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Various growth attributes of *Escherichia coli* cultures supplemented with *Aloe vera* as substrate

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Abstract

In present investigation, extracts of *aloe vera* plant organs incorporated into the TY-growth media and its effects on sub-merged *Escherichia coli* (*E. coli*) k_1 fermentation are studied. The 18-hours cultures maintained with extracts of different aloe vera fresh organs (12.5%, v/v) in TY_0 (1% Bacto-trypton, 0.5% NaCl, 0.5% yeast extract), TY_1 (⅛ TY_0), TY_2 (TY_1 + leaf-peel extract), TY_3 (TY_1 + root extract) and TY_4 (TY_1 + leaf-gel extract) medium. The cell multiplication observed high in leaf peel extract base TY_2 cultures. Among the fermented biochemical analysis, maximum reducing sugars observed in TY_4 than other medium ($p \geq 0.05$), while flavonoids in TY_3 medium non-signifiantly. The total phenolics noted higher in both TY_3 and TY_4 medium. Similarly hydrolytic enzymes have shown differential activities among the different cultures like as *amylases* activity in TY_2 (gel), *xylanases* in TY_4 (root) and *lipases* in TY_3 (peel) medium measured significantly high. Overall, it is concluded that aloe vera is the best fermentation substrate for the production of various extra-cellular enzymes and essential substances. Even aloe vera is anti-bacterial agent, while its variant concentration in the fermentation medium has differential impacts on the propagation of micro-organisms.

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Introduction

The aloe vera [*Aloe vera* var. *barbadensis* (Mill) L.] is thick and short stemmed shrub plant. From the last few decades *Aloe vera* has been topic of research regarding to its therapeutic properties. As it is made of wide range of organic compounds which could be grouped into complex sugars (inside the leaves in gel) performs immune - stimulating action, anthraquinones substance (present in skin of leaves) have laxative action and several other substance including minerals, vitamins, amino acids, and enzyme (Grundmann, 2012; Manoj Kumar *et al.*, 2011). With this specific composition of *Aloe vera* plant, it has showed-up antimicrobial activities (Grindlay and Reynolds, 1986; Sierra-García *et al.*, 2014). It may be used to treat minor skin-infection (Parvu and Parvu, 2011; Rosca-Casian *et al.*, 2007; Ueno, 2000), to inhibit growth of *Streptococcus, Mycobaterium* and *Shigella* species (Maccaferri *et al.*, 2012; Wang *et al.*, 2009).

It is also used as anti-inflammatory, antibiotic, anti-diabetic, regenerative and anti-cholesterolemic (Halteh *et al.*, 2016).

In spite of these activities, the parts of this plant especially their leaf gel is oxidize-able at various temperatures to initiate the fermentation process. Both hydro-conversions and pyrolysis are being low-cost and eco-friendly management methods of such agro-biomasses including their agriculture waste materials (Strubinger *et al.*, 2017). This phenomenon occurs in the phenolic compound, which is related to its defense mechanism (Kumar *et al.*, 2012). There oxidation of phenolic compounds involved to ablate the antimicrobial activity of *Aloe vera* afterwards to allow the bacterial growth. The growth of lactic acid bacteria in aloe flesh fermentation is already studied, while these bacteria proliferate after the deterioration of antimicrobial activities of aloe's tissues (Saibuatong and Phisalaphong, 2010).

The agro-wastes of aloe vera could be a new renewable and alternate energy source to solve energy needs and also reduce the agro-environmental problems. In comparisons to other agro-wastes, the *Aloe vera* has different biomass which could be the source of easiness for the conversion as well as production of fermentation metabolites (Strubinger *et al.*, 2017; Trabold and Babbitt, 2017).

Nowadays, the *Aloe vera* has been attracting both local as well as research community's interest significantly due to its nutritional and medicinal potential (Paez *et al.*, 2000). More than 300 genus *Aloe*'s species are most commonly known and cultivated (Cuvas-Limón *et al.*, 2016). It contains carbohydrates as mannose polymers (accemananos) and various vitamins (A, B1, B6 and C (García-Hernández *et al.*, 2006). Its soft drinks are enriching with healthy nutritional qualities containing amino acids and useful minerals. Both fresh as well as dry plant wastes of *Aloe vera* are rich with various nutrient components might be helpful in the manufacturing of the fermentation products. If the fermentation medium is supplemented with aloe plants parts could be helpful in the manufacturing of healthy foods with pharmacological properties (Baek *et al.*, 2010).

