

Short Communication

**Detection of Colicin genes by PCR in *Escherichia coli*
isolated from cattle in Shiraz-Iran**

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ABSTRACT

A variety of probiotic bacteria have been tested to control animal and foodborne pathogenic bacteria in livestock. The mechanism of inhibition of pathogenic bacteria for several of those probiotic microorganisms is mediated by the production of bacteriocins. Colicins are probably the group of bacteriocins that have been most thoroughly characterized. Colicins are antimicrobial proteins produced by one strains of *Escherichia coli* to suppress the growth of other strains of *E.coli*. The present study indicated the preparation of colicin from colicinogenic bacteria. A total of three hundred rectal and rumen swabs isolated from health and diarrheic calves located in Fars province feces. One hundred and fifteen strains were confirmed as *E.coli* by biochemical test. Polymerase chain reaction was used to determine the following genes encoding colicins. Nearly 100% of isolates were contained at least one gene of colicin. The frequency of several classes of colicin was determined. As a result the most detected gene was Ia Ib and the least detected gene was A.N.S₄. Colicin should be tested to control animal and foodborne pathogenic bacteria in livestock.

Keywords: *E. coli*, Colicin, PCR, Cattle

INTRODUCTION

The emergence of antibiotic-resistance in many bacteria relevant for animal and public health stresses the importance of decreasing use of antibiotics in animal production. The reduction of antibiotic application in livestock can only be achieved if

alternative antimicrobial strategies are available (Fuller *et al* 1999). A variety of probiotic bacteria have been tested to control animal and food borne pathogenic bacteria in livestock. The mechanism of inhibition of pathogenic bacteria for several of those probiotic microorganisms is mediated by the production of bacteriocins (Stahl *et al* 2004). Among the different types of bacteriocins, colicins probably have the greatest specificity (i.e. *E. coli* colicins). Colicins are antimicrobial proteins produced by one strains of

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Escherichia coli to suppress the growth of other relative strains of *E. coli* (Diez-Gonzalez 2007). Colicins are typically produced under stress conditions and more than 25 different colicins have been characterized and these can be classified according to their mode of action and their import pathways (Le Jeune et al 2001). Colicin E1 and K inhibit all macromolecular synthesis without arrest of respiration (Schamberger & Diez-Gonzalez 2004), colicins E2 E7 E8 and E9 cleave DNA (Schaller & Nomura 1976, (Toba et al 1986, Zakharov et al 2006), colicins E3 E4 and E6 hydrolyze rRNA (Stahl et al 2004, Boon 1971, Bowman et al 1971), colicins D and E5 cleave tRNA (Ogawa et al 1999, Tomita et al 2000), colicin A acts by making tiny pores in phospholipid bilayers thus allowing the leakage of ions across them (Schein et al 1978, Cavard 2004). There is no any information of colicin and colicinogenic bacteria in Iran.

The presences of colicin in the gut confirm that animal can resist against colibacillosis, when antibiotics are not used (Papavassiliou 1961, Russell et al 2002). The main objective of the present study was screened colicinogenic bacteria from gastrointestinal tract of calves. The prevalence of several classes of colicin was determined. Polymerase chain reaction was used to detect bacteria those posses colicin genes.

MATERIALS AND METHODS

Bacterial Strains. A total of 300 rectal and rumen (at slaughter house) swabs were collected from feces of health and diarrheic calves located in Fars province. Biochemical test being Voges-Proskauer negative, citrate negative, indole positive and methyl red positive were used to confirm the isolates as *E. coli*. The swab sample put into the TSB and the incubated at 37 °C over night. The samples were then streaked on Mac Conkey agar. Lactose positive colony was chosen for biochemical test.

DNA isolation and PCR amplification. In this study we used seven pairs specific primers were designed by Gene Bank sequences (Hall 1999, Benson et al 2006). Genotypes of all strains were verified by PCR. Polymerase chain reaction was used to detect bacteria those posses colicin genes (table 1).

