

Short Chain Fatty Acids from Fermentation By Endophytic Bacteria in *Aloe Vera* Leaf Rind and Gel

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INTRODUCTION

Anatomically, *Aloe vera* leaf can be divided into three zones: the rind, the mesophyll, and the gel (parenchyma). The rind contains multiple layers; waxy cuticle, chlorophyll-rich region below the waxy cuticle. The rind is rich in oxalic acid. Just below the rind mesophyll lies. This contains the xylem and phloem vascular bundles and phenolic anthraquinones and chromones in highest concentration of aloe leaf. The gel rich in nutrient, is in inner parenchyma portion of *Aloe vera* leaf. In order to understand clearly endogenous microbiota of *Aloe vera* leaf, it is needed to recognize that *Aloe vera* leaf contains three parts in addition to the gel which have significant viscosity in aqueous solution in the range of 0.05 to 0.5% total solids: (1) the exterior surface of the leaf; (2) the outer, sub-cuticular portion of rind; and, (3) necrotic portions of leaf. Each of these has its own unique microbiota well adapted to grow at the particular temperature and pH of that part of the leaf, and using the nutrients of that part of the leaf. The rind and attached mesophyll are readily stripped away from the gel parenchyma.

Aloe vera inner gel are very rich in carbohydrates, especially polysaccharide acemannan, which supply suitable sources for microbiota growth. Yagi A. and Yu BP. reported importance of microbiota for immunomodulator and aloe's putative efficacy based on the modulation of gut microbiota status^[1].

Since *Aloe vera* belongs to the group of crassulacean acid metabolism plant, most organic acids detected are malic, citric, succinic and tartaric acid due to C₄-CO₂ assimilation. In an earlier report cardiogenic constituent of *Aloe saponaria*, calcium isocitrate, showing positive inotropic effects on isolated cardiac muscle, was revealed^[2]. On microbiology of *Aloe vera*, Waller TA. group reported that the endogenous and exogenous organisms to reduce and destruct specific aloe-associated commensal organisms, such as *Micrococcus*

ABSTRACT

Aloe vera extracts have antimicrobial and anti-fungal activities, which may be able to treat minor skin infections. In spite of these antimicrobial 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 detected in the ether extract of the fermentation broth with the rind. Butyric acid was identified by GC/MSD analysis from ether extract of the gel fermentation broth, expecting multiple beneficial effects of butyric acid at intestinal and extra-intestinal level. Present investigation exhibits that daily intake of the butyric acid fermentation extract from *Aloe vera* inner gel with endophytic bacteria, may provide the possible potential preventive and therapeutic roles in human health.

Key words: Short chain fatty acids; Butyric acid; Endophytic bacteria fermentation; *Aloe vera* inner gel

sp., were screened based upon industrial importance of *Aloe vera* gel, and the organisms on or in *Aloe vera* were identified in nine aloe industrial preparations with frequency of isolation^[3]. Effect of bacterial growth upon the organic acid content of *Aloe vera* gel was examined by HPLC analysis, and malic acid and lactic acid were revealed as markers for freshness or spoiled aloe material in *Aloe vera* gel preparations by Pelley RP. group^[4]. Under poor handling conditions in the presence of lactic acid bacteria, malic acid can be broken down to form lactic acid^[5]. Evaluation of *Aloe vera* extract hydrolyzed (AHE) with *Thermoanaerobacter sp.* strain USBA-018 as a culture medium for lactic acid production was demonstrated by Gomez-Gomez JA. group and AHE exhibited the production of large quantities of lactic acid as a low-cost substrate^[6]. International Aloe Science Council determined that value of malic acid between 818 to 3,427 mg/L in *Aloe vera* gel preparations (solids, 105°C nonvolatile: $\geq 0.46\%$), an average amount of 2,029 mg/L, in 2000.

