

Research Article

Subtilisin Genes (SUB1-3) Presence in *Microsporum canis* Isolates with Human and Animal Source

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Abstract

Background: The presence of subtilisin genes (SUBs) coding for serine proteases in *Microsporum canis* DNA, contribute to the adherence of fungi to keratinized tissues. The aim of this study was identified the presence/absence of subtilisin gene family in *M. canis* isolated from human and animal source.

Materials and Methods: This cross-sectional study was performed on 24 samples of patients and pets with dermatophytosis from September to November 2017. Genomic DNA, pertaining to all pure colonies in Sabouraud agar with cycloheximide and chloramphenicol (SCC) and Sabouraud Dextrose agar(SDA), extracted, using rapid method without liquid nitrogen. *M. canis* were detected by molecular test. SUB1, SUB2 and SUB3 gene of *M. canis* amplified. The relative frequency of the genomic sequences also calculated.

Results: *M. canis* DNA were extracted from cats (n=6), dog (n=1) and human (n=3) with tinea corporis and showed a high percentage for SUB2 (90%; 9/10) and the absence of SUB genes in a *M. canis* isolated from dog with develop dermatophytosis. The same results were observed in the frequency of the SUBs presence from the colonies grown on SCC and SDA.

Conclusion: The presence of SUBs in *M. canis* isolates with human and animal source will be the basal to understand zoonotic infections. The absence SUBs in clinical isolate, indicates that they are indispensable for initiation of the infection.

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1. Introduction

Two important keratinophilic and keratinolytic characteristics of dermatophytes indicate their ability to digest keratin as a nutritive source in vitro (saprophytic stage) and in vivo

(parasitic stage) [1–3]. *Microsporum canis* is the main cause of dermatophytosis in cats and humans, the natural reservoir of which is pets [4, 5]. Methods of routine diagnostic mycology laboratory are used to identify dermatophytes, Sabouraud Cycloheximide Chloramphenicol Agar is medium selective for dermatophytes. It is a recommended medium with antibiotics for eliminate other microorganisms [6]. Chloramphenicol and cycloheximide effects on the synthesis of enzymes in eukaryotic cell [7] and in yeast [8]. Present a PCR-based method for identifying *M. canis*, using specific primer and rapid DNA extraction method from clinical samples and isolates, leading to appropriate and faster treatment and infection control [9]. Pathogenic *M. canis* is dermatophyte that has the ability to attack keratinized structures [10]. Study on mechanisms of dermatophyte infection focused mainly on secreted serine protease [11]. A gene family encode subtilisin-like proteases in *M. canis*. The role of secreted subtilisin has been identified, during the infection steps [12]. The aim of this study was identified the presence/absence of SUB1, SUB2, and SUB3 in *M. Canis* DNA isolated from human and animal source and effect of cycloheximide and chloramphenicol in Sabouraud agar on subtilisin genes presence.

2. Materials and Methods

2.1. Collection of human and animal samples

This cross-sectional study was performed on 24 samples of patients and pets with dermatophytosis from September to November 2017, 14 samples of cats and dogs with dermatophytosis were collected from three veterinary clinics in Kerman. 10 samples of skin lesions were collected from patients with dermatophytosis referred to Tehran's mycological laboratory.

2.2. Direct microscopic and laboratory examination

Skin and hair samples were examined by 10% KOH and Lacto phenol blue and cultured in Sabouraud agar with cycloheximide(0.5g/l) and chloramphenicol(0.05g/l)(SCC) (Quelab) and Sabouraud Dextrose Agar(SDA)(Quelab) [13]. In two separate groups, colonies were grown of *M. canis* (from human and animal infections) in SCC and SDA cultured in plates of 20 ml Sabouraud Dextrose Broth (Quelab) and incubated to 5 days at 25°C. The mycelium was grounded with a pestle in a mortar Surrounded by ice pack and without using liquid nitrogen and total DNA extracted by the rapid method [14].

2.3. PCR test for *M. canis* identification

The specific primers Mcfor (5' GTGTGATGGACGACCGTCCCCCT 3') and Mcrev (5' ATAATACATGGTGCCTTAGGCCAGCCTG 3') with NCBI Reference Sequence: NW_003299166, were prepared from Humanizing Genomics Macrogen Company [9] (Control positive was strain of *M. canis* (PTCC: 5069) and control negative was strain of *Trichophyton rubrum* (ATCC11404).

