

Bio-Stimulant Effects of the Fermentation Extract by Endophytic Microbiota from Outer Leaf Layer in Aloe Vera

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Abstract

The acidic solution from the fermented Aloe vera outer layer is applied as an organic Aloe booster for agricultural field.

Keywords: Bio-Stimulant; Fermentation; Endophytic Microbiota; Aloe vera

Introduction

Prof J. Rockstrom, the director of the Stockholm Resilience Centre said "Science and the world at large are really concerned over all the extreme climate events highly society across the planets. But what worries us, even more, is the rising signs of dwindling planetary resilience". He said this failing resilience could make restricting global heating to the 1.5°C climate goal impossible and could bring the world closer to real topping points. Scientist said that the world was on the brink of multiple disastrous tipping points. The severe unusual weather we are experiencing could be a result of global warming and it continues every year, and we have to solve these severe weather conditions specially in the agricultural fields.

On the other-hands, we concerning with Aloe business know that Aloe drinking juice (soft drink) was made from Aloe vera gel containing aloin less than 10 ppm and the large amounts of outer layers are back to field. We searched the use of Aloe outer layer for bio-stimulants: the fermentation in water of Aloe outer leaves after air-dry-powdering, producing water insoluble yellow compound aloe-emodin from water-soluble bitter aloin. We achieved the fermented bio-stimulant containing short chain fatty acids; acetic, propionic and butyric acid. Now, we are trying to apply the fermented sample solution as bio-stimulants for dried agricultural field.

Bio-stimulants are recently formulated as the 3rd Pesticides by Ministry of Agriculture, Forestry and Fisheries on April, 2025, in Japan. Bio-stimulants are positioned as one of the Natural Fertilizer products for agricultural suppliers in EU Bio-stimulant law. The world market of bio-stimulants is widely growing in EU and USA.

Endophytic microbiota of Aloe vera

Health between human and plant is closely combined with microbiota from the soil in front of garden to intestinal circumstance, and the hidden half of microbiota were expressed by MB. Montogomery and A. Bircke in their book: The hidden half Nature: The microbial

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Roots of Life and Health. In about 1940, the organic agriculture started and now, the activation of endo-phytic microbiomes in agriculture are stimulated and the research was largely achieved for immune regulation between microbiome and plants [1].

Endophytic bacteria have been identified in Aloe vera and some of these bacteria have been antibacterial activities against the growth of pathogenic bacteria: *Staphylococcus aureus* and *Escherichia coli* [2].

Aloe vera extracts have antimicrobial and anti-fungal activities, which may be able to treat minor skin infections. In spite of these anti-microbial activities, the inner leaf gel containing acemannan: non-digestible polysaccharide, leads to fermentation with endophytic bacteria and results in bacterial growth promotion while no short chain fatty acids were resulted in the ether extract of the fermentation broth with rind. Butyric acid was identified by GC/MSD analysis from ether extract of the gel fermentation broth, expecting multiple beneficial effect of butyric acid at intestinal and extra-intestinal level. The investigation exhibits that daily intake of the butyric acid fermentation extract from Aloe vera inner gel with endophytic bacteria may provide the possible potentiate preventive and therapeutic roles in human health [3].

Effect of endophytic bacteria (*Lactobacillus fermentum* and *L. helveticus*) in Aloe vera gel on human health was investigated [4] and it was very interested with Aloe vera endophytic microbiota which mediated between the human intestinal circumstance and the soil growing Aloe vera. Furthermore, the endophytic microbiota fermented polysaccharides producing short chain fatty acid, butyric acid [5].

Elucidation of the translation mechanism by microRNA in plants: Effect of endophytic microbiome in Aloe vera

microRNA(miRNA) is endogenous approximately 23nt RNAs that play important gene regulatory role in animal and plants by pairing to the mRNAs of protein-coding genes to direct their post-transcriptional repression. The review outlines the current understanding of miRNA target recognition of protein-coding genes [6].

Translational repression of miRNA in plant

miRNA is one of the small none-cording RNA which have not coding-protein molecule having small genome. miRNAs bind Argonaute protein in order to form RNA-induced silencing complexes (RISCs) that can silence the expression of complementary mRNA. Plant miRNAs can mediate the cleavage target mRNAs, as well as the repression of their translation. By *in vitro* system prepared from plant culture cells we biochemically dissect the mechanisms by which Arabidopsis thaliana ARGONAUTE1 RISC (AtAG1-RISC) has the ability to repress translation initiation without promoting de-adenylation or mRNA decay [7]. The translational repression mechanism of miRNA mediated gene regulation, which is common in animals but also exists in plants, as not well understood mechanically. Conversely, miRNA-directed mRNA cleavage by the RNA-induced silencing complex (RISC) is common in plants, but also occurs in animals [8]. Isolation of pre-mRNA-splicing (RNA-induced silencing, RID1) in translation mechanism is much important in specially, crown-growth of useful plants. Identification of RID1 in *Arabidopsis* was investigated [9]. miRNA has various activities modulating cell growth, development and differentiation concerning human diseases such as cancer and heart. Since 1995 when miRNAs were detected, the studies on miRNAs were widely developed for pathophysiological mechanisms [10], A Yagi., *et al.* reported miRNA regulation and case report with Aloe vera supplement [11] and H Miyagi., *et al.* reported possible prophylaxes of Aloe vera gel to miRNA regulation in case report [12].

Effects of endophytic microbiota in Aloe vera as an immunostimulant for human health

Polysaccharides are classified as follows: glycoproteins, glycosyl lipids, carboxyl chitosan, mucopolysaccharide inclusion and acemannan in Aloe vera gel is a mucopolysaccharide inclusion with negative charge like carboxylate function which contains protein, lectin and water. The lectin, having active glycoprotein (14 KD: protein 82% and carbohydrate 11%) on polyacrylamide electrophoresis, showed a proliferation promoting activity on human and Hamster cells *in vitro* [13].