Endophytes are microorganisms that include bacteria and fungi living within plant tissues without causing any immediate overt negative effects. Several bacterial genera found in the phyllosphere of *Aloe* have been reported. Amir H. group showed that the bacteria of the phyllosphere microflora from the blended *Aloe vera* leaf from New Caledonia was mainly presented with *Enterobacteria*, *Klebsiella*, *Lactobacillus* and *Streptococci* as the predominant genera^[7]. Prakash O. group proposed a yellow-strain-positive, non-motile, non-endospore-forming, spherical endophytic actinobacterium, isolated from *Aloe vera* inner leaf tissues collected from Pune, India, as a novel species of the genus *Micrococcus* and suggested the name *M.aloeverae sp. nov.*, with strain AE-6^T as the type strain of the species^[8]. Akinsanya MA. group applied the high throughput techniques of next generation sequencing (NGS) to the metagenomics study of endophytic bacteria in *Aloe vera* plant, and the use of novel shotgun 16S rRNA gene by NGS has revealed the overall richness and diversity of microbiota communities in plant tissues to encompass both the culturable and unculturable endophytic bacteria. The analyses revealed *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* as the predominant genera in three tissues; leaf, stem and root, in *Aloe vera*^[9]. Kim YW. group revealed that five novel *Lactobacillus brevis* strains; probiotics originating from *Aloe vera* leaf, were isolated from naturally fermented *Aloe vera* leaf flesh, and expressed high levels of the glutamate decarboxylase gene which produces a beneficial neurotransmitter, γ -amino butyric acid^[10].

Isolation of endophytic *Ralstonia sp.* from *Aloe vera* gel and its antimicrobial activity were studied by Sinha A. group. The study was conducted to determine the significance of endophytic bacteria in *Aloe vera* gel for the prevention and treatment of various infections caused by bacteria. Ribosomal database project 10.28 release showed that nearest neighbour for VITNARMJ-3 is *Ralstonia pickettii* (Phylum: *Proteobacteria*), accession no. AY741342 (sequence similarity 94.6%). The bioactive compounds obtained from isolate VITNARMJ-3 can be effectively used in food and pharmaceutical industries against various bacterial pathogens^[11]. Akinsanya MA. group revealed for the first time the endophytic bacteria communities from *Aloe vera*; *Pseudomonas hibiscicola*, *Macrocococcus caseolyticus*, *Enterobacter ludwigii*, and *Bacillus anthracis* that produce bioactive compounds with high 1,1-diphenyl-2-picryl-hydrazyl scavenging properties, (75-88%) and *Bacillus tequilensis*, *Pseudomonas entomophila*, *Chryseobacterium indologenses*, and *Bacillus aerrophillus* that produce bioactive compounds with antimicrobial activities against bacterial pathogens^[12].

Present study reveals the key to understand how an *in-vitro*-

synthesis of short chain fatty acid, butyric acid, plays important roles to understand the endophytic microbial-plant host interaction by the fermentation within *Aloe vera* inner gel and *Aloe vera* gel juice has a pivotal role in the context of gut/body health in quality of life.

MATERIALS AND METHODS

Aloe vera sample preparation

Aloe barbadensis Mill. (*Aloe vera*) leaves collected from the medicinal farm of the department of pharmacognosy, Faculty of Pharmacy, Tanta University. A voucher specimen was deposited at the international herbarium of Faculty of Science, Tanta University, Egypt. *Aloe vera* leaves (127 g) were washed with hypochlorite solution (0.02%) and rinsed with water. After trimming off butts and tips, and culling of diseased or damaged leaves, the rind is removed by hand, to produce gel fillets. These fillets are lightly ground and filtered cellulose filter to give crude *Aloe vera* gel juice. Depulping rind were cut into slice to fermentation.

Fermentation

Fermentation broth using MRS medium at 25°C was cultivated with gel juice (30 mL) and rind (18 g) at pH 4.2 and 4.2, respectively, for 72 hr, and extracted with ether. The ether extract layer was evaporated to give the ether extract 17 mg from rind broth and 2 mg from gel broth. Growth curves in the presence of the fermented *Aloe vera* gel or rind were drawn at different time intervals including 0, 2, 4, 6, 8, 24, 48, and 72 hrs.

GC-MSD analysis

Sample preparation: The ether extract of the fermentation broth was dissolved in 2 ml hexane, sonicated for 10 min, methylated by sodium methoxide, then clear hexane layer filtered through PTFE membrane. Sample were diluted 1:20 with hexane before injection under SIM conditions.

Instrument used: GC/MSD 5977A, Agilent, USA; Column used: Agilent, HB 5ms -60°C-325°C (350°C): 60 m × 250 μ m × 0.25 μ m; Oven program: 30°C for 3.7 min, then 5°C/min to 200°C; Inlet: Split/Splitless mode, Liner Agilent 5190-2294: 990 μ L.

