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Isolation and Identification of Tannin-degrading Bacteria from Native Sheep and Goat Feces in Kohgiloye and Boyer-Ahmad Province

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ABSTRACT

Oak trees cover a wide range of forests in Iran and most of them are located in Kohgiloye and Boyer-Ahmad province (K.B). The high tannin is one of the anti-nutritional characteristics of acorn for domestic animal. Wide range of gut microorganisms could tolerate tannin and reduce it by producing tannase enzyme. This study was carried out to isolate and identify the tannin resistance bacteria (tannase enzyme producing bacteria) from feces samples of domestic sheep and goat. For this purpose, twenty six feces samples of native sheep and goats were collected through the province. Firstly, samples were diluted and then 0.1 ml of each suspension (dilutions) was cultivated on the tannin-treated brain heart infusion agar media containing tannin and then was incubated at 37 C for 72 hours under anaerobic conditions. The colonies were isolated and identified by biochemical experiments and API (20 STREP) test. The results showed that five strains of *Streptococcus pneumonia* and 2 strains of *Streptococcus bovis* having tannin hydrolysis activity.

Key words: Isolation, Identification, Tannase, Sheep and Goat, Bacteria.

INTRODUCTION

Oak trees cover a wide range of forests in Iran. Every year, tons of acorns are produced in Iran Oak forests located in K.B (Bojar Pour et al., 2010). Because of high amount of available carbohydrate, acorn is used *ad libitum* as an energy source in ruminant's nutrition (Maldonado et al., 1996). One of the anti-nutritional characteristics of acorn is tannin having bitter taste and low palatability and harmful effects on animals (Makkar, 2003; Osawa, 1990). Tannins are secondary polyphenolic compounds known primarily for their ability to bind to and precipitate proteins and other macromolecules. The toxicity of phenolic compounds in the environment has fostered studies of bacteria that are able to tolerate and/or metabolize high levels of these compounds, particularly under anaerobic conditions (Allison et al, 1990; Brooker et al, 1994; Field et al, 1987; Huang et al, 1990; Nelson et al, 1995; Skene and Brooker 1995). Large groups of bacteria, fungus and yeasts can reduce tannin by producing tannase (Aguilar et al, 2007; Field et al, 1987). Bacteria capable of degrading or tolerating tannins have been isolated from the alimentary tracts of several animals, for example; koalas (*Phascolarctos cinereus*) (Osawa, 1992), goats (*Capra hircus*) (Brooker et al, 1994; Nelson et al, 1995), and horses (*Equus caballus*) (Nemoto, 1995). Eden and et al. (2005)isolated 12 species of tannin-resistance bacteria and identified from feces samples of ruminants which grazing on wild plants containing tannin. Likely, tannase producing bacteria might be found in the gut of domestic sheep and goats of K.B since they usually use the acorn forests as a food source. Thus,

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the aim of the current study was to isolation and identification of tannase producing bacteria from feces samples of native sheep and goats feces of K.B province.

MATERIALS AND METHODS

This study was conducted in September 2012. Twenty six feces samples of K.B. province ingenious sheep and goats were collected in sterile test tubes. These samplessuspend in sterilized phosphate buffer saline (Osawa, 1990) , thoroughly mixed with a homogenizer and a Vortex test-tube mixer (Heidolph–Germany) in the microbiology laboratory of animal science department in Yasouj University. Approximately 5 ml of filter-sterilized 2% tannic acid solution (Merck Company–Germany) was overlaid on a plate of brain heart infusion agar medium (Merck Company–Germany) containing 0.5% yeast extract (*Sigma-Germany*) for 20 min. After this treatment, the surface of the medium was highly opaque, the overlaid tannic acid solution was removed with an aspirator, and the surface of the medium was rinsed with sterile 0.85% phosphate buffer saline three times to ensure removal of residual tannic acids from the surface of the medium. 0.1 ml of each suspension (dilutions) was cultivated on the tannin-treated brain heart infusion agar media and incubated at 37^0C for 72 hours under anaerobic conditions (Gas pack A, Merck Company-German). After incubation and bacterial growth, tannin hydrolysing bacteria were identified by observation of clear zones around the colonies (figure 1). In order to prepare pure cultures of bacteria, Colonies harvested and subculture for two time. Different species of bacteria were recognized by identification of their color and gram reaction (figures 2 and 3), biochemical tests (The capability of the isolates to grow in different carbohydrate media was studied in broth-containing Glucose, Lactose, Inulin, Mannitol, Trehalose, Arabinose, Starch, Galactose, Sorbitol, Raffinose and other test contain Catalase; Oxidase; B-Hemolysis; Calicin; Bile Esculin; Optochin and Bile Susceptibility) and API 20 STREP test (Microgene Bioproducts, U.K) (table 1).