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Plant exome-like nanovesicles: Emerging therapeutics

The antioxidant effect of small extracellular vesicles (EV) is derived from Aloe vera peels for wound healing. The findings reveal that Aloe EV could activate the antioxidant defense mechanisms and wound healing process via the Nrf2 activation and suggest the Aloe EVs are promising as a potential agent for skin regeneration [14].

Aloe EV as carriers of photoinducible metabolites inhibiting cellular phototoxicity

This study investigates the phototoxic effects of EVs from *Aloe arborescence, A. barbadensis* and *A. chinensis* on the human melanoma cell line SK-MEL-5 for-cussing on their anthraquinone content, recognized as natural photosensitizers. These findings suggest that EVs from *A. arborescens, A. barbadensis* and *A. chinensis* hold promise as potential photosensitizers thus highlighting their potential for future application in photo-dynamic cancer and providing valuable insights into the possible utilization of plant-derived EVs for therapeutic purpose [15].

The potential of EVs derived from Aloe in reprogramming macrophage phenotypes was discovered. Pro-inflammatory macrophages undergo a transited towards an anti-inflammatory immune phenotype through phagocytosing and internalizing these Aloe vera-derived EV nanovesicles. This transition results in the release of anti-inflammatory IL-10 effectively curbing inflammation and fostering lung tissue repair. These finding firmly establish the immunomodulatory impact of Aloe-derived EV nanovesicle on macrophages, proposing their potential as a therapeutic strategy to modulate macrophage immunity in bacterial pneumonia [16].

Roles of microRNA-21 of aloin in cardioprotective and skin wound healing

The long non-coding RNA (Small nucleolar RNA host-gene: the long non-coding RNA SNHG1)/(miR-21 axis) mediates the cardioprotective role of aloin in sepsis through modulatory cardiac cell viability and inflammatory responses. The findings indicated that aloin exerts protective effects in sepsis-related myocardial damage through regulating cardiac cell viability and inflammatory responses via regulating the SNHG1/miR-21 axis [17].

Role of microRNA-21 in skin wound healing

Micro vesicles (MVs) have a diameter of 100 - 1,000 nm and are actively generated by a variety of cells, including epithelial cells. They mediate cell to cell communication by transferring microRNAs as chemokines and cell surface receptors from origin cells to target-cells. The promotion of the fibroblast differentiation, anti-inflammatory enhancement of collagen synthesis and the reepithelization of the wound. micRNA-21 may serve as a potential therapeutic target for wound healing [18].

Aloe vera increase mRNA expression of antioxidant enzymes in cryo-presented bovine ovarian tissue and promotes follicular growth and survival after *in vitro* culture

10% Aloe vera increase the expression of mRNA for PRDX, GPX-1 and SOD in vitrified ovarian tissues maintenance follicular survival and promotes activation and development of follicles after *in vitro* culture of vitrified bovine ovarian tissue [19].

Effects of Aloe vera extract on growth, viability, ultrastructure and expression of mRNA for antioxidant enzymes in bovine secondary follicles cultured *in vitro*

2.5% Aloe vera increases antrum formation in cultured bovine secondary follicles and 2.5% and 5% Aloe vera extract improves *in vitro* secondary follicle viability rate. Aloe vera 2.5% and 10% Aloe vera increases the levels of SOD and GPX1 mRNA. Follicles cultured with Aloe vera 2.5% extract have well presented in ultra-structure. 2.5% Aloe vera increases of mRNA for SOD in cultured secondary follicles, but higher concentrations of Aloe vera have negative effects of follicular ultrastructure [20].

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Application of the fermented extract of Aloe leaf dry-crushed powder for agriculture

Application of the fermented extract of Aloe vera leaf dry-crushed powders for agricultural behind the concept by DE Montogomery and A. Bickle. The hidden half of Nature. The microbial roots of Life and Health, and Microbiome Agriculture Beyond on Organic Farming. Case reports 1. Efficiency of endophytically fermented extract from twelve crude drugs of Kampo as official plant roots grown regulator (Aqua-life Modifier: 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 extract.

Case report 1: ALM green composed from 12 species of Syoyaku (herbal medicines) in Japanese Pharmacopeia, were incubated for 3 years in dark and closed box at room temperature to get a thin layer of microbiome, yeasts (Pichia membranifaciens) to get the anaerobic fermented solution at ~pH 3. The solution (ALM green) was authorized as a root- growth controller in following plants-roots by Japanese Ministry of Agriculture, Forestry, and Fisheries: weeds, rose and strow berry. Pesticide registration number: 17851.

Case report 2: The outer layer of Aloe vera leaves after the inner gel were scratched out to make Aloe vera soft drink, were dried and powdered at room temperature. The water solution of dried powders was fermented with the endophytic microbiota to get short chain fatty acids for three months at room temperature. The acidic solution with Aloe vera outer layer was applied as an organic Aloe vera booster or a bio-stimulant to agricultural field [21].

Conclusion

Bio-stimulants effects of the fermented by endophytic microbiota from the outer leaf layer in Aloe vera produced short chain fatty acids, such as acetic, propionic and butyric acids, are one of bio-stimulants for dried agricultural fields. Bio-stimulants, which are recently formulated as the 3rd pesticides in Japan, were positioned as one of the natural fertilization products applied as an organic Aloe booster for agricultural suppliers. Application of Aloe booster and ALM green may have a big impact on agriculture.

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