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Review Article

Application of the Fermented Extract of Aloe vera Leaf Rind Dry-Crushed Powder for Agriculture Behind the Concept of D. R. Montgomery and A. Biklè, the Hidden Half of Nature; The Microbial Roots of Life and Health and Microbiome Agriculture Beyond an Organic Farming. Case Reports-1: Efficacy of Endophytically Fermented Extract from Twelve Crude Drugs of Kampo as an Official Plant-Root Growth Regulator (ALM Green) in Japan, Case Report-2: Application of the Fermented Aloe vera Dry-Rind-Powder and ALM Green in Circulation-Type Agriculture, and Case Report-3: Prophylactic Role of Aloe vera Whole Leaf Extract Certified by International Aloe Science Council as the Purified Leaf Juice Extract

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Abstract

In case report-1 we exhibited the efficacy of fermented 12-Kampo extract (ALM Green, pH4.64) agreed with the concept of "Silent Spring", by Rachel Carson published on September 1962, has been authorized as an official plant root-growth regulator in Japan; a registered number: 17851, to turf grass, strawberry and rose roots, without any toxicity for rats, mice, rabbits, carps, water fleas, silk worms and honey bees, on May, 1991. In case report-2, the fermented *Aloe vera* leaf rind-dry-crushed powder showed rapid pH decrease at pH 4.39, and the stimulation of seed-germination and rapid root growth suggested that the application of *Aloe vera* rind in circulation type-agriculture behind the concept of D.R. Montgomery and A. Biklè; The hidden half of Nature; The microbial roots of life and health. Microbiome agriculture beyond an organic farming creates the product enhancing flavor and nutrition. In case report-3 the *Aloe vera* whole leaf extract in pH 4.16 by *Aloe* Life International Inc., showed that aloin A and B contents of 0.032 ppm, and total *Aloe*-polysaccharide contents 8.23%. The *Aloe vera* whole leaf extract was certified International *Aloe* Science Council (IASC) as a Purified *Aloe* leaf juice extract, containing the mitigating several clinical symptoms by K. Koch, a Clinical nutritionist on 1999.

Keywords *Aloe vera; Microbiome Agriculture; Organic Farming; Kampo; ALM Green; Dry-Rind-Powder*

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Introduction

Two substances from *Aloe vera* leave: polysaccharides complex (clear *Aloe vera* gel) and yellow latex (rind) have been used to commercial manufacture products. *Aloe vera* gel typically is to make topical medications for skin condition; cosmetics and health-maintenance beverages. *Aloe* latex obtained in dried yellow powders; called resin, are worldly used as an over-the counter (OTC)-drug for relief of constipation as National Formula. A compound found in the semi-liquid-latex of *Aloe* spp., was the common ingredient in OTC's laxative products, mainly aloin, in the USA until 2002 when the FDA banned it, because manufactures failed to provide the necessary safety data. *Aloe vera* latex has potential toxicity with side effects occurring at some dose levels when ingested and applied topically, even toxicity may be less when aloin is removed by processing, *Aloe vera* gel ingested in high amounts may induce side effects, such as abdominal pain. International *Aloe* Science Council (IASC) determined that the content of aloin in *Aloe vera* gel beverage is safe in less than 10 ppm.

Endophytes are microorganisms that include micro-bacteria and fungi living within plant tissues without causing any immediate overt negative effects. Several bacterial genes found in the phyllosphere of *Aloe* have been reported. In the previous report [1], we determined that the growth of endophytic bacteria in MRS broth was evaluated in the presence of *Aloe vera* gel or leave rind at different time intervals. And the marked increase of fermentation by endophytic bacteria in *Aloe vera* gel provided short chain fatty acids (SCFAs), acetic, propionic and butyric acid, from the ether extract by GC-MSD analyses from *Aloe vera* gel, while the less concentration of SCFAs from *Aloe vera* rind extract were detected. Furthermore, we identified the following endophytic microbiota; *Bacillus cereus, B. licheniformis, Lactobacillus paralimentarium*, Yeast; *Clavispora lusitaniae*, in the gel [2].

