

***Tannerella forsythia***

(2011 / 10/ 31 2011/ 9 /14 )

Chronic

(48)

(60-20)

periodontitis

*Tannerella forsythia*

Catalase

Esculine-

*Tannerella*

Boiling method

DNA

.bile salt medium

(1.8-1.6)

/

( 6.5-5.2)

*for sythia*

*Tannerella forsythia*

PCR

(%20.8)

*Tannerella forsythia*

PCR

(%43.7)

.16SrRNA

641 bp

*Tannerella forsythia*

:

## Comparison of Classical and Molecular Identification for *Tannerella forsythia* from Chronic Periodontitis Infections

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### ABSTRACT

The study included (48) Chronic Periodontitis samples were collected from both sexes with ages between (20-60) years, in addition to determination the average of pocket depth. Morphological and Classical characteristics of *Tannerella forsythia* using light and fluorescent microscopy were carried , in addition to biochemical tests. The species shown to be catalase and indol negative and without acid from glucose but Esculin hydrolysis test was positive while no growth was noted on esculin bile salt medium. DNA was extracted from *Tannerella forsythia* using the boiling method approximately (5.2-6.5) µg/ µl was obtained with (1.6-1.8) purity. polymerase chain reaction using species specific primer to amplify 641bp fragment from 16SrRNA gene was performed. It was found to be a more effective method for the detection of *Tannerella forsythia* in clinical specimen as it gave (43.7) positive isolates compared with (20.8) by using conventional methods.

**Keywords:** periodontitis, *Tannerella forsythia*, PCR.

Inflammatory disease

Periodontitis

%35

.(Niwa *et al.*, 2011; Tortora *et al.*, 2010)

(700)

Dental plaque

(400)

*Tannerella forsythia*

*Treponema denticala* *Aggregatibacter actinomycetemcomitans* *Dialister pneumosintes*

Infectious agents

*Porphyromonas gingivalis*

.(Boyanova *et al.*, 2009 ; Yoo *et al.*,2007)

.....

(*Bacteroides forsythus* ) *Tannerella forsythia*

*Bacteroides* (1986) Tanner

*Bacteroides* *Bacteroides* (1989) Shah and Collins

*Bacteroides* *fragilis*

(1994) Paster .*Bacteroides* *Bacteroides forsythus*

*Bacteroides forsythus* 16SrRNA

*Porphyromonas* *Bacteroides forsythus* *Bacteroides*

16SrDNA " (%86) 16SrRNA

.(Sakamoto *et al.*, 2002) *Tannerella*

.(Brooks *et al.*, 2010 ; Kawasea *et al.*, 2010)

*Porphyromonas gingivalis* *Tannerella forsythia*

.(van Winkelhoff *et al.*, 2002)

*Tannerella forsythia*

Immunosuppressive Adhesions

(BspA) Antiphagocytic factors

Epithelial Fibronectin and fibrinogen binding Coaggregation

attachment and invasion

(Saito *et al.*,

.2009)

.(Gomes *et al.*, 2006)

DNA probes

ELISA

Indirect immunoflouorescence assay

(Boyanova *et al.*, Benzoyl - DL- arginine- haphthylamide

. 2009)

Real time- PCR

.(Saito *et al.*, 2009)

*Tannerella forsythia*

PCR

. DNA

Periodontitis

48

3 ≤ Periodontal pocket

: (Loo *et al.*, 2009)

- 
- 
- 
- 

.Periodontal probe

Paper point

Normal saline<sup>3</sup> ( 0.5)

.(Zuger *et al.*, 2007)

.PCR

:  
:

Trytic soy agar (TSA)

(1)

%(0.4)

( %5)

<sup>3</sup> /

(5)

(%5)

Brucella agar

(Sabet *et al.*, 2003) K

.....

