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Isolation and identification of tannin-degrading bacteria from deer gut and potency for improving nutritional value of tannin rich plants

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Abstract

This experiment was conducted to isolate and identify tannin-degrading bacteria from deer gut and to use these bacteria in improving of the nutritive value and gas production parameters of conocarpus and eucalyptus leaves. Isolation, tannase enzyme activities and processing of eucalyptus and conocarpus leaves with these isolates was conducted for 10 days. The result of molecular identification based on DNA sequencing indicated that isolates of A1, A2, A3, A4, A5, A7, A8 and A9 were similar to *Klebsiella pneumoniae* while A6 isolate belonged to *Acinetobacter* sp. *Acinetobacter* sp. and *K. pneumoniae* A2 had the highest and lowest enzymatic activity of tannase, respectively. Bacterial fermentation decreased tannin of conocarpus and eucalyptus leaves and the lowest was for processing with *K. pneumoniae* A7 and *Acinetobacter* sp A6. Bacterial processing had no effect on the gas production potential of conocarpus leaves, but the gas production rate was increased; while ferementation caused an increase in the gas production potential and rate of eucalyptus leaves. The highest fermentation parameters were found in conocarpus and eucalyptus leaves processed with *K. pneumoniae* A9. These results indicated that *K. pneumoniae* and *Acinetobacter* sp. as tannin degraders isolated from deer gut improved fermentation parameters of conocarpus and eucalyptus leaves and can be used to enhance the nutritive value of tannin rich plants.

Key words: Tannin-degrading bacteria, Conocarpus, Deer, Eucalyptus, Tannin, Nutritive value

Introduction

Deers can use low quality materials such as twigs, leaves, and end buds of trees and shrubs, high efficiently. Its revealed that sika deer often graze on bark and twigs in the winter which are rich source of hydrolyzable tannins (Hiura et al., 2010). Conocarps and eucalyptus trees as tropical trees are in abundance in Southwest of Iran that are grazed by ruminants such as deer in this area.

Plants produce various secondary compounds like tannins that have ability to

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bind with proteins and other nutrients (Hiura et al., 2010). The tannin-protein complex is resistent to protein-digesting enzymes including trypsin, which may cause a nutrient loss, negative nitrogen balance and weight losses in animals (Hiura et al., 2010). Tannins act as a nutrient substrate for some microorganisms (fungi, bacteria, some yeasts) that utilize its by hydrolytic enzymes such as tannase (Belur et al., 2010).

Some animals have adapted to tannins through the presence of tannin-degrading microorganisms. Osawa (1990) reported koalas that only graze on some eucalyptus spp. possess tannin degrading bacteria in their feces. Furthermore, the Japanese large mouse that preferably feeding on acorns rich in hydrolysable tannin, have *Lactobacillus* (Sasaki et al., 2005). Kohl et al. (2016) isolated and characterized tannin degrading bacteria from the feces of the wild rodent (*Neotoma lepida*). Therefore, several tropical trees can be introduced in ruminant farming systems by reducing the negative effects of tannins. This could improve the nutritional status and productivity of animals, in addition to allowing a more environmentally friendly ruminant production.

The processing of acorn with *Streptococcus spp* as tannin degraders (Mosleh et al., 2014), and oak leaves by *S. pulverulentum* (Babaei et al., 2015) were decreased phenolic compounds and tannins. Also Tahmourespour et al., (2016) reported isolates of goat feces degrade 72% tannin that influences digestibility and fermentation by rumen microorganisms.

The gas production technique are widely has been considered to assess fermentation, digestibility and the nutritional value of foods for animal. However, gas produced in derived directly from carbohydrates (carbon dioxide and methane) or indirectly by the buffering of short-chain fatty acids (carbon dioxide from the bicarbonate buffer) (Makkar & Becker, 1997).