RESULT AND DISCUSSION

Acorn Anti-nutritional substances having harmful effect on animals (Makkar, 2003). Some bacteria can produce tannase enzyme and reduce tannin, so these bacteria can be adapted to this tannin-rich diet and become part of the micro flora of ruminant gastrointestinal tract. According to our results (figures 1 and 2), isolated bacteria were gram-positive cocci species. Based on the morphology, tannin-hydrolysing ability, carbohydrate utilization capacity, a total of two groups of bacteria were identified. The first group: MN1, MN15, MN18, MN20 and EE23, second group: MN22 and MN24 isolates from sheep and goat feces, shared similar features in most of the parameters. Among the isolated species, 2 strains (MN22 and MN24) of *Streptococcus bovis* were identified which was similar to results of Nitiema et al (2010) Eden et al. (2005) and Osawa (1990) isolated and identified some species of *Streptococcus.* 5 strains (MN1, MN15, MN18, MN20 and MN23) had a diplococci arrangement were identified.

Conclusion

According to the results of this study, it can be conclude that some *Streptococcus pneumonia* and *Streptococcus bovis* strains which were isolated from feces samples of domestic goat and sheep can hydrolyse acorn tannin in rumen and reduce negative effects of tannin on animal. Presently to our knowledge, there was no research conducted on to isolate *Streptococcus pneumoniae* so far and our research could be assumed a first study that way.

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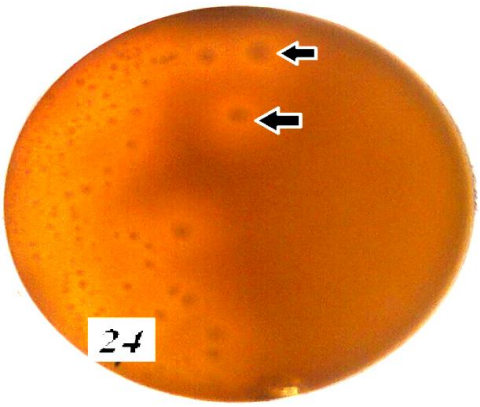

Figure 1: tannin hydrolysis bacteria growth on rich-tannin media and observing clear zones around the colonies

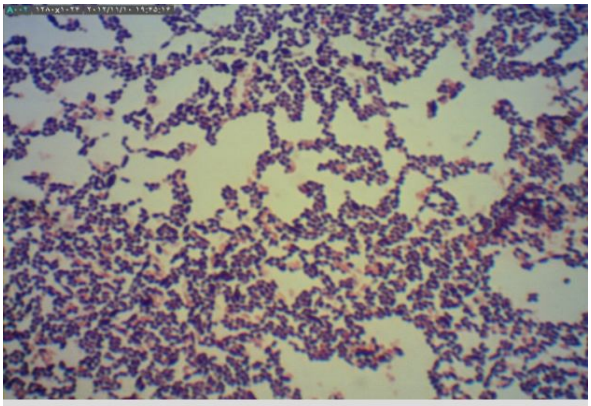

Figure 2: species MN 23 bacterium gram-positive diplococci staining

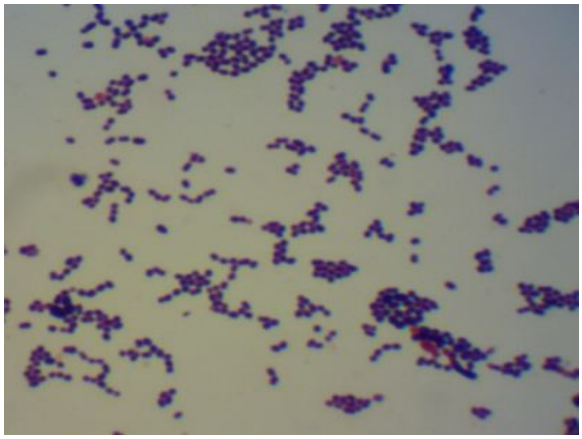

Figure 3: species MN 24 bacterium gram-positive Cocci,Chsins staining

Table1: The identification of bacteria isolated from feces

Strain	Isolation from the Feces	Find Identification by API 20 STREP	Gram Stain	Morphology	Tannin-degrading	clear zone	Aerobic Growth	Anaerobic Growth	Catalase	Oxidase	B- Hemolysis	Glucose	Lactose	Inulin	Mannitol	Trehalose	Arabinose	Starch	Galactose	Sorbitol	Raffinose	Calicin	Bile Esculin	Optochin and Bile Susceptibility
MN-1	Goat	*S.Pneumoniae*	positive	Diplococci	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	V	-	S
MN-15	Sheep	*S.Pneumoniae*	Positive	Diplococci	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	V	-	S
MN-18	Goat	*S.Pneumoniae*	Positive	Diplococci	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	V	-	S
MN-20	Sheep	*S.Pneumoniae*	Positive	Diplococci	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	V	-	S
MN-22	Goat	*S.Pneumoniae*	Positive	Diplococci	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	V	-	S
MN-23	Goat	*S. Bovis*	Positive	Cocci,Chsins	+	+	+	+	-	-	-	+	+	+	-	V	+	+	+	-	+	+	+	R
MN-24	Goat	*S. Bovis*	positive	Cocci,Chsins	+	+	+	+	-	-	-	+	+	+	-	V	+	+	+	-	+	+	+	R

Symbols: S, Sensitive; R, Resistant; +,Positive; -, Negative; V, Variable Reactions.