By keeping in view the above findings, aims of the present study are to investigate the *E. coli* fermentation cultures supplemented with various parts of *Aloe vera* plants as a carbon source. The analysis of various enzymatic products in the aloe's organ based fermentation cultures might be useful in the way to search out the resources for production of human health based beneficial compounds. Moreover, it could be a source of more beneficial products as well as cost effective probiotic productions with enhanced therapeutic valued end-products from *Aloe vera*.

Materials and methods

Preparation of fermentation substrate

The 3-4 months old plants of *Aloe vera* were collected from plant nursery located in the vicinity of university. These fresh plant materials washed with tap-water to remove the dirt. Different organs or parts of plants were excised and weighted exactly 50g leaf-peels (without gel), root and leaf-gel also. The roots and leaf-peel were divided into pieces with a fine knife.

Each was crushed with pestle and mortar in equal volume of sterilized dH_2O. After grinding, mixture was centrifuged at 4,000 rpm at room temperature for 10 min. The supernatant was stored in @ 4°C for next use, while pallet was discarded.

Preparation of aloe vera based fermentation culture

The aloe vera based fermentation cultures were raised in liquid nutrient TY-medium (10g l^{-1} Bacto-trypton, 5g l^{-1} yeast extract, 5g l^{-1} NaCl, pH 7.0]. The 12.5% of *Aloe vera* extracts (leaf-peel, roots and leaf-gel) was maintained in ⅛ strength of TY-medium. The TY-medium itself was used as standard positive control as well as ⅛ TY-medium as minimum nutrient control culture (Table 1). All of these media were sterilized for 20 minutes at 121°C and cool down to room temperature before use.

Table 1. Composition of different medium used for *Escherichia coli* growth supplemented with aloe vera as substrate.

SN	Medium	Composition of medium
01.	TY_0	1 % Bacto-trypton, 0.5% NaCl, 0.5% yeast extract in dH_2O (w/v)
02.	TY_1	⅛ TY_0 in dH_2O (v/v)
03.	TY_2	TY_1 + 12.5% leaf-peal extract (v/v)
04.	TY_3	TY_1 + 12.5% roots extract (v/v)
05.	TY_4	TY_1 + 12.5% leaf-gel extract (v/v)

Note: Each culture maintained in 4 replicates and volume of each replicate adjusted 50 ml before autoclave.

Micro-organism and preparation of inoculum

The *Escherichia coli* (*E. coli*) k_1 was used as a fermentation organism from glycerol stock. It was activated in 2ml TY_0 medium with incubation at 37°C with constant 250 rpm shaking for overnight. Its 100µl was sub-cultured in 5ml TY-medium and incubated at same conditions for 30 minutes and it was used as a master culture. With this culture, the media enlisted in table 1 were inoculated to rise with final OD600 0.02. After inoculation, cultures were incubated for 18 hours at 37°C with 250 rpm constant shaking.

Harvesting of cultures

After 18 hrs, the incubated cultures were harvested. Before going to harvest the cultures, their OD600 was taken. The cultures were centrifuged at 5,000 rpm for 10 minutes.

The supernatant of cultures transferred to the clean dark-colored glass-bottles and pallet was discarded. The supernatant was stored at 4°C for next use. In actual it is used as a sample for the measurements of various biochemical and activities of enzyme produced during fermentation. These samples were kept at 4°C till the completion of this experiment.

Biochemical analysis

The collected supernatant was subjected for various biochemical analyses. Like as, the total sugars were determined by mixing 1ml supernatant with 2.50ml concentrated H_2SO_4 and 5ul 80% phenol in a test tube. Stand the mixture at room temperature for at least 10min than absorbance was read at 485nm (Dubois *et al.*, 1956).

The reducing sugars were measured by mixing 1ml sample with 2.0ml of DNS reagent. After heating in boiling water bath for 15min, its OD 540 was read (Miller, 1959). Similarly, protein contents were measured by following (Lovrien and Matulis, 2004). Exact 1ml sample was mixed with 2.5ml alkaline copper reagents than shacked thoroughly at room temperature. After 10min, 0.25ml folin reagents (1:1) were added and absorbance was read at 750nm.

The free proline (Abrahám *et al.*, 2010), glycinebetaine (Grieve and Grattan, 1983; Valadez-Bustos *et al.*, 2016) and total flavonoids (Ira *et al.*, 2014) were also analyzed.