Additional researches must be carried out to explore the benefits of tropical tree leaves (high tannin) in animal feed. The aim of the current study was to isolate and identify of tannin degrading bacteria from the deer gut and evaluate effect of these bacteria on the nutritive value and *in vitro* ruminal fermentation of rich tannin plants (i.e. conocarpus and eucalyptus) leaves.

Materials and Methods

Sampling of deer gut

The fresh sample of rumen fluid was collected from 4 European fallow deers (2 males and 2 females, 1.5 years of age), that were maintained at Dez wildlife Park of Dezful in Southwest of Iran. They were grazed twice a day on pasture forages available there (*Conocarpus erectus, Albizia lebbeck, Olea europaea* and *Eucalyptus microtheca* leaves), barley and wheat bran and had free access to water. The fresh samples were collected into a plastic bottle and transported to the laboratory under anaerobic and cooled conditions. All animal management and sampling procedures conducted according to The Care and Use of Agricultural Animals in Research and Teaching guidelines (FASS, 2010).

Isolation of tannin degrading bacteria

For isolation of tannin degrading bacteria, three media including enrichment, screening and selective media were used and samples were cultured separately and followed individually for tannase producing bacteria. Initially, enrichment media: 50 ml of Tryptic Soy Broth (TSB; Merck, Germany) media was prepared [1.5 g × 50 ml distilled water] and autoclaved at 121ºC for 15 min. Amount of 100 µl of the gut was added to 5 ml TSB media, and incubated in a shaker incubator for 37°C for 24 h, and rate of 150 rpm. Then, screening media: 100 µl of turbid broth was cultured on Mueller-Hinton Agar (MHA; Merck, Germany) media (19 g MHA was solved in 500 ml distilled water and autoclaved at 121ºC for

15 min) and incubated for 37°C for 24 h and sub-cultured 3 times in anaerobic condition. Selective media: Discrete colonies were transferred on Tannic Acid Agar (TA) media and incubated for 37°C for 24 h. TA media prepared according to Belur *et al.* (2010).

After incubation and bacterial growth, tannin hydrolyzing bacteria were identified based on the observation of clear zones around the colonies. After 24 h incubation (37ºC), colonies with different morphologies were selected as candidate tannic acid degrading strains. In order to prepare pure cultures of bacteria, colonies were harvested and sub cultured at 6 times, then subjected to primary identification using phenotypic tests: Gram stain, snot test, oxidase, catalase reaction (Hemraj *et al.*, 2013) and growth on MacConkey Agar (MA. Allen, 2005).

Tannase activity assay

Erlenmeyer flasks (100 ml) containing 10 ml TA broth media were prepared and was inoculated with 100 µl each isolate under shaking (150 rpm) for 37°C for 24 h, and centrifuged for 2 min (18894 ×*g*). The supernatant was used for tannase activity. The enzyme activity assay was carried out according to the procedure described by Sharma *et al.* (2000), but only in this method the incubation was set at 37ºC.

DNA extraction and amplification of 16S rRNA gene

Genomic DNA of selected isolates (A1-A9) was extracted after examination of some procedures, by the boiling method as described by Ausubel *et al.* (1992). All bacterial DNA were stored at −80 ºC. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) in a Bio-rad i-cycler (Bio-rad, USA). Bacterial DNA was amplified using universal PCR primers as described by Weisburg et al. (1991). The PCR mixture consisted of 1.0 µl of template, 1.5 U Taq DNA polymerase, 2.5 µl buffer (10×), 200 mM dNTPs, 1 µl of 10 ρmol concentration of forward and reverse primers, 2 mM $MgCl_2$ and deionized water for a total reaction volume of 25µl. The primers were F (5´-CCGAATTCGTCGACAACAGAGATTTGATCCTGGCTCAG-3´) and R (5´-CCCGGGATCCAAGCTTACGGTTACCTTGTTACGACTT-3´). The cycling conditions were as follows: initial denaturation at 94ºC for 5 min; 30 cycles consisting of template denaturation at 94ºC for 1 min, primer annealing at 58ºC for 40 s, and extension at 72ºC for 150 s; and a final extension at 72ºC for 20 min. The PCR products were visualized on 1% agarose gel stained with DNA safe stain and band was 1500 base pair (Fig.1) and were also sequenced and compared with Gen bank data available in the National Center for Biotechnology Information (NCBI) using the BLAST algorithm and registered in Gene bank. The phylogenetic tree of sequences was drawn by MEGA 5.2 software (Tamura *et al.*, 2007).

