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CRYPTOBACTERIUM CURTUM: A NOVEL BACTERIA WITH A POSSIBLE SIGNIFICANT INVOLVEMENT IN PERIODONTAL DISEASE. A REVIEW.

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ABSTRACT

Periodontal disease is considered to be connected with some very well-known anaerobic etiological agents. Many studies are analysing the associations between Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Tannerella forsythia with periodontal disease. Still, the prevalence of this disease is increased. In this review we aimed to present a possible new bacteria connected with periodontal disease: Cryptobacterium curtum. We performed a search in PubMed and we identified 8 articles, published between 1999 and 2018. Here we present a summary about this bacterial species regarding its microscopic, cultivation and genomic features. New molecular biology techniques, such as NGS (next generation sequencing) or mRNA technology, identified this bacteria to be associated with acute dental abscesses, and with chronic periodontitis. The virulence factors of this bacteria are not yet identified, but with the recent highly sensitive techniques, there is hope to completely establish the role of C. curtum in the pathogenesis of periodontal disease.

Keywords: *periodontal disease, etiologic agents, modern technology, NGS, mRNA*

1. INTRODUCTION

Periodontitis is a polymicrobial disease caused by complex interactions between distinct pathogens.[1] At the biofilm level we can observe the occurrence of destructive phenomena on periodontal tissues.[2,3] It is unanimously accepted in the literature that certain microorganisms, which are still poorly understood, may be involved in the onset or progression of periodontitis [4]. For several decades, oral microbiology research has failed to identify the composition of the subgingival microbiota due to technical limitations.[5]

In the last decade, due to paradigm shifts involving the use of molecular biology and sequencing techniques, the detection of novel periodontal pathogens has become feasible.[4-6] It is therefore evident that, in addition to conventional periodontal pathogens, other microorganisms could be significantly involved in the occurrence and progression of periodontal and peri-implant pathological processes.[7,8]

Among the new periodontopathogenic microbial agents we can list *Cryptobacterium curtum, Dialister pneumosintes, Filifactor alocis, Mitsuokella dentalis, Slackia exigua, Selenomonas sputigena, Solobacterium moorei, Treponema lecithinolyticum and Synergistes.*[9]

2. *CRYPTOBACTERIUM CURTUM* - GENERALITIES

The name *Cryptobacterium curtum* derives from the Greek words "Kryptos," which means "hidden" and "curtum," which means "shortened." These aspects led to its inclusion in the genus *Eubacterium*, but subsequent reclassification was necessary into a new genus called *Cryptobacterium.*[10-11] We can point out that it belongs to the domain *Bacteria*, phylum Actinobacteria, class *Actinobacteria*, order *Coriobacteriales*, family *Coriobacteriaceae*, genus *Cryptobacterium*, and species *Curtum*. Morphologically, they exhibit characteristics pathognomonic of short gram-positive bacilli, obligate anaerobic, lacking motility and spores, mesophilic, non-sucrolytic and can range in size from 0.8 μm to 1.0 μm. Ultrathin sections showed a single-layered cell wall, with a thickness of 10 nm, in which no pili or flagella were identified (Fig. 1).

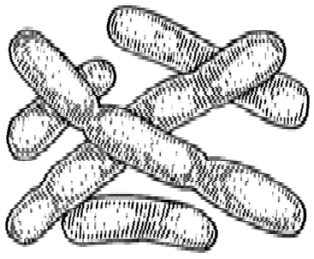

Figure 1. Schematic representation of *Cryptobacterium curtum*

On specific and favourable culture media, the formation of small translucent non-haemolytic colonies with a < 1 mm (0.3-0.5 mm) diameter can be observed.[9-11]
Growth of *C. curtum* in broth with glucose-supplemented peptone yeast extract can be optionally augmented with 0.5% arginine, derived by enzymatic degradation of arginine deaminase-associated peptides. At the same time, *C. curtum* shows poor growth due to limited substrate utilisation for metabolic activity.[10-13]
Cryptobacterium curtum is a relatively recently identified and characterized species that has been isolated from the gingival sulcus of patients with periodontal disease.[14] There is, however, limited scientific evidence demonstrating more frequent detection of *T. socranskii* in human subjects with periodontitis, although the observed differences were not statistically significant.[15] At the same time, we can recall that the prevalence of the bacterium was higher with the increasing of probing depth and the exceeding of the physiological threshold. [16]

3. DESCRIPTION OF CRYPTOBACTERIUM GEN. NOV.

Cryptobacterium (Crypt. 0.b ac. te'ri.um. Gr. n. kryptos hiding; Gr. n. bakterion a small bacillus; M.L. neut. n. *Cryptobacterium* a bacteria hiding in the form of a bacillus). The cells may present as short Gram-positive bacilli, occasionally Gram-variable, when in the stationary phase. They are obligately anaerobic, lacking motility and non-spore-forming. Biochemically, they are catalase-negative, asaccharolytic and without volatile end products in glucose-supplemented peptone yeast extract. From an analytical

perspective we can mention that the G + C DNA content is 50-51 mol%. According to the almost complete 16s rDNA sequence, the genus groups with the general actinomycete group of Gram-positive bacteria and the type species is *Cryptobacterium curturn.*[14]