The total phenolics were also measured by adding 1ml sample with 1ml 5% Na_2CO_3 and 0.5ml folin reagents. The absorbance was taken at 760nm (John *et al.*, 2014). For ascorbic acid analysis, 1ml sample mixed with 1.25ml asco-buffer and mixture was incubated at room temperature for 15min. The 0.25ml H_2O_2 was added and absorbance was read at 290nm immediately (Lucas, 1944). The antioxidants were determined by mixing 0.2ml supernatant with 2ml buffer (mixed 5.88ml conc. H_2SO_4, 0.4g sodium phosphate, 0.078g ammonium molybodate in 100ml dH_2O). The reaction sample was kept in water bath at 95°C for 90min than OD695 was read (Pisoschi and Negulescu, 2012). The phosphates analyzed by following method of (He and Honeycutt, 2005).

Measurements of enzyme activities

The *amylases* activity was determined by using the supernatant as a crude enzyme mixture. The 1ml supernatant mixed with 1ml of known substrate (1% soluble starch in dH_2O) and incubated at 37°C for 15min. The 2ml DNS reagent was added and kept in boiling water bath for 5min. After cooling the mixture, the absorbance was read at 540nm (Afiukwa *et al.*, 2009). For *xylanses* activity, 1ml sample was mixed with its 1ml substrate (1% xylose in dH_2O).

The 2ml DNS was added than kept in boiled water bath for 5min and absorbance was read at 540nm (Kamble and Jadhav, 2012). The *lipases* activity was measured by following (Montero *et al.*, 2012) method.

Statistical analysis

The collected data during the present study was comprised on means of four replicates of each culture. Data significance was computed with a computer based program "CoStat" version 3.03 [CoHort software, Berkeley, USA] and significant values at 5% were further subjected for Duncan Multiple Range (DMR) analysis (Behrens, 1997; Henley, 1983; Quinn and Keough, 2002).

Results and discussion

The aloe vera plant has great remedy for human health due to its properties for being rich with antioxidant and anti-bacteria widely used to treat burns (Maenthaisong *et al.*, 2007). It also uses for treating canker sores, reducing dental plaque and reducing constipation (Kumar *et al.*, 2010). Despite it have strong antibacterial properties, when it is concentrated, while its dilutions might lead it to inappropriate usage.

The lesser antibacterial stringency in diluted aloe vera cultures may allow the bacterial species to grow (Quezada *et al.*, 2017). Further its fermentation weakened the antibiotic activity of effective ingredients in leaf gel after oxidation. Fermentation of concentrated aloe vera extracts has already reported at about pH 3.8, there acid-tolerant bacterial species like *L. plantarum*, *L. pentosus* and *L. acidophilus* (Jiang *et al.*, 2016; Kim *et al.*, 2014).

Its diluted extracts are helpful in the growth of other bacterial species. Like as the *E. coli* cultures showed variant growth rate among the *Aloe vera's* extract supplemented bacterial cell cultures (Fig 1).

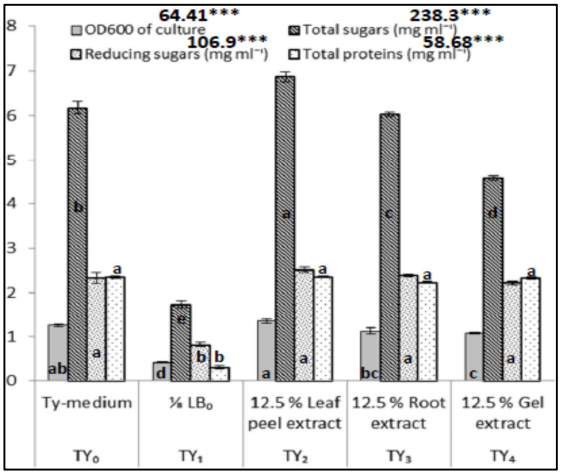

Fig. 1. The cell growth of *Escherichia coli* cultures supplemented with aloe vera as substrate and existed levels of total proteins and sugars after 18 hrs of incubation.