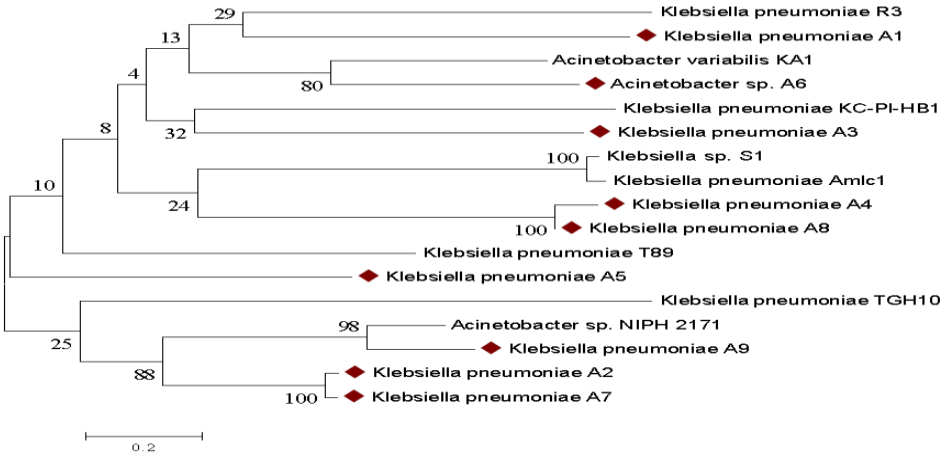

Fig 1. The phylogenetic tree of tannin degrading bacteria isolated from deer gut
The red dots are all strains that we identified after sequencing

Inoculation procedure

The leaves samples were collected from *Conocarpus erectus* and *Eucalyptus microtheca* trees and were cut to 3-4 cm and incubated with the isolated bacteria at 37ºC for 10 days. The treatments were including leaves of eucalyptus and conocarpus processed with *K. pneumoniae* A1, A2, A3, A4, A5, A7, A8 and A9 *Acinetobacter* sp (3 replicates per each treatment). Before incubation, all isolates were cultured on TA broth media and incubated at 37ºC for 24 h. Then, 250 ml nutrient broth medium per each flask was prepared and transferred equally to Erlenmeyer flasks and autoclaved at 121ºC for 15 min. Then the flasks were inoculated with isolates (3 ml of each isolate were cultured on Tannic Acid broth media, 37°C for 24 h, and incubated with continuous shaking at 37°C for 24 h under aerobic conditions. Then processing of leaves samples (500 g) with 250 ml bacterial suspension (10^{7} CFU) was conducted in thick plastic bags that closed and placed in normal conditions at 37°C for 10 days. After 10 days, the processed leaves were air-dried. The control treatments were conocarpus and eucalyptus leaves separately as these leaves treated in liquid medium without addition of isolated bacteria. Total tannins amount of smaples was measured according to AOAC (1990).