2.4. Molecular studies for SUB genes

The primers were purchased from Humanizing Genomics Macrogen Company based on the nucleotide sequences of the SUB1-3 genes in the GenBank database for *M. canis* [15]. All PCR amplification were performed in a Thermal Cycler (Eppendorf) in a final volume of 25 μ l. PCR cycling consisted of 94°C for 5 min followed by 35 cycles of 30 seconds at 94°C, 1 min at 58-62°C and 45 seconds at 72°C and a final extension step of 7 min at 72°C. Each PCR product was electrophoreed on 1% agarose gel in TBE buffer at 90 V for 1 h [14].

2.5. Statistical analysis

The relative frequency of subtilisin genes and their frequency distribution among isolates were analyzed by SPSS software version 16 and chi-square test.

3. Results

3.1. Isolation and identification of *M. canis* strains

Dermatophytic lesions were identified in 58% of animal samples (14/24), based on microscopic and macroscopic examination. On the other hand, yeast (n=1) and saprophytes (n=9) isolated from fungal infection [10]. *M. canis* were detected from human (n=3), cats (n=6) and dog (n=1) whit tinea corporis by observing the fragment 176 bp from PCR products in the 2% agarose gel (Fig. 1).

3.2. Identification of SUB1-3 genes

Positive results of PCR products were observed different fragment of SUB1(1540bp), SUB2(1496bp), and SUB3(1388bp), indicated the presence of subtilisin virulence genes

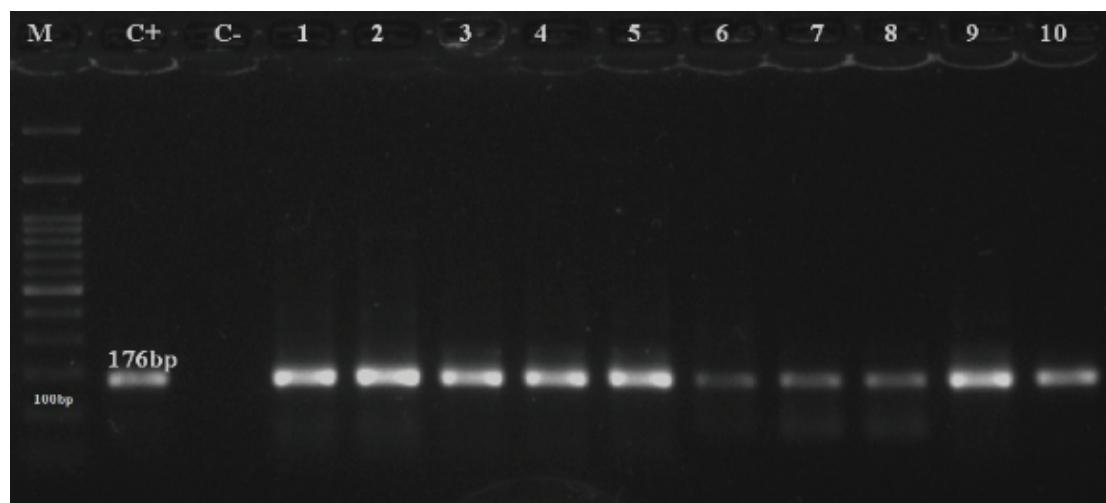


Figure 1: Positive results of 176 bp fragment of ITS1 sequence of the ribosomal DNA were obtained for 10/10 samples containing *M. canis*; Lane M – 100 – 3000 bp DNA molecular weight markers. Lanes 1- 3- infected human. Lanes 4- 8 and 10- infected cat. Lane 9 - infected dog. +C- Control Positive is *M. canis* isolate (PTCC: 5069). -C - Control Negative is *Trichophyton rubrum* isolate(ATCC11404).

in *M. canis* DNA (Fig. 2, 3, 4). The relative risk to develop infection associated with the presence of SUBs, SUB2 (9/10) and SUB1 (8/10) seem to be higher in isolates obtained from dermatophytosis and SUB3 gene was observed in a low percentage (70%; 7/10) of the isolates. Screening for the presence of SUB1-3 in isolates, representing 60% (6/10), were positive for SUBs, while in 10% (1/10) none of the SUBs was observed (Table. 1). In comparing SCC to SDA for isolation of fungi, SCC as an inhibitory (Bacteria and Saprophytic fungi) medium is preferable to SDA for investigate of subtilisin genes presence in *M. canis*.