Aloe arborescens is a native to southern Africa and is a vernacular name of shrubbery *Aloe*. In Japan where it was first introduced in the 17th century, it became naturalized. The taxonomy of *A. arborescens* is complicated by occurrence of interspecific hybrids both in the wild and in cultivation. On *A. arborescens var. natalensis* (kidachi *Aloe* in Japanese), Reynolds concluded that it is regarded *A. arborescens* as being a variable species, that to attempt to uphold varietal names, *A. arborescens var. natalensis* on overseas growth forms. In our previous report [3], we found that acetic, propionic, butyric acid were produced from the fermented extract of *A. arborescens* leaves with endophytic bacteria by GC/MSD analysis. Furthermore, quantitative analysis of phenolic compounds, specially aloin was found to be 14 ppm based on previous analysis. Reduction of aloin concentration (from ~600 ppm into 14 ppm in fresh tissue) and production of butyric acid were clearly indicated, while the phenolic materials were detected by GC/MS analysis, suggesting the potential efficacious role of endophytic bacteria in *A. arborescens* leaves to human clinical and medical homeostasis.

The utilization of *Aloe vera* leaf rind to produce a bioethanol could underpin the *Aloe vera* gel processing industries toward circular bioeconomy as an option of waste to wealth. Rajeswari and Jacob [4] investigated the previously delignified by lactase to have better accessibility holocellulose and an attempt has been made through this study to optimize enzymatic hydrolysis using crude cellulase produced from *Aspergillus* sp. Fermentation of saccharified liquid to ethanol using *Saccharomyces cerevisiae* (yeast) was performed using three different modes of inoculum application; Ca-alginate immobilized yeast suspension and immobilized yeast loaded in a packed bed reactor (PBR) under uniform process condition. Immobilized yeast in PBR has resulted in maximum ethanol yield with a fermentation efficacy of 76.14%.

In the present review article, we expressed the amino acid profiles by *Bacillus* sp. fermented with *Aloe vera* rind supplying nearby in Japan, and metabolomics profiling during biofilm development of *Bacillus licheniformis*, and *B. cereus*, and phosphate-solubilization with *Bacillus* sp. Furthermore, the efficacy of possible spore forming *B. licheniformis* as bio-stimulants for enhancing plant (maize) water under both normal and drought stress conditions was discussed. And we have been described fermented butyric acid from endophytic microbial in *Aloe vera* gel as an immunomodulator to cancer therapy and brain homeostasis.

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In case report-1 we discussed the efficacy of endophytically fermented extract, ALM green, from 12 crude drugs Kampo as a plant-root growth regulator (Pesticide) for 3-years in facultative anaerobic fermentation, an officially registered number in Japan; 17851, to turf grass, strawberry and rose roots, without any toxicity for rats, mice, rabbits, carp, water fleas, silk worms and honey bees on 1991. In case report-2 we demonstrated the pH comparison fermentation between small-cutting (A), crushed powder (B) and dry-crushed-powdered of *Aloe vera* rind (C) with ALM green at room temperature with supplying syrup in a sealed bottle with comparing to ALM-green for 5-months in winter. In case report-2, we strongly showed that the efficacy of *Aloe vera* dry rind powder (C) as a plant-seedling stimulator of germination and rooting in circulation-type agriculture, under the concept that "The hidden half of nature; the microbial roots of life and health" by DR. Montgomery and A. Biklè. Microbiome agriculture beyond an organic farming creates the products enhancing flavor and nutrition. In case reports-3 the *Aloe vera* whole leaf extract (*Aloe* Life International) in pH 4.16, demonstrated that aloin A and B contents of 0.032 ppm and total *Aloe*-polysaccharide contents 8.23%. The *Aloe vera* whole leaf extract was certified IASC as a purified *Aloe vera* leaf juice extract mitigating several clinical symptoms on 1999.