(Meurman *et al.* , 1997) K<sup>3</sup> / (10) Hemin  
 7- 5 37 (Oxiod) Gaspak anaerobic system

Yeast-Cysteine-Blood agar (YCB)

Hemin %(0.001) K %(0.0001) (Gersdorf *et al.*, 1993)

.(Yoo *et al.*, 2007)

.Light Microscope -1

.( Tortora *et al.*, 2010)

.Fluorescent Microscope -2

Acridin orange  
/

.(Tortora *et al.*, 2010)

Esculine bile salt

.(Braham and Moncla., 1992 ; Gersdorf *et al.*, 1993)

Boiling method

DNA

DNA

(Freschi *et al.*, 2005)

UV-Transilluminator

DNA

.(Sambrook *et al.*, 1989)

BIONEER

Primers for <i>T. forsythia</i>	size
Tf- F 5- GCG TAT GTA ACC TGC CCG CA-3 Tf- R 3- TGC TTC AGT GTC AGT TAT ACC T-5	641bp

96.9 (Forward)  
 133.9 (Reverse) / 100  
 (Stock solution) / 100  
 / 20 Working solution  
 .(Loo *et al.*, 2009)

: 25

1X	12.5µl	Go Taq®Green master mix, 2X
0.1- 1.0µ	0.25- 2.5µl	Up stream primer, 10µM
0.1-1.0µ	0.25- 2.5µl	Down stream primer, 10µM
<250 ng	1-5µl	DNA template
N.A	25µl	Nucleuse- Free water to

Thermal Cycler

° 60 30 ° 95) 35 ( ° 95)  
 .( 7 ° 72) ( ° 72 30

:

DNA Ladder

100 PCR

60 100

%1

260 UV-Transilluminator

(%100) 48

48

(2,1)

(3)

.(2007)

Martinez

(%27.3)

(30-20)

(40-31)

(%54.5)

(%29.4)

(%11.8)

.....

*Tannerella forsythia*

7-5 37

Brucella agar Tryptic soy agar

Yeast- cysteine- blood agar (YCB)

(YCB) 1

.(1)

(Zuger *et al.*, .(2)

2007)

(3) ( ) "

.(de Lillo *et al.*, 2004 ; Leys *et al.*, 2002) *Tannerella forsythia*

*Tannerella forsythia*

Esclin-bile

(Sakamoto *et al.*, 2002 ; Braham and Bacteriodes salt

.Moncla., 1992)

(%20.8) (10) *Tannerella forsythia*

.(%79.2) (38)

*Tannerella forsythia*

(Loo *et al.*, 2009; Suzuki *et al.*, 2004)

N-acetylmuramic acid

PCR

(2007) Slots

*Tannerella forsythia* ( 2011) Honma

N- Trypsin-Like protease Sialidase

BspA S-layer benzoly – Val-Gly- Arg-*p*- nitroanilide- specific protease

( 5.9)

(Kishi *et al.*, 2010)

(2009) Boyanova

*Tannerella forsythia*

( 7-5)

(2005) Freschi

(6.5- 5.2) DNA *Tannerella forsythia* DNA  
 .(1.8-1.6) /

DNA

(%43.7)

( 21)

PCR

(27)

16SrRNA

(641bp)

*Tannerella forsythia*

DNA

(%56.3)

DNA

(641bp)

*Tannerella forsythia*

*Invitro*

PCR

.(4)

DNA

*Tannerella*

PCR

*for sythia*

PCR

(1997)

Meurman

(3)

PCR

(%37.9)

(%89.7)

5

PCR

(2007)

Tanner

*Tannerella*

*for sythia*

PCR

( 4.8)

PCR



.....

( 7-5) (%50) ( 5-3) (2009) Boyanova (% 37.5)  
 . 7 (% 37.5)  
 (266) (2009) Loo  
 PCR (% 26.3) (70)

: 1

		<b>48=</b>	
(%78.1)25	(%21.9)7	(%66.7)32	
(81.2)13	(%18.8)3	(%33.3)16	
		(60-22)35	
		5.9	

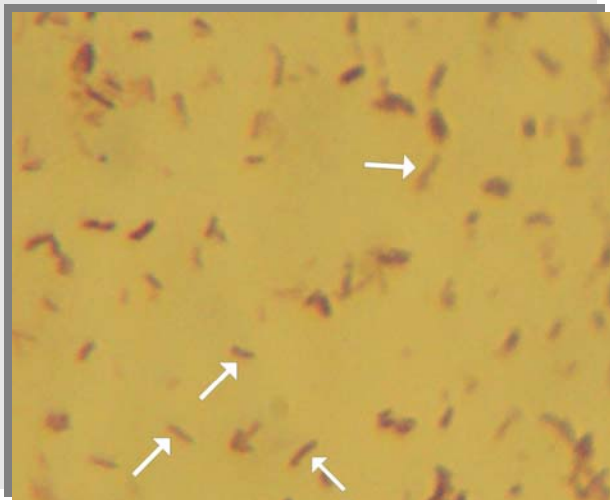
.(PCR)