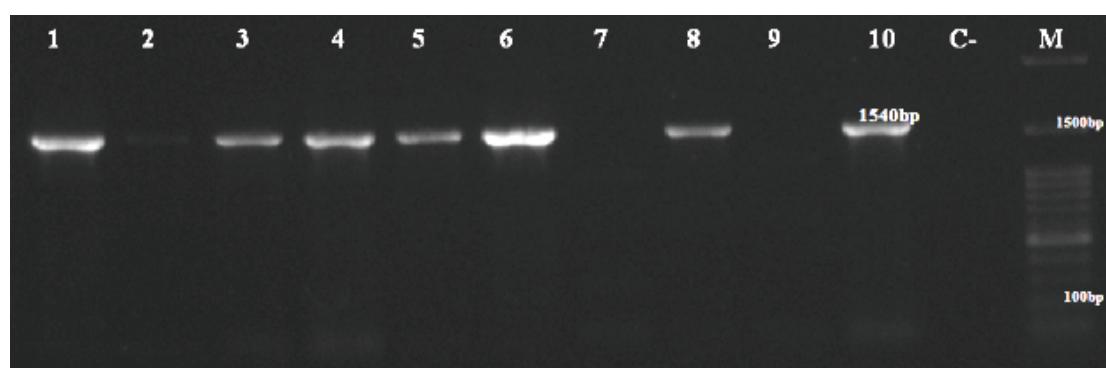


Figure 2: Positive results of 1540 bp fragment of SUB1 genes were obtained for 8/10 samples containing *M. canis*; Lanes 1- 3- infected human. Lanes 4- 8 and 10- infected cat. Lane 9 - infected dog. -C - Control Negative is *Trichophyton rubrum* isolate. Lane M – 100 – 3000 bp DNA molecular weight markers.

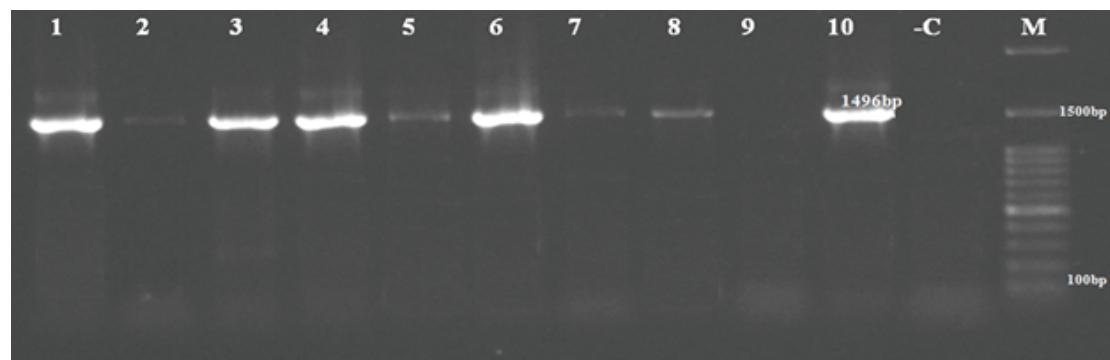


Figure 3: Positive results of 1496 bp fragment of SUB2 genes were obtained for 9/10 samples containing *M. canis*; Lanes 1-3- infected human. Lanes 4- 8 and 10- infected cat. Lane 9 - infected dog. -C - Control Negative is *Trichophyton rubrum* isolate. Lane M – 100 – 3000 bp DNA molecular weight markers.