Metabolomics profiling during biofilm development of *Bacillus licheniformis* isolated from milk powder

Bacillus licheniformis is a major source of microbial contamination to daily industry, and biofilm formation by this spoilage bacterium aggravates the safety issues. Wang., *et al*. [5] reported that metabolomics analysis the dynamic changes of the metabolites and their roles during process of biofilm development of *B. licheniformis* at 55°C for 24h. Amino acid metabolism was quote active, with cooperation from lipid metabolism; carbohydrate metabolism and nucleotide metabolism. Amino acid biosynthesis provided significant contributions especially during early biofilm development from 8 to 12h. Dynamic metabolic process was established during biofilm formation by *B. licheniformis* isolated from milk powder.

The heat resistance of spores from biofilms of Bacillus cereus grown in tryptic soy broth and milk

Bacillus cereus is a concern for the daily industry due to the biofilm and spore formation during the manufacture of daily products, causing contamination in the final products. Heat stable spores of *B. cereus* are an important source of contamination for milk-derived products, such as milk powder and instant food formulas. Huang., *et al*. [6] suggested that more severe heat treatments are needed to control *B. cereus* spores from biofilm in the daily industry.

Phosphate solubilizing microorganisms and their potential for agricultural use

Microorganisms capable of solubilizing insoluble mineral phosphates in soil by the production of organic acids was reviewed by Nishio and Kimura [7]. The importance of the presence or addition of a sufficient amount of available organic matter was emphasized for the activation of inoculated or native microorganisms to promote the solubilization of insoluble phosphates in soil. The importance of the following process was also underlined: most of the solubilized phosphates are incorporated into the soil microbial biomass in the presence of organic matter and plants utilize the available phosphates later released from the microbial biomass after its turnover. The practical use of phosphate-solubilizing organisms in agriculture is discussed.

Tyrosine phosphatase TpbA controls rugose colony formation in Pseudomonas aeruginosa by dephosphorylating diguanylate cyclase TpbB

In biochemistry, dephosphorylation is the removal of a phosphate (PO_4^{3-}) group from an organic compound by hydrolysis. It is a reversible post-trans-stational modification. Dephosphorylation and its counterpart, phosphorylation, activate and deactivate enzymes

by detaching or attaching phosphoric esters and anhydrides. A notable occurrence of dephosphorylation is the conversion of ATP to ADP and inorganic phosphate. Tyrosine phosphatase TpbA in *Pseudomonas aeruginosa* PA14 is a negative regulator of the diguanylate cyclase TpbB. In activation of TpbA caused rugose colony morphology which is related to cell persistence in clinical infections. Pu and Wood [8] showed that TpbA controls phosphorylation of TpbB at both Tyr and Ser/Thr residues *in vivo* as detected by Western blot analysis. In activating the tyrosine phosphatase TpbA of *Pseudomonas aeruginosa* PA14 induces biofilm formation by 150-fold via increased production of the second messenger cyclic diguanyl acid (c-di-GMP). Ueda and Wood [9] showed the TpbA mutation reduces extracellular DNA (eDNA) and that increased expression of TpbA increases eDNA; hence eDNA is inversely proportional to c-di-GMP concentrations.

Identification of the genes controlling biofilm formation in the plant commensal Pseudomonas protegens Pf-5

Ueda., *et al*. [10] investigated the determinant genes controlling biofilm formation in a plant commensal bacterium, *Pseudomonas protegens* Pf-5, were identified by transposon mutagenesis. Comprehensive screening of 7500 transposon-inserted mutants led to the isolation of four mutants exhibiting decreased and five mutants exhibiting increased biofilm formation. The authors showed that sessile-motile lifestyle is regulated by divergent regulatory genes in *P. protegens* Pf-5.

Population-distribution of phosphate-solubilizing microorganisms in agricultural soil

Phosphorus (P) is an essential macronutrient for plant growth and is mainly present in agricultural soil in unavailable forms. Phosphate-solubilizing microorganisms (PSMs) increase soil P availability. Djuuna IAF., *et al*. [11] presented the population and type of PSMs and their relationships with soil characteristics in the agricultural soil of Manokwari. Twenty-one composite soil samples (0 - 20 cm) were collected at the rhizospheres of plants in the Prafi and Masni Districts. A dilution technique and plate count method on Pikovskayas agar medium were used to examine the PSM population, phosphate-solubilizing index (PSI), and various soil properties. The results obtained showed that the total population of phosphate-solubilizing bacteria ranged between 25 x 10^3 and 550 x 10^3 CFU/g of soil at all locations. The PSI of the isolates ranged between 1.1 to 3.6 mm, with the most efficient and highest PSI being obtained for *Bacillus* sp. and the lowest for *Pseudomonas* sp. Six isolates found at all locations were identified at the genes level: *Chromobacterium* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Caulobacter* sp., and *Aspergillus* sp. A correlation was observed between the number of PSMs and the level of soil P availability and moisture content, indicating an increase in soil P availability with a greater abundance of PSMs in soil.