:2

		<b>48 =</b>	
(%53.1)17	(%46.9)15	(%66.7)32	
(%62.5)10	(%37.5)6	(%33.3)16	
		(60-20)35	
		4.8	

:3

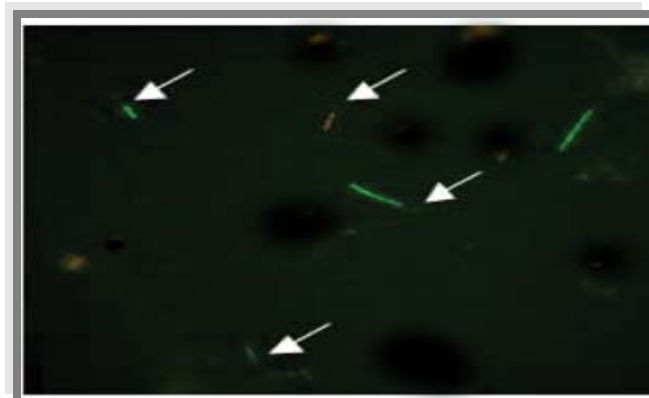
(%45.5)10	(%54.5)12	(%72.7)16	(%27.3)6	22	30-20
(%70.6)12	(%29.4)5	(%88.2)15	(%11.8)2	17	40-31
(%60)3	(%40)2	(%80)4	(%20)1	5	50-41
(%50)2	(%50)2	(%75)3	(%25)1	4	60-51
(56.3) 27	(43.7) 21	(79.2) 38	(20.8)10	48	



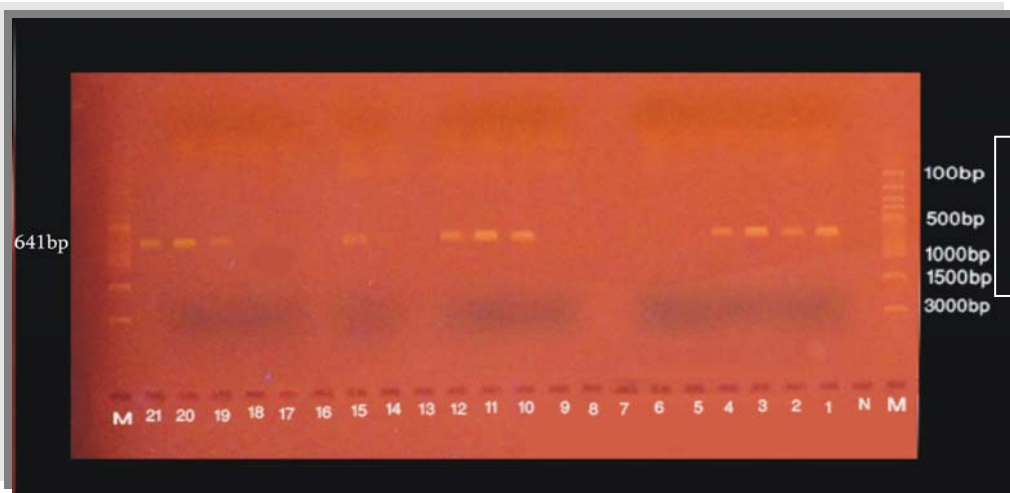
الصورة 2: خلايا جراثيم *Tannerella forsythia* تحت المجهر الضوئي (1000 X).



1: *Tannerella forsythia* .YCB



الصورة 3: خلايا جراثيم *Tannerella forsythia* تحت المجهر المتألق (400 X).