Gas production assay

Rumen fluid was supplied from four sheeps (one years old with average live weights of 35 ± 1.5 kg) fed with 40:60 concentrate: forage for 2 weeks; prior to the morning feeding. Filtered rumen liquor was purged with CO_2 and added to the anaerobic mineral buffer solution (1:2 v/v). About 200 mg experimental samples (1.0 mm screen, triplicates) incubated with 30 ml rumen buffer mixture in 100 ml glass vials based on modified procedure that was described by Makkar & Becker (1997). Gas production was recorded at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h by using a digital pressure gauge (Model SDPG0015PG5, SenSym ICT, Honeywell Inc., Morris NJ) fitted with a 21 mm gauge needle. *In vitro* gas production values were fitted to the modified exponential non-linear model of Orskov & McDonald (1979); $Y = b (1 - e^{-ct})$, where b is the gas production (ml) from the fermentable fraction, c is the rate of gas production (ml/h), t is the incubation time (h) and Y is the volume of gas produced at time. The vials contents were filtered, then the residues were dried and used to calculate the cell wall degradability. The partitioning factor, microbial biomass and truly digested organic matter were calculated by the method of Makkar & Becker (1998).

Statistical analysis

All data were analyzed using the GLM procedure of SAS (2005) based on the statistical model: Yij = μ+ Ti+ eij , where Yij is the observation, μ is the general mean, Ti is the effect of treatment on the observed parameters, and eij is the standard error of term. Means were compared by the Duncan multiple comparison test at $p < 0.05$.

Results

The results of molecular identification, based on DNA sequencing and the phylogenetic tree were indicated in Fig. 1. These results revealed that the A1, A2, A3, A4, A5, A7, A8 and A9 isolates were similar with *K. pneumoniae* in 100, 99, 99, 99, 91, 99, 99 and 97%, respectively. The A6 isolate belonged to *Acinetobacter* sp. with 97% similarity. This is the first isolation report *of K. pneumoniae* as tannin degrader from deer gut, as well as the first report of *Acinetobacter* sp. as tannase producer in the ruminants.

Culturing of rumen fluids on enrichment and selective media revealed that all isolated organisms (i.e., A1- A9) could grow on TA agar-media as the sole source of energy and carbon. Results of biochemical tests (Table 1) showed that all 9 isolates were gram-negative, facultative anaerobes, catalase-positive, oxidase-negative and snot-positive. Except for isolate A6 which showed a negative response to the lactose test, other isolates could hydrolyze lactose. The results of enzymatic assay indicated the greatest and lowest activity belonged to A6 and A2 isolates, respectively.

Table 1. Results of biochemical tests of bacteria isolated from deer gut

Bacteria isolates	FA	GR	SN	CA	OA	LA	GM	Facultative anaerobic	Tannase activity, (μmol of product min^{-1})
A8	+	-	+	+	-	+	-	+	60.59 8
A6	+	-	+	+	-	-	-	+	157.42 6
A7	+	-	+	+	-	+	-	+	57.537
A1	+	-	+	+	-	+	-	+	17.70 1
A4	+	-	+	+	-	+	-	+	14.48 4
A5	+	-	+	+	-	-	-	+	41.42 5
A3	+	-	+	+	-	+	-	+	46.20 3
A2	+	-	+	+	-	+	-	+	6.81 2
A9	+	-	+	+	-	+	-	+	32.14 9

Note. FA, fermentative activity; GR, gram stain; SN, snot test; CA, catalase activity; OA, Oxidase activity; LA, lactose fermentative; GM, growth on MacConkey agar

Bacterial fermentation decreased tannin of conocarpus and eucalyptus leaves and the lowest was for processing with *K. pneumoniae* A7 and *Acinetobacter* sp A6 (Table 2) ($P<0.05$).

The result indicated that processing of samples with isolated bacteria did not affect the gas production potential of conocarpus leaves ($P>0.05$), but the gas production rate was improved ($P<0.05$). The highest gas production rate and microbial biomass efficiency (Table 3) was seen for conocarpus leaves processed with *K. pneumoniae* A3 and *K. pneumoniae* A8, respectively ($P<0.05$). Processing with isolated bacteria did not improve the gas production potential and rate of eucalyptus leaves ($P>0.05$). The highest truly digested organic matter, partitioning factor, microbial biomass, efficiency of microbial biomass and degradability of cell wall (Table 4) were found in conocarpus and eucalyptus leaves processed with *K. pneumoniae* A9 ($P<0.05$).

