
6 (IWAKI Co. Ltd., Tokyo, Japan). The nitrate ion concentrations in the starter
7 hydroponic solutions were 420 mg/L (CSL) and 189 mg/L (fish-based fertiliser). At the
8 start of cultivation we added 560 g oyster shell lime to each hydroponic solution as a
9 minor-nutrient supplement. For 22 days from the start of cultivation we added 30 mg
10 N/plant daily as CSL or fish-based fertiliser; after that we added 45 mg N/plant daily.
11 The organic fertilisers were added directly to each hydroponic solution. Daily from 28
12 June, wood ash was added to the hydroponic solution as a potassium supplement at 0.34
13 g with 1 g of CSL, or at 0.932 g with 1 g fish-based fertiliser. For the control
14 experiment, conventional hydroponics was conducted with chemical fertiliser solution
15 containing 260 mg/L N, 120 mg/L P_2O_5, 405 mg/L K_2O, 60 mg/L MgO, 1.5 mg/L MnO,
16 1.5 mg/L B_2O_3, 230 mg/L CaO, and 2.7 mg/L Fe. The amount of fertiliser added to the
17 system was adjusted to make it the same as in the organic hydroponics system.

18 **Plant inoculation**

19 A microbial ecosystem was constructed at an ambient temperature of 30°C in a
20 container, length 65 × width 23.5 × height 19 cm, filled with 15 L water inoculated with
21 50 g nursery soil as a microbial inoculum; we added CSL at 0.2 g/L daily for 8 days to
22 the water. When a nitrate ion concentration of more than 200 mg/L had been achieved
23 and the ammonium ion concentration had decreased to below 10 mg/L, we eliminated
24 the nursery soil from the solution and added 150 g oyster shell lime as a minor-nutrient
25 supplement. We then cultured 16 seedlings of 2-week-old ‘Ponderosa’ tomato (Asahi-
26 nouen Co., Ltd., Aichi, Japan) in the container at 32°C in a temperature-controlled

cabinet (Koitotron, Yokohama, Japan). Three days after the start of culture, 10 mL of 1.56 to 2.38 × 10^8 cells/mL of *R. solanacearum* MAFF 03-01487[43] was inoculated into the hydroponic solution. The hydroponic solution was collected 18 days after pathogen inoculation and examined for *R. solanacearum* by plate culture with selective tetrazolium-chloride medium plates[21]. The plates were incubated at 32°C for 6 days; colonies were then counted to determine the density of the bacterium in the hydroponic solution.

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9

10

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15 **Author Contributions**

16 M. Shinohara entirely carried out this study and wrote the paper. H. Ohmori assisted
17 tomato cultivation. Y. Uehara suggested properties of nitrifying bacteria. All authors
18 discussed the results and commented on the manuscript. M. Shinohara supervised all of
19 the work.

20

1 Fig. 1 Mineralisation by microbial inocula from various sources.

2 We examined the suitability of various inoculum sources for mineralisation of
3 organic fertiliser to nitrate-nitrogen: 5 g/L of field soil, nursery soil or bark
4 compost was added as microbial inoculum in 100 mL of distilled water
5 containing fish-based soluble fertiliser at 1 g/L. Seawater was substituted for
6 distilled water without other inoculum. Distilled water without inoculum was used
7 as a control. Vertical bars represent the means and standard deviation of three
8 replications.

9

10 Fig. 2 Inoculum requirements.

11 We determined the optimum concentration of bark compost inoculum for
12 mineralisation of organic nitrogen from corn steep liquor to nitrate-nitrogen. Bark
13 compost was added at 0, 0.5, or 5 g/L as a microbial inoculum to 100 mL
14 distilled water containing 2.5 g/L of corn steep liquor. Vertical bars represent the
15 means and standard deviation of three replications.

16

17 Fig. 3 Organic fertiliser requirements.

18 We determined the optimum concentration of organic fertiliser for nitrification in

19 the presence of 5 g/L bark compost inoculum. Fish-based soluble fertiliser was

20 added at 0.5, 2.5, or 5 g/L daily for 7 days from the start of the experiment.

21 Vertical bars represent the means and standard deviation of three replications.

22

1 Fig. 4 Tomato growth study.

2 Growth of tomato plants with three kinds of fertiliser: chemical inorganic fertiliser
3 (a), corn steep liquor (b) and fish-based soluble fertiliser (c). Upper: tomato
4 plants just before harvesting; middle: tomato roots on 15 June 2006 during
5 cultivation; lower: roots submerged in hydroponic solution.

6

7 Fig. 5 Phytopathogen inoculation.

8 Susceptibility to bacterial wilt disease of tomato was examined by inoculation of
9 the culture solution with *Ralstonia solanacearum* MAFF 301487. Upper: tomato
10 plants cultivated with chemical fertiliser (top container) and with corn steep
11 liquor (bottom container) as organic fertiliser. Lower: graph shows that more
12 than half of the plants grown with chemical fertiliser (white squares) died from
13 bacterial wilt disease; there were no wilted tomato plants among those grown
14 with corn steep liquor (black squares). Vertical bars represent the means and
15 standard deviation of three replications.