Inlet temperature: 250°C, Auxiliary temperature: 250°C.

MS information: Acquisition Mode: SIM/SCAN, Solvent Delay: 3.7/min, Scan parameters: Low Mass: 29, High Mass: 550.00.

Environmental condition: Temp. : 24°C, Humidity: 51%.

RESULTS AND DISCUSSION

Butyric acid from ether extract of the fermentation broth with *Aloe vera* gel juice (AVJ) were identified by GC/MSD analysis. Butyric acid in the ether extract of *Aloe vera* gel fermented, showed 91% of library matching with an authentic sample at retention time: 13.625, while no short chain fatty acids were detected in the ether extract of the fermentation broth with *Aloe vera* rind. Recent paper by Cushing K. group evaluated the basic immunologic effects on mucosal inflammation of butyrate and a role in maintaining healthy colon barrier function, which prevents the flux of potentially pathogenic microbes across the epithelium^[13]. Butyrate produced from the fermentation of dietary fibers by microbiota was reported as an histone deacetylase inhibitor by Khan S. and Jena G. group and butyrate has a pivotal role in the context of "gut-body health"^[14]. Berni Canani R. group reported that butyric acid production is dependent on diet and intestinal microflora composition, and it is also able to modulate intestinal microflora through regulation

of lumen pH and to exert many beneficial extra-intestinal effects through epigenetic mechanisms^[15]. Ji J. group showed that the large bowel microbial fermentation product, butyrate, facilitates M2 macrophage polarization, *in vitro* and *in vivo*^[16]. Chang PV. group exhibited that butyrate can modulate the function of intestinal macrophages and the most abundant immune cell type in the lamina propria^[17]. *Aloe vera* has been used widely in herbal edible plants and the flesh rind contains phenolic compounds, such as barbaloin, showing antibacterial and anti-fungal activities. Yagi A. reviewed that *Aloe vera* inner gel rich in carbohydrates is prone to oxidation and fermentation, due to deprivation of plant-defensive phenolics in the rind^[18].

It was important to ascertain the growth time of the lactic acid bacteria in order to determine the stop point of fermentation. Therefore, growth of endophytic bacteria in MRS broth was evaluated in the presence of *Aloe vera* gel or leaf rind at different time intervals. As shown in Figure 1, marked increase in the optical density (660 nm) of endophytic bacteria grown in the presence of *Aloe vera* gel was recorded. Pogribna *et al*^[19] and Pelley *et al*^[4] reported that enhanced growth response to *Aloe vera* supplementation was noticed at 24 h for the bacterial cultures. Similarly, Kim *et al*^[10] reported that *Lactobacillus brevis* strains isolated from naturally fermented *Aloe vera* leaf flesh expressed high levels of the glutamate decarboxylase (GAD) gene which produces a beneficial neurotransmitter, γ -aminobutyric acid (GABA).

Fermentation by endophytic bacteria in *Aloe vera* gel provided butyric acid from the ether extract by GC-MSD analysis, and suggested that the daily application of fermentation extract of *Aloe vera* gel may be beneficial to putative prophylaxis of butyric acid for health and QOL as an immune modulator. Our findings present a big evidence to *Aloe vera* gel as one of brain therapy in daily diet.

Further research will concern about the identification of microbiota producing butyric acid in the fermentation.

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CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

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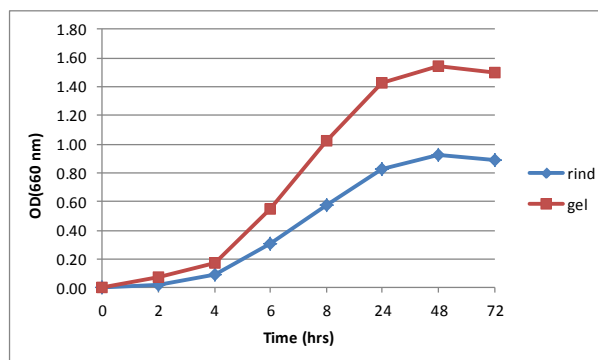


Figure 1 Growth curves of endophytic bacteria from aloe vera gel and rind showing changes in the biomass.

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