A study of absorption and accumulation of amino acids in a rice seedling, using doubly labelled (15N and 13C) glutamine and valine

Nihei., *et al*. [12] studied the absorption and distribution of two kinds of amino acids, glutamine and valine, in a rice seedling to study the difference of availability of organic nutrients. When doubly labeled (^{15}N and ^{13}C) amino acids were applied, absorption and accumulation amount of ^{15}N in the rice seedling was the same but especially, in the case of glutamine, accumulation amount of ^{13}C in the seedling was lower than that of absorbed amount. The results suggest that a part of amino acids assimilated in the plant was lost as carbon dioxide through respiration and the assimilation of glutamine occurred more smoothly than that of valine.

Genetic background behind the amino acid profiles of fermented soybeans produced by four Bacillus spp

Jang., *et al*. [13] reported that strains of four *Bacillus* spp. were respectively inoculated into sterilized soybeans and the free amino acids profiles of the resulting cultures were analyzed to discern their metabolic trails. After 30 days of culture, *B. licheniformis* showed the highest production of serine, threonine, and glutamic acid; B. subtilis exhibited the highest production of alanine, asparagine, glycine, leucine, proline tryptophan, and lysine. *B. velezensis* increased the aminobutyric acid concentration to ≥ 200% of that in the control sample.

B. sonorensis produced a somewhat similar amino acid profile with *B. licheniformis*. Comparative genomic analysis of the four *Bacillus* strains and the genetic profiles of the produced free amino acids revealed that genes involved in glutamate and arginine metabolism were not common to the four strains.

Bacillus licheniformis FMCH001 increases water use efficiency via growth stimulation in both normal and drought conditions

Increasing agricultural losses due to biotic and abiotic stresses caused by climate change challenge food security worldwide. A promising strategy to sustain crop productivity under condition of limited water availability is the use of plant growth promoting rhizobacteria. Akhtar., *et al*. [14] investigated the effect of spore forming *Bacillus licheniformis* (FMCH001) on growth and physiology of maize (*Zea may* L. cv Ronaldinho) under well-watered and drought stressed conditions. The pot experiments were conducted in the automated high-throughput phenotyping platform PhenoLab and under greenhouse conditions. The author showed that seed coating with gram positive spore forming *B. licheniformis* could be used as bio-stimulants for enhancing plant water use efficacy under both normal and drought stress conditions.

Vinegar: A cheap and simple way to help plants fight drought

Water deficit caused by global climate changes seriously endangers the survival of organisms and crop productivity, and increases environmental deterioration. Plants resistance to drought involves global reprogramming of transcription, cellular metabolism, hormone signaling and chromatin modification. Kim., *et al*. [15] reported that an essential drought-responsive network in which plants trigger a dynamic metabolic flux conversion from glycolysis into acetate synthesis to stimulate the jasmonate (JA) signaling pathway to confer the drought tolerance. In *Arabidopsis*, the ON/OFF switching of whole network is directly dependent on histone deacetylase. In addition, exogenous acetic acid promotes *de novo* JA synthesis and enrichment of histone H4 acetylation: which influences the priming of the JA signaling pathway for plant drought tolerance. External application of acetic acid successfully enhanced the drought tolerance in *Arabidopsis*, rapeseed, maize, rice and wheat plants.