Table 2. Effects of bacteria isolated from deer gut on total tannin of conocarpus and eucalyptus leaves

Treatment	Total tannin of conocarpus leave (g/100g DM)	Total tannin of eucalyptus leave (g/100g DM)
Control (no bacteria)	6.32^{a}	10.95^{a}
K. pneumoniae A8	1.86^{e}	4.10^{ef}
Acinetobacter A6	1.11^{a}	2.19^{g}
K. pneumoniae A7	2.59^{d}	3.37^{ef}
K. pneumoniae A1	3.48^{b}	8.09^{b}
K. pneumoniae A4	3.80^{c}	9.80^{a}
K. pneumoniae A5	3.14^{cd}	5.22^{de}
K. pneumoniae A3	2.87^{cd}	6.17^{cd}
K. pneumoniae A2	5.85^{a}	10.30^{a}
K. pneumoniae A9	3.27^{cd}	7.13^{bc}
*SEM	0.30	0.42
**P-Value	0.001	0.001

Note. *K. pneumonia: Klebsiella pneumonia*. *SEM, standard error of means; **P-Value-in each column, values with different superscript letters (a,b,c) are significantly different (P < 0.05).

Table 3. Parameters of fermentation and gas production of conocarpus leaves processed with isolates

Treatment	^{a}GPP(ml)	^{b}GPR(ml/h)	^{c}CWD(%)	^{d}OMD(g/kg)	^{e}PF(mg/ml)	^{f}MB(mg)	^{g}MBE(%)
Control (no bacteria)	71.21	0.04^{ab}	26.18^{g}	160.25^{h}	5.08^{b}	90.95^{g}	56.75^{b}
K. pneumoniae A8	74.68	0.02^{b}	28.47^{fg}	183.40^{g}	5.39^{a}	108.60^{f}	59.20^{a}
Acinetobacter A6	75.15	0.05^{ab}	31.98^{ef}	201.30^{f}	5.37^{a}	118.80^{e}	59.00^{a}
K. pneumoniae A7	76.80	0.04^{ab}	35.00^{e}	222.76^{e}	5.37^{a}	131.46^{d}	59.01^{a}
*K. pneumoniae*A1	78.02	0.07^{a}	43.31^{d}	241.60^{d}	5.31^{ab}	141.50^{c}	58.54^{ab}
*K. pneumoniae*A4	83.86	0.05^{ab}	46.77^{cd}	248.55^{cd}	5.17^{ab}	142.95^{c}	57.51^{ab}
*K. pneumoniae*A5	89.32	0.06^{ab}	50.20^{c}	258.65^{c}	5.22^{ab}	149.75^{c}	57.89^{ab}
*K. pneumoniae*A3	92.42	0.08^{a}	58.48^{b}	273.16^{b}	5.30^{ab}	159.86^{b}	58.52^{ab}
*K. pneumoniae*A2	93.29	0.06^{ab}	61.80^{ab}	277.40^{b}	5.28^{ab}	161.90^{b}	58.35^{ab}
K. pneumoniae A9	98.67	0.06^{a}	65.00^{a}	296.30^{a}	5.39^{a}	175.30^{a}	59.16^{a}
*SEM	14.68	0.013	1.39	3.20	0.073	3.20	0.57
**P-Value	0.90	0.01	0.0001	0.0001	0.02	0.0001	0.02

Note. ^{a}GPP, Gas production potential; ^{b}GPR, Gas production rate; ^{c}CWD, cell wall degradability; ^{d}OMD, Truly digested organic matter; ^{e}PF, Partitioning factor; ^{f}MB, Microbial biomass; ^{g}MBE, Microbial biomass efficiency; *SEM, standard error of means; **P-Value: in each column, values with different superscript letters (a,b,c) are significantly different (P < 0.05).