4. DESCRIPTION OF CRYPTOBACTERIUM CURTUM SP. NOV.

Cryptobacterium curtum (cur'tum. L. neut. adj. shortened curtum, a shortened cell of this organism). This description is based on the study of two strains isolated from the oral cavities of patients with periodontal disease. The cells are very short bacilli, Gram-positive, obligately anaerobic, non-motile and non-spore-forming.[11-15] The cells may be individual or may appear as arranged in clusters. Older cultures sometimes stain Gram-negative, due to changes that may occur in the bacterial cell wall. Tiny colonies, less than 1 mm in diameter, form on culture media and are circular, convex and translucent even after prolonged incubation in an anaerobic glove box. Growth in broth media is poor with or without carbohydrates. No hemolysis occurs on blood agar plates with brain heart infusion blood. Cells are inert in most biochemical tests.[23] Starch and esculin are not hydrolysed; nitrate is not reduced. No liquefaction of gelatin occurs. Indole, urease and catalase tests are also negative. Ammonia is produced from arginine. The strains are non-fermentative and do not utilise adonitol, amygdalin, arabinose, cellobose, erythritol, aesculin, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose or xylose.[24-26]

No metabolic end product is detected in glucose-supplemented peptone yeast extract medium or glucose-supplemented peptone yeast extract broth. It was isolated from the periodontal pocket of a human adult with periodontal disease. The G + C DNA content is 50-51 mol%.[14]

5. GENOME PROPERTIES

The genome has a length of 1 617 804 bp and comprises a main circular chromosome with 50.9% GC content (Table I). Of the 1422 predicted genes, 1364 were protein-coding genes and 58 were RNA-coding genes.[27-29] A total of 7 pseudogenes were also identified. Among the majority of protein-coding genes (78.5%) were assigned with a putative function, while the rest were noted as hypothetical proteins.[30-31] Genome properties and statistics are summarised in Table I. The distribution of genes into COG functional categories is shown in Table II.[10]

Table I: Genes of *Cryptobacterium curtum* identified by complete genome sequencing (adapted from Mavrommatis et al., 2009)

Category of gene	% of Total
RNA genes	2.37%
rRNA operons	
Protein-coding genes	95.92%
Pseudo genes	0.49%
Genes with function prediction	78.55%
Genes in paralog clusters	5.41 %
Genes assigned to COGs	77.57%
Genes assigned Pfam domains	77.64%
Genes with signal peptides	19.37%
Genes with transmembrane helices	14.46%

Table II. The most representative genes of *Cryptobacterium curtum* (adapted from Mavrommatis et al., 2009)

Description of gene function	%
Translation, ribosomal structure and biogenesis	**9.4**
RNA processing and modification	**0.1**
Transcription	**6.9**
Replication, recombination and repair	**5.5**
Chromatin structure and dynamics	**0.1**
Cell cycle control, mitosis and meiosis	**1.1**
Nuclear structure	**0.0**
Defense mechanisms	**1.5**
Signal transduction mechanisms	**4.7**
Cell wall/membrane biogenesis	**5.1**
Cell motility	**0.1**
Cytoskeleton	**0.1**

Other genes identified related to energy production and conversion, carbohydrate transport and metabolism, amino acid transport and metabolism, nucleotide transport and metabolism, coenzyme transport and metabolism, lipid transport

and metabolism, inorganic ion transport and metabolism. This full complete genome analysis performed with old and new NGS technologies offered the possibility of properly classification of this bacterial species, from *Eubacterium* (*Firmicutes*) to *Actinobacteria,* close to the *Coriobacteriaceae.* [10]

6. ASSOCIATION OF *C. CURTUM* WITH PERIODONTAL DISEASE

Kumar et al., 2003, [32] using PCR amplification of the 16S rDNA and the downstream intergenic spacer region (ISR) for bacterial species and phylotypes detection, identified *C. curtum with a prevalence of* 33% in healthy persons and with a prevalence of 64% in periodontitis patients, with a p = 0.0005, which suggest its significant association as a possible etiologic agent. Using 16S gene sequenced on the Ilumina MiSeq platform, Lopez-Oliva et al., 2018, [33] identified *Cryptobacterium curtum*, from subgingival plaque of individuals with periodontal disease. These two very sensitive molecular biology techniques have the advantage of detecting new bacterial species associated with periodontal disease, but only after larger studies very well conducted, would it be possible to confirm its pathogenicity and its virulence factors associated with periodontal diseases.

The diversity and complexity observed are likely due to several factors, including the interdependence of bacterial species within commensal communities as well as inter-individual variation in microbial aetiology and host susceptibility.[34,35] Elucidation of the pathogenesis mechanisms of periodontal and peri-implant diseases requires the initiation of future studies focusing on obtaining quantitative information on the proportions of these newly identified species especially at active disease sites.[7]

7. CONCLUSION AND PERSPECTIVES

The microbial aetiology of both chronic and acute periodontal and peri-implant processes is more complex than the information provided in the current literature and a large number of bacterial species, appear to show a possible association with disease. This premise necessitated the expansion of the list to include several uncultured species but recently identified, by ribosomal sequence analysis.

Longitudinal studies may play a key role in understanding the natural history of this chronic disease. At the same time, bacterial species found in a healthy subgingival environment deserve further study to be able to predict the identification of new microbial periodontal pathogens.

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