Acetic acid treatment enhances drought avoidance in Cassava (Manihot esculenta Crantz)

The external application of acetic acid enhances survival of drought in plants such as *Arabidopsis*, rapeseed, maize, rice, and wheat has been reported. On increased drought tolerance in woody plants such as a tropical cassava, the effect of acetic acid application on increased drought tolerance was investigated by Utsumi., *et al*. [16]. The acetic acid-treated cassava plants had a higher level of drought avoidance than water-treated control plants. Specially, higher leaf relative water content, and chlorophyll and carotenoid levels were observed as soils dried out during the drought treatment. Leaf temperatures in acetic acid-treated cassava plants were higher relative to leaves on plants pretreated with water and an increase of abscisic acid (stress hormone; ABA) content was observed in leaves of acetic acid-treated plants, suggesting that stomatal conductance and the transpiration rate in leaves of acetic acid-treated plants decreased to maintain relative water contents and to avoid drought. The external application of acetic acid enhances drought avoidance in cassava through the upregulation of ABA signaling pathway genes and several stress responses- and tolerance-related genes. The data support the ideas that adjustments of the acetic acid application to plants is useful to enhance drought tolerance, to minimize the growth inhibition in the agricultural field.

Rice acclimation to soil flooding: Low concentrations of organic acids can a barrier to radial oxygen loss in roots

Monocarboxylic acids produced by anaerobic microorganisms in waterlogged soils promote development of a barrier to radial O_2 loss

(ROL) in roots of some wetland species. Environmental cues triggering root ROL barrier induction, a feature that together with tissue gas-filled porosity facilitates internal aeration, are important to elucidate for knowledge of plant stress physiology. Colmer., *et al*. [17] investigated the comparatively low, non-toxic, concentrations of acetic, propionic butyric acids induce root ROL barrier formation in rice. Respiration in root tissues was not, or moderately, inhibited. Beyond a narrow concentration range, respiration declined exponentially and the order for EC_{50} was butyric, propionic, and acetic acid.

Role of acetic and/or butyric acid in the mechanisms of biological soil disinfestation

Biological soil disinfestation (BSD), or reductive soil disinfestation; achieved by amendment with organic materials such as wheat bran followed by flooding and covering the soil surface; has been used to control some soilborne diseases including *Fusarium wilt* and bacterial wilt of tomato by Momma., *et al*. [18]. During a BSD treatment, accumulation of acetic and/or butyric acid was detected with high-performance liquid chromatography. Survival of *Fusarium oxysporum* f. sp. *lycopersici* or *Ralstonia solanacearum* was suppressed by these organic acids.

Beneficial role of histone deacetylase inhibitor, butyric acid, as an immune-modulator and an adjuvant in cancer immunotherapy, Kampo, and brain homeostasis

In previous papers we described the medical and clinical efficiencies of fermented butyrate from endophytic microbiota in *Aloe vera* gel. And we have discussed the beneficial roles of butyrate, histone deacetylase inhibitor, as an immune-modulator and an adjuvant in the field of cancer immunotherapy, Kampo, and brain homeostasis [19].

Infectious history, maintaining that status within new contexts in which germs and hosts interact in new ways almost certainly will require us to bring ever more sophisticated technical wit, meta-genomics, and social intelligence to the contest: Super life Form, by J. Lederberg' concept [20].

Case report-1: Efficacy of endophytically fermented extract from twelve crude drugs of Kampo as an official plant-root growth regulator in agricultural fields

Twelve crude drugs (Herbal drugs in Japanese traditional medicine) in Kampo included in Japanese Pharmacopeia 18th are incubated in closed water container at room temperature for 3 years, and the fermented extract with endophytic yeast; *Pichia membranifaciens* in facultative anaerobiotic fermentation, showed pH 3.5, and was examined by gas-chromatography analysis to give acetic, propionic and butyric acid and other carboxylic acids. The water extract containing 3.5 - 3.8% from the 12 crude drugs (Kampo) exhibited plant hormone-like bioactivity at 0.1 nM concentration to turf grass, straw berry and rose roots as a root-growth stimulant without any toxicity for rats, mice, rabbits, carps, water fleas, silkworm and honey bees. And the mixed 12-crude-drug extract (Kampo), was registered by Ministry of Agriculture, Forestry and Fisheries of Japan as a plant-growth-regulator; A registration number: 17851 on May, 1991. Since then, the Kampo crude extract (ALM Green) agreed with the concept of "Silent Spring"; the environmental science book by Rachel Carson published on September 1962, has been widely recognized as a plant-root growth promoter in Japanese agricultural fields, and got the patents: 2004-28568 (2004-28568A) as "The plants use fertilizer" in Japan.