Table 4. Parameters of fermentation and gas production of eucalyptus leaves processed with isolates

Treatment	^{a}GPP(ml)	^{b}GPR(ml/h)	^{c}CWD(%)	^{d}OMD(g/kg)	^{e}PF(mg/ml)	^{f}MB(mg)	^{g}MBE(%)
Control (no bacteria)	62.46	0.069	18.87^{i}	150.06^{j}	4.67^{h}	78.56^{j}	52.36^{h}
*K. pneumoniae*A8	73.00	0.034	21.53^{h}	181.31^{i}	4.73^{g}	98.81^{i}	54.59^{g}
Acinetobacter A6	77.40	0.036	24.19^{g}	197.97^{h}	5.01^{f}	111.07^{h}	56.10^{f}
*K. pneumoniae*A7	93.92	0.036	27.24^{f}	251.70^{g}	5.78^{e}	156.00^{g}	61.98^{e}
*K. pneumoniae*A1	95.39	0.042	32.75^{e}	275.31^{f}	5.98^{d}	174.11^{f}	63.24^{d}
*K. pneumoniae*A4	103.74	0.048	38.97^{d}	295.61^{e}	6.09^{d}	188.91^{e}	63.90^{d}
*K. pneumoniae*A5	95.09	0.054	40.71^{cd}	329.66^{d}	6.46^{c}	217.46^{d}	65.96^{c}
*K. pneumoniae*A3	98.18	0.074	42.22^{c}	355.43^{c}	6.58^{bc}	236.63^{c}	66.57^{bc}
*K. pneumoniae*A2	105.54	0.062	54.53^{b}	391.22^{b}	6.78^{ab}	263.62^{b}	67.38^{ab}
K. pneumoniae A9	118.97	0.078	60.42^{a}	407.60^{a}	6.79^{a}	275.60^{a}	67.60^{a}
*SEM	16.62	0.014	0.59	2.97	0.05	2.97	0.30
**P-Value	0.45	0.26	0.0001	0.0001	0.0001	0.0001	0.0001

Note. ^{a}GPP, Gas production potential; ^{b}GPR, Gas production rate; ^{c}CWD, cell wall degradability; ^{d}OMD, Truly digested organic matter; ^{e}PF, Partitioning factor; ^{f}MB, Microbial biomass; ^{g}MBE, Microbial biomass efficiency; *SEM, standard error of means; **P-Value: in each column, values with different superscript letters (a,b,c) are significantly different (P<0.05).

Discussion

The present study showed that *K. pneumoniae* and *Acinetobacter* sp. as tannin degraders bacteria were identified in the gut of European fallow deers. The researchers reported that some *Klebsiella* sp. strains C2A showed the capability to degrade 40–60% tannic acid within 24h (Jadhav et al., 2011). Tahmourespour et al. (2016) isolated *K. pneumoniae* strains from goat's feces fed with a tannin rich diet. Some Klebsiella strains with tannase activity have been isolated from wastes of oil mill (Pepi et al., 2013). According to other studies, *K. pneumoniae MTCC 7162* was isolated from tannery effluent (Sivashanmugam and Jayaraman, 2011), and a Klebsiella sp. strain was isolated from garden soil (Jadhav et al., 2011). In the study of Sharma et al. (2017), three rumen bacterial isolates producing tannase were identified as *K. variicola PLP G-17 LC, K. variicola PLP S-18* and *K. pneumoniae PLP G-17 SC.* Kumar et al. (2015), also used *K. pneumonia* for tannin degradation. Manson et al. (2016) stated that the bacteria genus Acinetobacter is frequently associated with both aspen foliage and gypsy moth consuming that tissue, and one isolate, Acinetobacter sp. R7-1 utilized condensed tannins but not phenolic glycosides or glucose as carbon sources.

Also, in another study, *Bacillus* and *Pseudomonas* genera as tannin degrading isolates isolated from pistachio soft hulls by Tahmourespour et al. (2017). *Streptococcus gallolyticus, waius* and *bovis* as tannin degradaer was isolated from sheep's feces fed on tannin rich diets (Babaei et al., 2015).