The PCR reactions were in a 25 µL volume and comprised 1µL of MgCl₂ (15mM), 0.5µL of 2.5 mM dNTP, 2.5µL of Tag buffer (10×), 0.5µL of each primer (2.5pM each), 0.1µL of Tag DNA polymerase and 2 µl of the DNA sample. Sterile filtered water was used to bring the final reaction volume to 25µL. The thermocycler cycling conditions were 1 cycle of denaturation at 94 °C for 2 min, annealing at the primer

Table 1. Oligonucleotides (5'-3') sequences of colicin genes according to farward and reverse primers.

Target gene of colicin	Primer name	Primer Sequences (5' to 3')	PCR product Size bp	Annealing temperature °C
A.N.S ₄	NS4: r NS4: f	CGT AGC TAT AAT GAA GCA ATG GCT TCA ACC TCC AAC AGG AGA GGT CCC CAG TT	225	57
V	V: f V: r	CAC GCC CTG AAG CAC CAC CA CCG TTT TCC AAG CGG ACC CC	400	68
Ia, Ib	Iab: f Iab: r	GCA CAA CAG GCC CGT CTG CTC CAC CTT CCA CAT CCT CTG TCA CC	385	68
E2.E3.E4E5.	Mix: f	CGA CAG GCT AAA GCT GTT CAG GT	219	60
E6.E7E8.E9	Mix: r	TGC AGC AGC ATC AAA TGC AGC CT		
U, Y	Yu: f Yu: r	GTG AAC GGA CAG AAA CCC GCC CAA TCT GTC TGA CAG CCT CTC CC	243	68
5,10,K	510K: f 510K: r	AAA GCT GAA CTG GCG AAG GC CAA CTC ATC ATC CCC TAT GTA AGA AG	803	60
E ₁	E1: f E1: r	ACG GGA GTG GCT CTG GCG G CTC TTT ACG TCG TTG TTC TGC TTC CTG	389	68

specific T_m for 1min, elongation at 72 °C for 1 min, then 35 cycles of denaturation at 94 °C for 1 min. The PCR reaction products were stored at -20 °C or immediately separated on 2% agarose gels in a 0.5×TBE buffer (per liter: 10×TBE is 108 g of Tris base, 55 g of boric acid and 40mL of 0.5 MNa₂EDTA) (Setia 2009).

Statistical Analysis. Data were recorded using the SPSS version 13. Statistical analysis was performed using ANOVA office software (Microsoft).

Table 2. Sources of *Escherichia coli* and distribution of colicinogenic *E. coli* isolates

Source of isolates	Number of isolates (%)	<i>E. coli</i> isolates (%)	Colicinogenic strains (%)
Rumen	55(18.33)	14(4.66)	12(85.71)
Feces	245(81.66)	101(33.66)	101(100)
Total	300(100)	115(38.33)	115(100)

RESULTS AND DISCUSSION

Bacterial Strains. One hundred and fifteen samples were confirmed as *E. coli* by biochemical test (Voges-Proskauer negative, citrate negative, indole positive and methyl red positive). According to a previous study these strains were not pathogen and they were negative for F5, F41, STa and Shiga toxin (Tahamtan et al 2011, Shams et al 2010). Of the 115 colicinogenic *E. coli* (CGEC) strains, 100% were found from feces, while 85.71 % detected in rumen (table 2).

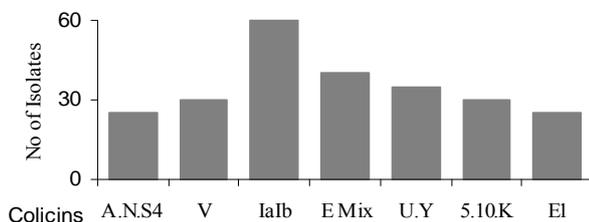


Figure 1. All colicinogenic *E. coli* strains harbored at least one colicin gene in their genum. IaIb was the dominant among the colicins.