Case report-2: In the comparison of fermentation starting on 24, June, 2023 between small-cutting-rind (A, pH 4.64), crushed rind

(B, pH 4.64), and dry-crushed-powdered rind (C, pH 4.64) of *Aloe vera* with ALM green (D, pH 4.64) treating with syrup, the dry-crushed-powdered rind extract (C) showed pH at 4.43 and ALM green, pH 4.44 with biofilm formation after three months on 26, September. After 4-month on Nov.13, pH changed to A. 4.41, B. 4.42, C. 4.39. and D. 4.45. The germination and rooting experiment of *Brassica rapa var. perviridis*, an edible plant Komatsuna in Japanese were performed by using of the plant germination testing paper at 22°C for 5 days, in the concentration of 500 times dilution of C, and the mixture of C and D showed the strong germination and root-length stimulation. Then, germination and root-length stimulation experiment of *Spinacia oleracea*, Spinach, one of hard rooting plants in Japan, were performed for 10 days. The dry-powdered rind extract (C) and (C) with ALM green(D) mixture in 500-times concentration on the test paper made it possible to obtain rooting and germination stimulation of the mixture C and D at 53.42 cm, and C 66.39 cm, in 300 times dilution, while the other tested samples A, B and water did not show strong rooting. The safe and tasty agricultural products beneficial to health and had good quality as to make a contribution to balanced soil-making and the establishment of environmental circulation-type agriculture. The strong pH changing with biofilm-formation and decrease in aloin of the crush-dry-powdered of *Aloe vera* rind extract (C) in 300-times dilution and (C) with ALM green (D) in 500 times dilution could produce good-enough rooting of Komatsuna and Spinach. Water soluble bitter aloin in *Aloe vera* rind is easily oxidized into water-insoluble *Aloe*-emodin with microbiota and air-oxidation in facultative anaerobic fermentation [3].

The proposed title to patent cooperation treaty and patent application and accepted date; Dec. 25, 2023; Application of fermented extract of *Aloe vera* leaf rind crushed powder as plant growth regulator for the microbiome circulation-type agriculture.

Case report-3: The *Aloe vera* whole leaf extract in pH 4.16, demonstrated that Aloin A and B contents of 0.032 ppm, and the total *Aloe*-polysaccharide contents 8.23% by high performance liquid chromatography. The *Aloe vera* whole leaf extract was certified IASC as a purified *Aloe vera* leaf juice extract. The gift of nature: Whole leaf *Aloe vera* juice. Clinical Observations and Applications written by Ms. K. M. Koch, Clinical Nutritionist, with over 17-years of experience counseling patients in medical clinics, was first edited in 1999 in USA, in which the wide health maintenance with *Aloe vera* whole leaf extract was widely mention.

Aloe vera leaves has the phytoremediation activity to remove heavy metals from contaminated soil polluted with metal such as As^{3+}, Cd^{2+}, $Cr^{3+/6+}$, Cu^{2+}, Pb^{2+}, Hg^{2+}, and Ni^{3+}, due to the great reduction of these metals in contaminated soils [21].

In our previous articles we discussed that *Aloe vera* gel have been world-widely applied for medicinal and heath food [22-28]. On the other hand, *Aloe vera* rind containing a lot of yellow latex have been wasted out in the processing of *Aloe vera* gel products, because the rind contained a lot of water soluble aloin.

Bacillus licheniformis and *B. cereus* strains which are identified as the endophytic microbiota of *Aloe vera* gel, produced short chain fatty acids, butyric acids (histone deacetylase inhibitor). Furthermore, phosphate-solubilizing *Bacillus* sp. and water use efficacy via growth stimulation in normal and drought conditions with *B. licheniformis* and the molecular regulation of rice acclimation to soil flooding with acetic, propionic and butyric acid were widely discussed.