According to the current study, processing with bacterial isolates decreased the tannin content of conocarpus and eucalyptus leaves and the lowest content was for *Acinetobacter* sp A6. The reason is this strain has higher enzymatic activity. Mosleh et al. (2014) reported following 5 and 10 days of processing of acorn with *Streptococcus pneumoniae and Streptococcus bovis*, its phenolic compounds were reduced.

Also, fermentation of oak leaves for 10 days with *S. pulverulentum* decreased content of total phenols and condensed tannins by 58 and 66%, respectively (Lotfi and Rouzbehan, 2011). The amounts of hydrolysable and condensed tannins in pistachio waste silage significantly reduced during 30 and 60 days ensling, likely due to the formation of larger internal polymers or tannin oxidation (Bagheripour et al., 2008). Information about the effects of tannin degradating bacteria isolated in current study on digestibility, gas production and fermentation of tannin rich feeds in ruminants is scarce. On the basis of the obtained results, processing of conocarpus leaves with isolated bacteria did not affect gas production but only improved the gas production rate of leaves. The lack of effect of isolates on gas production maybe was because of the inoculum amount of isolates and/or processing time with isolates was not enough. The compositions of leaves, tannase activity and fermentation time may influence microorganisms growth, cell solubility, digestibility and fermentation.

It is possible that improvement of gas production rate of conocarpus leaves by processing with bacterial isolates, due to the decrease of tannin content of conocarpus might have increased digestibility and fermentation. In relation with other research reported reduction of the phenolic compounds by processing of acorn with strains of *Streptococcus pneumonia* and *S. bovis* that increase digestibility and gas production (Mosleh et al., 2014). Rakesh et al. (2000) reported 30% decrease in tannins of black locust leaves after 30 days incubation with tannase (produced by fungi) and can change digestibility and fermentation. It is observed that fungal tannase (*Aspergillus niger* and *Penicillium* sp. strains) isolated from Mexican deserts degrade 76–79% catechin tannin that can increase digestibility (Aguilar et al., 2004).

The great effect of K. pneumoniae A9 strain on the fermentation parameters of conocarpus leaves may be due to its high enzymatic activity that can improve fermentation and digestibility. Differences in gas production volume are reported among the current isolates which could be due to their genetic differences (Babaei et al., 2015). In contrast to our results, researchers reported that OMD in fresh conocarpus leaves was higher than processed leaves that is probably due to an increase of soluble carbohydrates and decreased crude fiber content compared to processed conocarpus leaves (Al Koaik et al., 2014).

Processing with isolated bacteria did not change the gas production potential and rate of eucalyptus leaves which can be due to the bacterial inoculum amount or processing time with isolates. Rakesh et al. (2000) observed change in tannin amount by incubation of black locust leaves for 30 days. Also reduction of phenolic compounds of acorn was observed by processing with *Streptococcus* for 10 days (Mosleh et al., 2014). Bagheripour et al. (2008) reported tannins of pistachio silage reduced during 30 days. In all these studies decreasing of tannins can change digestibility and gas production. Although, ensiled leaves of eucalyptus had a negligible content of condensed tannin, so the gas production was not affected. However, plants rich in condensed tannin have shown to reduce methanogenesis in sheep and cattle, probably due to the direct effect of tannins on methanogen activity and indirect effect via fiber digestion (Sallam et al., 2010). Tannins can disrupt the membrane function of bacteria by inhibiting the electron transport chain and inhibit the cell wall entry of substrates by decreasing permeability (Smith et al., 2005). Microbial inhibition by tannins may reduce the potential ability of enzymes to degrade lignocellulose and hence reduce fiber utilization by ruminants.

According to the current results, processing with isolated bacteria increased microbial biomass and highest was found in conocarpus and eucalyptus leaves processed with *K. pneumoniae* A9. The reduction in microbial biomass was associated with a reduction in the efficiency of microbial protein synthesis in tannin rich feeds. But proccesing with bacteria can overcome tannin inhibition by modification, degradation, and inactivation of tannin, membrane modification and metal ion sequestration (Smith et al., 2005).