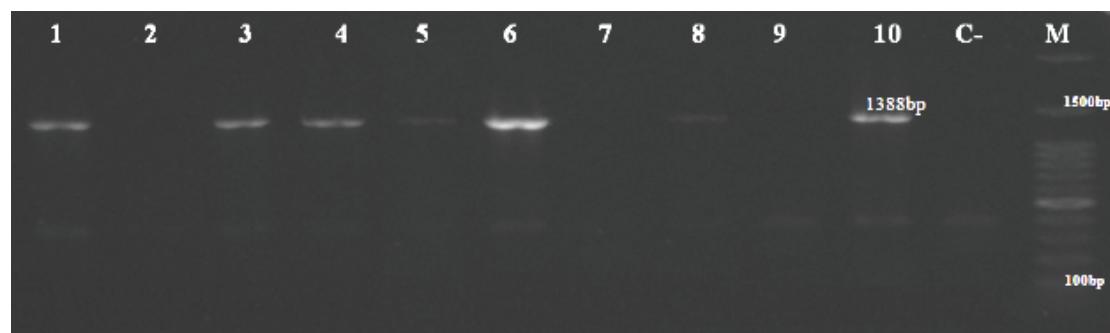


Figure 4: Positive results of 1388 bp fragment of SUB3 genes were obtained for 7/10 samples containing *M. canis*; Lanes 1-3- infected human. Lanes 4- 8 and 10- infected cat. Lane 9 - infected dog. -C - Control Negative is *Trichophyton rubrum* isolate. Lane M – 100 – 3000 bp DNA molecular weight markers.

TABLE 1: Distribution of SUBs on the analyzed Dermatophyte Infections.

		Hosts * SUBs Crosstabulation						Total	
Dermatophyte Infections		SUBs				SUBs absent(n=6)%			
		SUB1 (n=8)%	SUB2 (n=9)%	SUB3 (n=7)%	SUBs absent(n=6)%				
Hosts	Human	37.5	33.3	28.6	16.7			30.0	
	Animal	62.5	66.7	71.4	83.3			70.0	
Total		100	100	100	100			100.0	

4. Discussion

fungal infections are one of the most common skin infections in humans and animals. Dermatophytes and saprophytic fungi are the most important fungal agents in the development of these infections [16]. *M. canis* serve as the zoophilic dermatophyte and its infection has considerable zoonotic importance that transmitted from pets to their owners diagnosed with dermatophytosis [17]. The most common isolated dermatophytes were *M. canis* by Cafarchia et al. (2006) in Bari, southern Italy [18]. Basiri et al. (2012) were

reported the highest incidence of dermatophyte infection in dogs related to *M.canis* [19]. Katiraee et al. (2006) were investigated epidemiological factors of *M.canis* that transmitted from cats to their owners diagnosed with dermatophytosis [17]. Dermatophytes are pathogenic fungi that infect healthy people and the production of subtilisin proteases in vivo infection is known as an indicator of their importance in virulence of dermatophytes [20, 21]. A serine protease(SUBs) inhibitor, which significantly inhibits the adherence of *M. canis* arthroconidia to stratum corneum [22]. This study showed that a high percentage for SUB2 and SUB1virulence genes of *M. canis* isolates. Lemsaddek et al. (2010) were reported that SUB2 (82%) and SUB1(79%) detected in a high percentage of *M. canis* isolates [15]. At least the expression of SUB3 for keratinolytic activity in fungal infection is necessary [11]. SUB3 expression in *M. canis* DNA is a crucial event in initial contact [23, 24]. We reported that SUB3 gene was observed in a low percentage. Lemsaddek et al. (2010) were reported that SUB3 detected in a low percentage (68%) of *M. canis* isolates. These results suggested that SUB3 is not related to invade. In contrast, anti-SUB3 specific antibody(IgG)

could be detected [11, 25]. However, the expression of SUBs were shown during both adherence and early invasion [10]. In the present study, The absence of SUB 1, SUB2 and SUB3 in a *M. canis* isolated from dog with develop infection while in14% none of the SUB genes was reported by Lemsaddek et al [15].

5. Conclusion

SUBs coding serine proteases on *M. Canis* give it the ability to digest keratinized tissues (skin and hair) in humans and animals and zoonotic attribute. In the present study, the absence SUBs in clinical isolate, indicates that they are indispensable for initial contact and adherence of fungi to host cells.

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Conflict of Interest

None declared.

Ethical Approval

[All samples were collected using aseptic methods and in conformity with the ethical guidelines of the 1975 declaration of Helsinki].

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