In "The hidden half of nature; the microbial roots of life and health", DR. Montgomery and A. Biklè discovered starting insights into the similarities between plant roots and the human gut. They said that we are not what we eat. We are all--for better or worse--the product

of what our microbes eat; The hidden half of nature; The microbial roots of life and health. They argue that earth's smallest creatures, microbes, could fundamentally change how grow food, what we eat and how we practice medicines. They uncovered stunning similarity between the root of a plant and the human gut that could transform the practice of agriculture and medicine. Taking readers deep into the science and history of agriculture and immunology, they showed microbes can strongly provide powerful solutions to the problems plaguing agriculture as well as our own bodies [29]. And the authors expressed that the part of the long-running controversy over nutritional differences between organic and conventional crops appears to arise from different definitions of what constituents a nutrient; the conventional definition of dietary constituents necessary for growth and survival, or a broader one that also encompasses compounds beneficial for maintenance of health and prevention of chronic disease. For assessing the effects of farming practices on nutrient density, soil health adds a more needed dimension; the provision of micronutrients and phytochemicals that support human health [30].

In case report-1 we described the efficacy of the mixed 12 crude drug Kampo extract (ALM Green) as a plant-root growth regulator, in agricultural fields without any toxicity for rats, mice, rabbits and carp, water fleas, silkworm and honey bees. In case report-2 we established the environmental circulation type of agricultural application uses under the concept of "The hidden half of Nature, the microbial roots of life and health" by DP. Montgomery and A. Biklè. Behind the concept of DP. Montgomery and A. Biklè, we discussed about the beneficial roles of microbiota fermented by endophytic microbiota in *Aloe vera* rind, producing short chain fatty acids; such as acetic, propionic and butyric acid, and amino acids, biofilm, phosphate-solubilizing bio-stimulants, which give us a deep insight for development of nutrient and agriculture applications. The beneficial properties of the endophytically fermented extract from *Aloe vera* rind, which is nearby supplied in Makino-hara city, Shizuoka, Japan could provide us a possible marketing opportunity in agricultural projects. In case report-3 the *Aloe vera* whole leaf extract in pH 4.16 (*Aloe* Life International), demonstrated that aloin A and B contents of 0.03ppm, and total *Aloe*-polysaccharide contents 8.23%. The *Aloe vera* whole leaf extract was certified IASC as a purified *Aloe vera* leaf juice.

Amino acid profiles produced by fermented *Bacillus* sp. in *Aloe vera*, metabolomics profiling during biofilm development of *B. licheniformis* and *B. cereus*, and phosphate-solubilization with *B.* sp. were widely discussed and reviewed. And water use efficacy via growth stimulation in normal and draught conditions with *B. licheniformis* was world-widely and seriously discussed. These beneficial properties of the fermented extract from *Aloe vera* rind, which is nearby supplied by *Aloe* Land Inc. in Makino hara-city, Shizuoka, Japan could provide a possible marketing research project in agricultural field. And *Aloe vera* rind extract with ALM Green, are widely used for agricultural soil. Microbiome agriculture beyond organic farming offers healthy plants production with micronutrients and contributes to soil health, and be used to increase yield, tackle pest and disease, and enhances flavor and nutrition. Based on above idea, we proposed the Patent Cooperation Treaty, the patent application date: Dec 25, 2023, in which beneficial uses of *Aloe vera* leaf rind for microbiological activities as a plant growth regulator; germination and rooting length stimulation, are stressed in circulation-type agriculture.

In an era of ecosystem degradation and climate changes, maximizing microbial functions in agroecosystems has become a prerequisite for the future of global agriculture. Interdisciplinary research strategies to optimize microbiome functions in agroecosystems provide novel ways to capitalize on core microbiomes for increasing resource-efficacy and stress-resistance of agroecosystems in circulation-type-agriculture [31].

Conclusion

The development of effective and rationally designed microbiome technologies for sustainable agriculture will provide new knowledge

on the key ecological and evolutionary interactions between plant species and their microbiome that can be harnessed for increasing agriculture productivity [32].

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