The negligible amount of condensed tannin and high contents of cell wall and higher lignified fiber fractions which reduce fiber digestibility and methanogenesis may have an important role in reducing fermentation (Sallam et al., 2010). These isolates probably enhanced pectinase and cellulase activity by changing pH and reduced toxic effects of tannins; and DM and OM degradation were improved. The decreasing of phenols and tannins will increase digestibility by rumen microorganisms. In a similar study, Raghuwanshi *et al.* (2014) observed that the use of microorganisms producing tannase in feed preparations would increase the bioavailability of nutrients by hydrolyzing phenols as anti-nutritional factors.

Klebsiella pneumoniae and *Acinetobacter* sp. were found as tannin degraders bacteria in the gut of European fallow deer for first time. The processing of conocarpus and eucalyptus leaves with these isolated bacteria (particulary *Klebsiella pneumonia* A9), from deer feces improved nutritional value of tannin rich plants such as conocarpus and eucalyptus leaves. Therefore, in the tropics and subtropics, processing tree leaves rich in tannin with tannase is very important subject in practical feeding systems for animal.

Conflict of interest

The authors declare that they have no conflict of interest.

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جداسازی و شناسایی باکتری‌های تجزیه کننده تانن از دستگاه گوارش گوزن و پتانسیل آن‌ها در بهبود ارزش تغذیه‌ای گیاهان غنی از تانن

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چکیده

این آزمایش برای جداسازی و شناسایی باکتری‌های تجزیه کننده تانن از دستگاه گوارش گوزن و استفاده از این باکتری‌ها در بهبود ارزش تغذیه‌ای و فراسنجه‌های تولید گاز برگ‌های کنوکارپوس و اکالیپتوس انجام شد. جداسازی، فعالیت آنزیم تاناز و فراوری برگ‌های اوکالیپتوس و کنوکارپوس با این جدایه‌ها برای ۱۰ روز انجام شد. نتایج تشخیص ملکولی بر اساس توالی یابی DNA نشان داد که جدایه‌های A1، A2، A3، A4، A5، A7، A8 و A9 با *کلبسیلا پنومونیه* و *جدایه* A6 با *اسینتو باکتر* شباهت داشتند. جدایه *اسینتوباکتر* A6 بالاترین و *کلبسیلا پنومونیه* A2 پایین‌ترین فعالیت آنزیمی را داشتند. تخمیر باکتریایی، میزان تانن برگ‌های کنوکارپوس و اوکالیپتوس را کاهش داد و کم‌ترین مربوط به فراوری با *کلبسیلا پنومونیه* A7 و *اسینتو باکتر* بود. فراوری باکتریایی هیچ اثری بر پتانسیل تولید گاز برگ‌های کنوکارپوس نداشت اما نرخ تولید گاز افزایش پیدا کرد. در حالی که فراوری باعث افزایش پتانسیل و نرخ تولید گاز برگ‌های اوکالیپتوس شد. بالاترین فراسنجه‌های تخمیری مربوط به برگ کنوکارپوس و اوکالیپتوس فراوری شده با *کلبسیلا پنومونیه* A9 بود. نتایج نشان داد که *کلبسیلا پنومونیه* و *اسینتوباکتر* به عنوان تجزیه کننده‌های تانن جداسازی شده از شکمبه گوزن فراسنجه‌های تخمیر برگ کنوکارپوس و اوکالیپتوس را بهبود دادند. بنابراین این جدایه‌ها برای ارتقای ارزش تغذیه‌ای گیاهان حاوی تانن بالا استفاده می‌شوند.

کلمات کلیدی: باکتری‌های تجزیه کننده تانن، اوکالیپتوس، کنوکارپوس، گوزن، تانن، ارزش تغذیه‌ای

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