Meanwhile, *Aloe vera* promotes growth of the *E. coli* when supplied with particular concentrations along the standard culture media (Table 1). In present study, the effect of *Aloe vera* extracts (@ 12.5% in v/v) on cell growth and analysis of subsequent extra-cellular productions were studied after 18 hrs of incubation at 37°C with continuous orbital shaking at 250 rpm. The highest cell growth was observed in TY_2 (TY_0 medium supplemented with leaf peel extract) than TY_0 (standard TY medium of *E. coli* growth), while lowest in TY_1 (⅛ TY medium) comparatively (Fig 1). Along the series of cultures from TY_0 to TY_2, TY_3 and TY_4, the culture growth found relatively higher than TY_1 medium. It could be suggested that fresh extracts of various organs of *Aloe vera* have no cell growth limiting effect. The nutritional composition of this plant contains mainly carbohydrates and various vitamins is expected for promotion of microbial growth culture (Lakhvinder, 2017) and same has also been observed in this study (Nagpal *et al.*, 2012).

With the growth of *E. coli* cells among the nutrient cultures, the total sugars observed higher in the all

cultures in comparison to TY_1. The TY_1 is the nutrient deficit medium without agriculture waste as a substrate. The reducing sugars were higher in TY_2, while total proteins measured relatively high in TY_4 cultures (Fig 2). These above final concentrations after 18 hrs of culture could have been passed through a number of alteration in the their biosynthesis due to the changing composition as well as conditions of the cultures. During the experiment there could be retarding effect of substrate on the growth of fermentation organism at initial or at final growth stage with the accumulation of various secondary metabolites. In this work, systematic impacts of fermentation parameters are not studied which can address the additional points for the improvement to utilize this fermentation substrate.

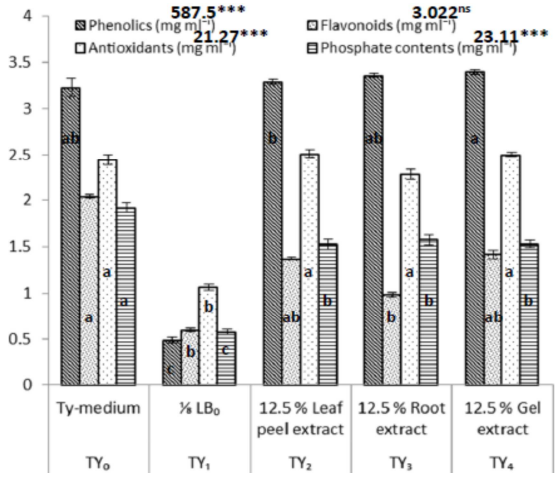

Fig. 2. Analysis of various bio-components produced along the growing cultures of *Escherichia coli* supplemented with aloe vera as substrate after 18 hrs of incubation.

The plants are rich with complex carbon sources, while their organ specification for the production of various enzymes in the fermentation culture is getting high importance. It is because of the composition of organs, which plays a decisive role in the economics production of respective extra-cellular enzymes (Christov *et al.*, 1999). Well when a plant is a source of numerous ingredients of therapeutic secondary metabolites including the derivatives of anthraquinones, alkylbenzenes, polysaccharides, dehydrabietic acid, salicylic acid, lignin, carotenoids, lectin, saponins etc (Abreu *et al.*, 2012; Coman *et al.*, 2012; Radha and Laxmipriya, 2015; Wynn, 2005). It could be adoptive and helpful in the induction of the biosynthesis of specific compounds as well as their respective enzymes (Udatha *et al.*, 2012). These ingredients might be helpful in the biosynthesis of proline, glycinebetaine and ascorbates (Fig 3), either when the fermentation organism is growing under the influence of growth retarding substrate. In positive sense, it could be an adaptation of the organism to grow with beneficial conversion of bio-compounds to the therapeutic compounds in *Aloe vera* supplied cultures.

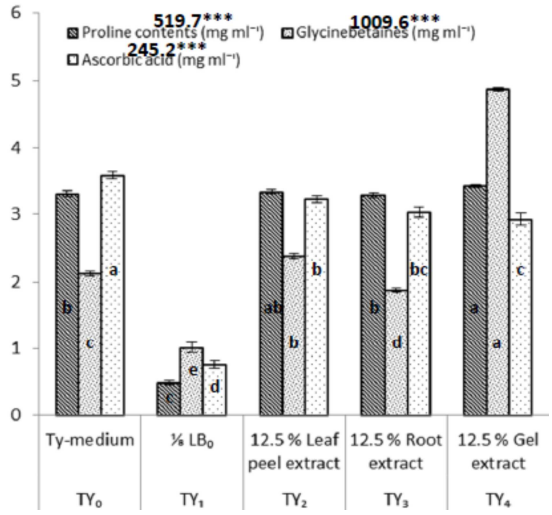

Fig. 3. Biosynthesis of free amino acids and vitamins in the *Escherichia coli* cultures supplemented with aloe vera as substrate after 18 hrs of incubation.