*Tannerella forsythia* DNA

:4

M  
N

- Boyanova, L.; Setchanova, L.; Gergova, G.; Kostyanov, T.; Yordanov, D.; Popova, C.; Kotsilkov, K.; Mitov, I. (2009). Microbiological diagnosis of the severe chronic periodontitis. *J. IUAB.*, 89- 94.
- Braham, P.H. ; Moncla, B. J. (1992). Rapid presumptive identification and further characterization of *Bacteroides forsythus*. *J. Clin. Microbiol.*, **30** (3), 649-654.
- Brooks, G. F.; Carroll, K. C.; Butel, J. S.; Morse, S. A.; Mietzner, T.A. (2010). Jawetz, Melnick and Adelberg's "Medical Microbiology". 25th edn., McGraw- Hill Companies, Inc., USA, pp. 274- 275.
- de Lillo, A.; Booth, V.; kyricou, L.; Weightman, A. J.; Wade, W. G. (2004). Culture-independent identification of periodontitis – associated Porphyromonas and Tannerella populations by targeted molecular analysis. *J. Clin. Microbiol.*, **42**, 5523- 5527.
- Freschi, C.R.; Carvalho, L.F.S. ; de Oliveira, C.J.B. (2005). Comparison of DNA- extraction methods and selective enrichment broths on the detection of *Salmonella typhimurium* in swine feces by PCR. *Braz. J. Microbiol.*, **36**, 363-367.
- Gersdorf, H.; Meisner, A.; Pelz, K.; Krekeler, C.; Gobel, U.B. (1993). Identification of *Bacteroides forsythus* in subgingival plaque from patients with Advanced periodontitis. *J. Clin. Microbiol.*, **31**(4), 941-946.
- Gomes, B.P.; Jacinto, R. C.; Pinheiro, E.T.; Sousa, E. L.; Zaia, A. A. ; Ferraz, C. C. ; Souza-filho, F. J. (2006). Molecular analysis filifactor alocis, *Tannerella forsythia*, and *Treponema denticola* associated with primary endodontic infections and failed endodontic treatment. *J. Endod.*, **32**, 937- 940.
- Honma, K. ; Mishima, E. ; Sharma, A. (2011). Role of *Tannerella forsythia* NanH sialidase in epithelial cell attachment infection and immunity. *J. Med. Microbiol.*, **79**(1), 393- 401.
- Kawasea, N.; Kishib, J. I.; Nakamura, H.; Hayakawab, T. (2010). Collagenolytic activity in sonic extracts of *Tannerella forsythia*. *J. Oral. Sci.*, **55**(8), 545- 549.
- Kishi, M.; Nemoto, Y.O.; Takahashi, M.; Kishi, K.; Kimura, S. ; Yonemitsu, M. (2010). Relationship between oral status and prevalence of periodontopathic bacteria on the tongues of elderly individuals. *J. Med. Microbiol.*, **59**, 1354- 1359.
- Leys, E. J.; Lyons. S. R.; Moeschger, M.L.; Rumpf, R.W. ; Griffen, A.L.(2002). Association of *Bacteroides forsythus* and a novel Bacteroides phylotype with periodontitis. *J. Clin. Microbiol.*, **40**, 821-825.
- Loo, W.T.Y.; Tin, L.T.; Cheung, M.N.B. ; Dou, Y.D. (2009). Detection of *Bacteroides forsythus* and *Porphyromonas gingivalis* in infected root canals during periapical periodontitis by 16Sr DNA. *Afr. J. Biotechnol.*, **8**(10), 2021-2026.
- Martinez, M.C.; Restrepo, D.P.; Isaza, D.M.; Orozco, L.M. ; Tobon, S. I. (2007). Detection of *Treponema denticola* in saliva obtained from patients with various periodontal conditions. *Clin. Ord. investigations*, original article.
- Meurman, J. H.; Wahlfors, J.; Korhonen, A.; Alakuijala, P.; Vaisaner, P.; Torkko, H. ; Janne, J. (1997). Identification of *Bacteroides forsythus* in subgingival Dental plaque with the Aid of a rapid PCR method. *J. Dent. Res.*, **76** (7), 1376-1380.
- Niwa, D.; Nishikawa, K.; Nakamura, H. (2011). Ahybrid two- component system of *Tannerella forsythia* autoaggregation and post- translational modification of surface proteins. *FEMS Microbiol lett.*, **318**(2), 189- 196.
- Paster, B. J.; Dewhirst, F. E.; Olsen, I.; Fraser, G. J. (1994). Phylogeny of *Bacteroides*, *Prevotella*, and *Porphyromonas spp.* and related bacteria. *J. Bacteriol.*, **176**, 725- 732.