PCR analyses. PCR was used to determine the following genes encoding the colicins. Genotypes of 115 strains were verified by PCR. Approximately

100% of isolates was contained at least one colicin gene (figure 1). The colicins detected in the isolates by PCR were E1, V, Ia Ib, E2 E3 E4 E5 E6 E7 E8 E9, UY, 5.10.K, A.N.S₄ (figure 2). The data indicated 39.19% of isolates harbored at least one colicin gene, while 21.73, 17.42, 13.04 and 3.34 present of isolates posses two, three, four and five gene of colicin repectively.

E. coli is the major species in the fecal coliform group (Daines & Paterson 2008). Coliform bacteria normally present in the intestine produce colicins which protect intestinal tract from pathogenic bacteria (Cascales et al 2007). The normal flora prevents colonization of pathogenic microorganisms by production of colicins. The colicinogenic bacteria play an important role in preventing the colonization of gastrointestinal bactrria in livestock. As results of using antibiotic, the balance of these normal flora in the colon may become disrupted and consequently, the enteric pathogen adhere to colon. The use of antibiotic has many limitations such as antibiotic resistance.

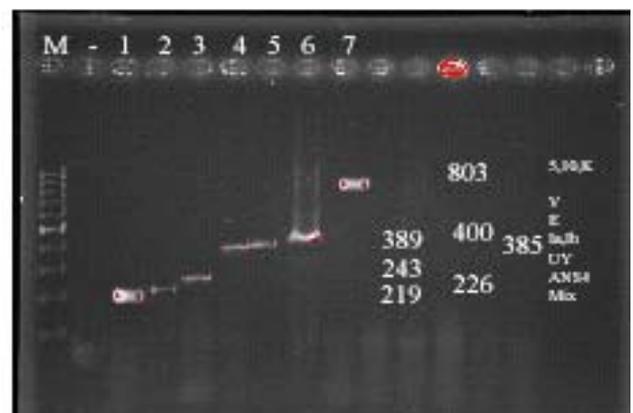


Figure 2. Single PCR for colicin gene. M: 100 bp marker, -: Negative control, Lan 1 to 7: E Mix, ANS4, UY, IaIb, E1, V, and 5 10 K.

Gastrointestinal tract can also acquire and serve as long term reservoirs for resistance genes. Theoretically these genes can be transferred to humans. Indeed the FDA and CDC are already moving to restrict the use of antibiotics in animal agriculture (Majeed et al 2011). Because of these limitations, colicinogenic bacteria are good alternative and effective replacement.

For the first time in Iran we reported colicinogenic strain from cattle feces and the prevalence of several classes of colicin was determined by PCR method. Nearly 100% of the isolates in the present study had at least one gene of colicins and some of the isolates had more than two colicins. The most detected gene was Ia Ib and the least detected gene was A.N.S₄. Setia et al (2009) indicated no rumen isolates evaluated were effective at inhibiting K88, but isolates from cattle feces were effective. This is rather interesting because it suggests that even though all isolates were phenotypically *E. coli*, there are subtle differences between those inhabiting the rumen compared with those in the hind-gut (Schamberger and Diez-Gonzalez. Schamberger and Diez-Gonzalez in USA screened isolates of *E. coli* from the feces of cats, cattle, chickens, deer, dogs, ducks, horses, humans, pigs, and sheep. They found colicinogenic strains from all sources, but the greatest number was from cats and sheep (Schamberger & Diez-Gonzalez 2002). However, many factors that might influence the differences animal isolates and the type of colicin, including colicin diversity, the use of other experimental designs, effectiveness of probiotic organisms, animal species, diet, and geographical region (Murinda et al 1996, Brashears et al 2003).

The presence of gene does not indicate whether or not gene expression because, under conditions of stress a fraction of colicinogenic bacteria are induced to produce colicin proteins. The type of stress causing the inductive activity needs to be better characterized (Diez-Gonzalez 2007, Kuhar et al 2001). Over use of antibiotics is the key factor to eliminate the bacteria particularly colicinogenic strains from gastrointestinal tract in animals and humans (Diez-Gonzalez 2007). The colicin will be use to prevent ETEC or VETC infection. Further investigation is need to known about the inhibitory activity of colicinogenic *E. coli* against pathogen bacteria.

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