The 18 hrs crude culture of *E. coli* also subjected for the analysis of the activities of different enzymes. The effect of different extracts of different organs of *Aloe vera* on the amylases, *xylanases* and *lipases* was studied (Fig 4). It is noted that maximum production and activity of amylases and lipases in TY_2 culture (supplemented with leaf peel extract) than other cultures. Comparatively low activities of *xylanases* and *lipases* than amylases and lipases observed among the cultures. The continuous increase in growth of cultures is due to the production of these enzymes by fermentation organism for its nutrition through conversion of complex agri-carbon wastes to simples carbon molecules (Frick and Wittmann, 2005; Jenkins, 2016; Nakamura *et al.*, 1993).

Even both cellulose and hemicellulose are being large portion of plant biomass including *Aloe vera*. Enzymatic degradation to free up the fermentation raw materials remains preferred inexpensive and eco-friendly technique and same has been studied in the medicinal aloe vera plant.

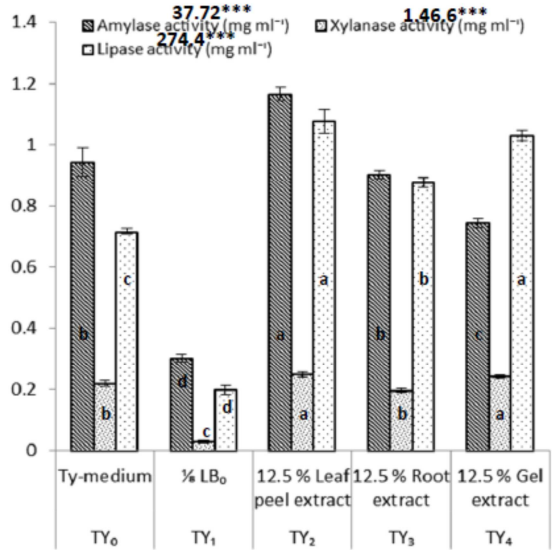

Fig. 4. Biosynthesis of various fermentation enzymes in the *Escherichia coli* cultures supplemented with *Aloe vera* as substrate after 18 hrs of incubation.

With reference to previous studies, agro-industrial residues are potential cost-effective energy source and is being rich with a variety of carbohydrates, minerals, proteins, lipids and plant growth regulators. Including the *Aloe vera* plant that provides succulent cell nutrients for good microbial growth and their extra-cellular productions.

Variation in these production by microbial cultures depends on the source of nutrition. Maximum extra-cellular enzymes production has been noted in mineral medium supplemented with very expensive pure beef and yeast extracts (Mukherjee *et al.*, 2008; Vijayaraghavan *et al.*, 2014). Currently, researchers are targeting the low cost and easily accessible nutrient sources like as agro-industry for bacterial fermentation.

The *Aloe vera* could also be a good choice for the production of fermented compounds due to its nutritional qualities and other tonics. The prebiotic property of *Aloe vera* is due to its nutritional composition (Nagpal and Kaur, 2011), in particular the presence of acemanane, mannose polymers (accemananos), glucomannan, vitamins (A, B_1, B_6, C) etc. These are the precursors of various anti-bacterial compounds and their fermented products could be cost-effective.

Conclusions

The *Aloe vera* has been used and is being a popular anti-microbial medicinal plant for thousand years. The lactic acid bacteria uses acemanane and glucomannan from *Aloe vera* for their growth and production of antimicrobial metabolites that could involves in the bacterial inhibition (Castañeda, 2018; Young and Huffman, 2003). Meanwhile the *Aloe vera* promotes the in-vitro growth cultures of probiotic lactobacilli, when supplied at a particular concentrations. As *Aloe vera* is thick-short stem and long leaved plant. Its leaves are full with gelatinous substance rich with numerous bioactive compound including free amino acids, vitamins and antioxidant. In this study, growth of *E. coli* remains good in leaf-peels cultures, while production of phyto-compounds in leaf gel while enzymes activities in leaf-peels. The *Aloe vera* as a substrate is found as full with requ

ired major phyto-compounds including other cell nutritive substances required for bacterial growth and needed by various pharmaceutical industries.

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