- Sabet, M.; Lee, S.W.; Nauman, R. K.; Sims, T.; Um, H. S. (2003). The surface (S-layer) is a virulence factor of *Bacteroides forsythus*. *J. Clin. Microbiol.*, **149**, 3617-3627.
- Sabeti, M.; Simon, J. H.; Slots, J. (2003). Cytomegalovirus and Epstein- Barr virus are associated with symptomatic periapical pathosis. *Oral Microbiol. Immunol.*, **18**, 327-328.
- Saito, D.; Coutinbo, L.L.; Saito, C.P.B.; Tsai, S. M.; Hofling, J. F.; Goncalves, R.B. (2009). Real-time polymerase chain reaction quantification of *Porphyromonas gingivalis* and *Tannerella forsythia* in primary Endodontic infections. *J. Endod.*, **35**(11), 1518- 1524.
- Sakamoto, M.; Suzuki, M.; Umeda, M.; Ishikawa, I. ; Benno, Y. (2002). Reclassification of *Bacteroides forsythus* (Tanner *et al.*, 1986) as *Tannerella forsythensis* corrig., gen. nov., *Comb. Nov. J. Syst. Evol. Microbiol.*, **52**, 841- 849.
- Sambrook, J.; Fritsch, E.F. ; Manatis, T. (1989). "Molecular Cloning: A Laboratory Manual". 2th edn., Cold spring Harbor laboratory press, Cold Spring Harbor. N.Y.P. 957p.
- Shah, H. N. ; Collins, M. D. (1989). Proposal to restrict the genus *Bacteroides* (*castellani* and *chalmers*) to *Bacteroides fragilis* and closely related species. *J. Syst. Bacteriol.*, **39**,85-87.
- Slots, J. (2007). Herpesviral- bacterial synergy in the pathogenesis of human periodontitis. *Curr. Opin. Infect. Dis.*, **20**, 278- 283.
- Suzuki, N.; Yoshida, A.; Saito, T.; kawada, M.; Nakono, Y. (2004). Quantitative microbiological study of subgingival plaque by Real- Time. PCR shows correlation between Levels of *Tannerella forsythensis* and *Fusobacterium spp.* *J. Clin. Microbiol.*, **42**(5), 2255- 2257.
- Tanner, A.C.R.; Listgarten, M. A.; Ebersole, J. L.; Strzempko, M.N. (1986). *Bacteroides forsythus* sp. Nov., a slow- growing, fusiform *Bacteroides* sp. From the human oral cavity. *J. Syst. Bacteriol.*, **36**, 213- 221.
- Tanner, A. C. R.; Paster, B. J.; Lu, S. C.; Kanasi, E.; Kent, R.; van Dyke, T. ; Sonis, S.T. (2007). Subgingival and tongue microbiota during early periodontitis. *J. Dent. Res.*, **85**(4), 318-323.
- Tortora, G. J.; Funke, B.R.; Case, C. L. (2010). " Microbiology". 10th edn., Pearson Education, Inc., USA, pp. 324- 325.
- van Winkelhoff, A. J.; Loos, B. G.; van der Reijden, W. A.; van der Velden, U. (2002). *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *J. Clin. periodontol.*, **29**, 1023- 1028.
- Yoo, J.Y.; Kim, H. C.; Zhu, W.; Mikim, S.; Sabet, M.; Handfield, M.; Hillman, J.; progulskefox, A.; Lee, S.W. (2007). Identification of *Tahnerella forsythia* antigens specifically expressed in patients with periodontal disease. *FEMS Microbiol lett.*, **275**: 344- 352.
- Zuger, J.; Schaller, H. L.; Gmur, R. (2007). Uncultivated *Tannerella* Buo45. and Buo63 are slim segmented filamentous rods of high prevalence but low abundance in inflammatory disease – associated dental plaques. *Microbiology.*, **153**, 3809-3816.