16

17

Supplementary methods

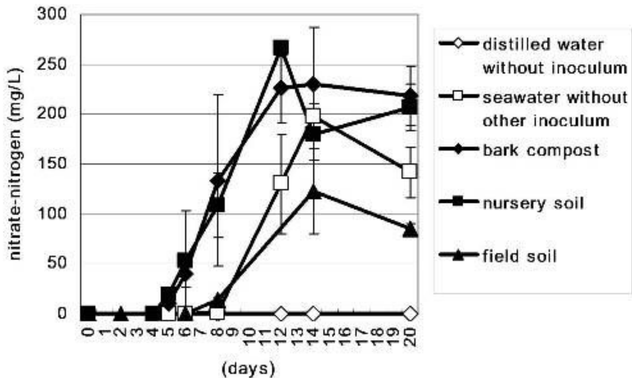
Equipment and settings

Fig. 4

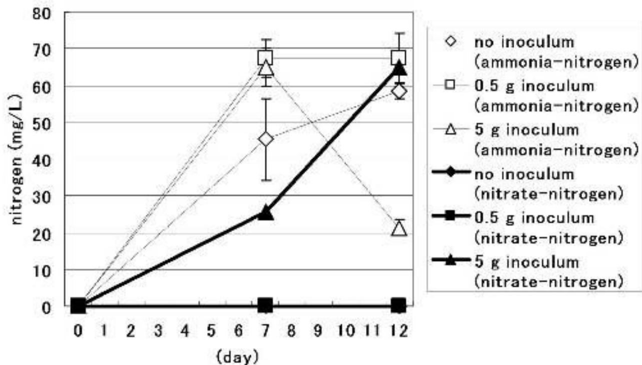
Tomato plant photos (i) were taken on 8 August 2006. Root pictures (ii) were taken on 15 June 2006. Enlarged pictures of roots (iii) were taken on 26 June 2006. Photos were taken with a digital camera (FinePix F440; Fujifilm, Tokyo, Japan) and resized with Microsoft Office Picture Manager software (Microsoft, Redmond, Washington WA, United States). The photos constructed were sized to 2160 × 2174 pixels (width × height).

Fig. 5

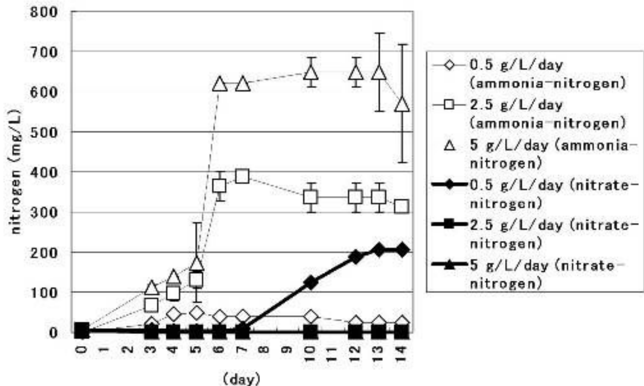
Tomato plant photo was taken on 5 October 2005 with a digital camera (FinePix F440; Fujifilm, Tokyo, Japan). It was resized with Microsoft Office Picture Manager software (Microsoft). The figure constructed with the photo and graph is 2160 × 3213 pixels (width × height).



nitrate-nitrogen (mg/L)
300
250
200
150
100
50
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
(days)
distilled water without inoculum
seawater without other inoculum
bark compost
nursery soil
field soil



nitrogen (mg/L)
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
11
12
(day)
no inoculum
(ammonia-nitrogen)
0.5 g inoculum
(ammonia-nitrogen)
5 g inoculum
(ammonia-nitrogen)
no inoculum
(nitrate-nitrogen)
0.5 g inoculum
(nitrate-nitrogen)
5 g inoculum
(nitrate-nitrogen)



800
700
600
500
400
300
200
100
0
nitrogen (mg/L)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
(day)
0.5 g/L/day (ammonia-nitrogen)
2.5 g/L/day (ammonia-nitrogen)
5 g/L/day (ammonia-nitrogen)
0.5 g/L/day (nitrate-nitrogen)
2.5 g/L/day (nitrate-nitrogen)
5 g/L/day (nitrate-nitrogen)

a(i)

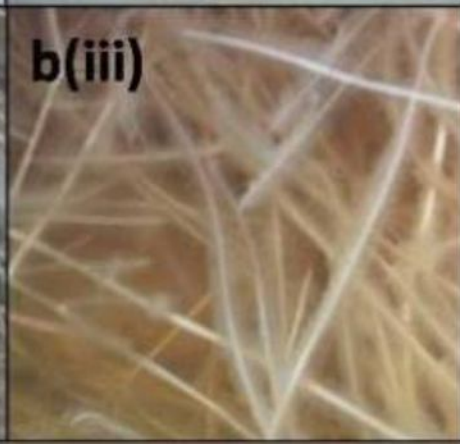
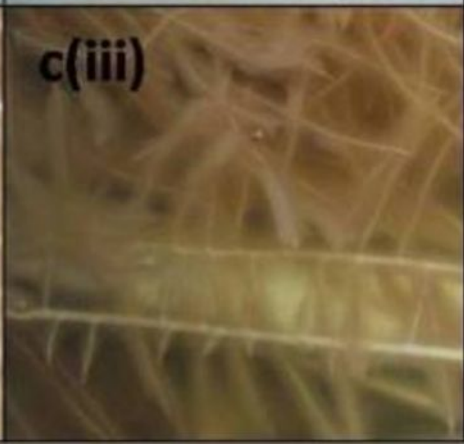
b(i)

c(i)

a(ii)

b(ii)

c(ii)

a(iii)

b(iii)

c(iii)

wilted tomato plants

16
14
12
10
8
6
4
2
0

0 1 2 3 4 5 6 7 8 9 10 11

(days)