

microbial analysis of saliva

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Introduction

- Saliva has attracted attention as a diagnostic fluid due to the association of oral microbiota with systemic diseases.
- Around 600 bacterial species and a still undetermined number of fungal species inhabit the oral cavity of humans
- Oral microbial cells arrange in organized biofilm structures on non-shedding surfaces such as teeth.

Aims

- This lecture aims to get to know microbial agents isolation from saliva and causes different disease which include :
 - 1- bacteria
 - 2- parasite
 - 3- fungus

Bacteria

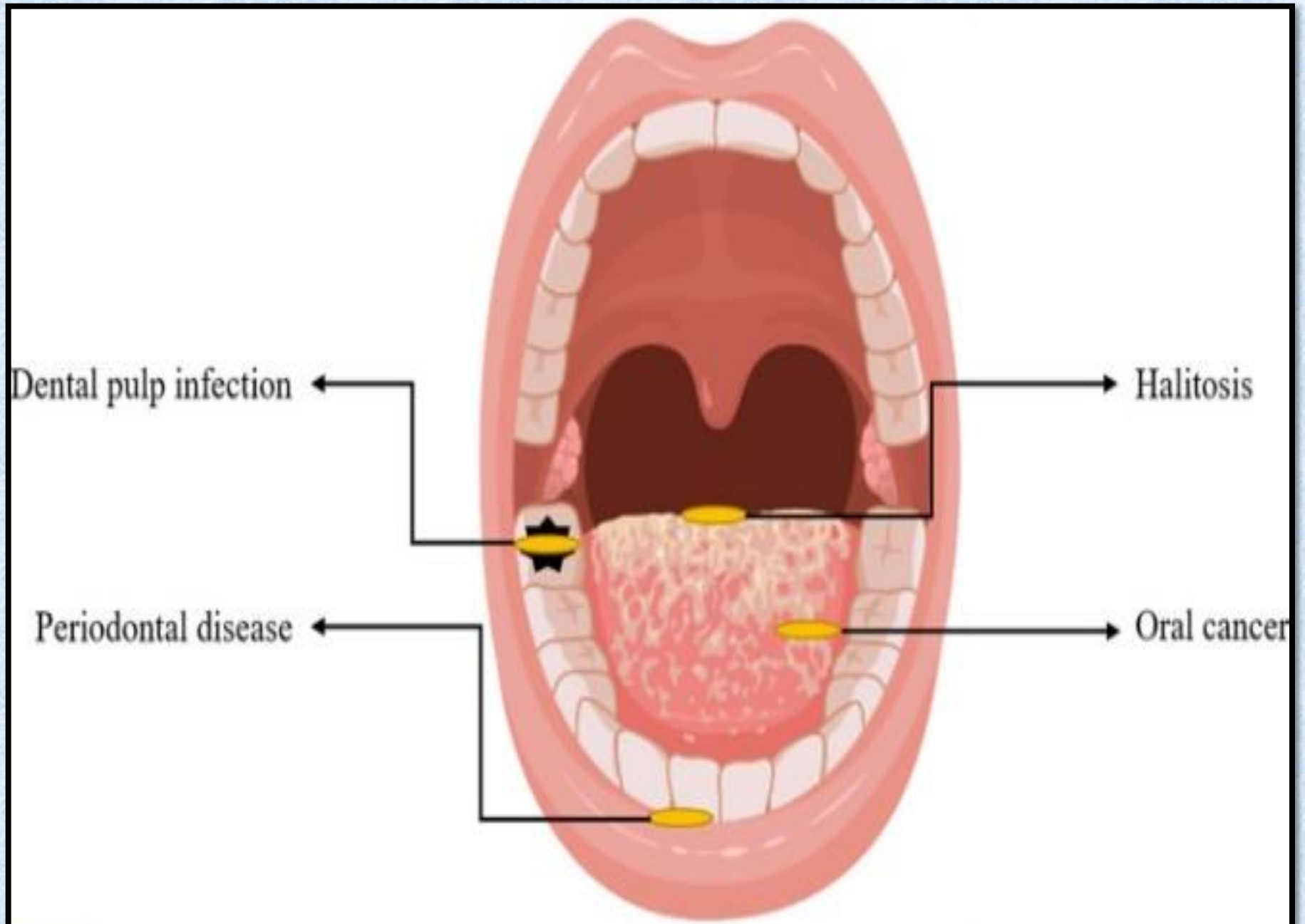
- **Fusobacteria**


- The genus *Fusobacterium* includes **gram-negative anaerobic rods**, which are part of the **natural bacterial flora** of the upper respiratory, digestive and genital tracts. Pathogenic species include *F. necrophorum* and *F. nucleatum*.

- **F. nucleatum** is part of **dental plaque**, invasiveness is enabled by the ability to adhere to both G-negative and G-positive biofilm. Bacteria found in the **healthy gingival** and **can also cause inflammation of the periodontium.**

- **F. necrophorum** causes serious infections of children and adolescents, a severe condition is **necrotizing tonsillitis** accompanied by the formation of abscesses.

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 *Fusobacterium nucleatum*

❖ **Characteristics and isolation:**

- Gram-negative, strictly anaerobic, **cigar-shaped bacilli** with **pointed ends** (Cells often have a central swelling.



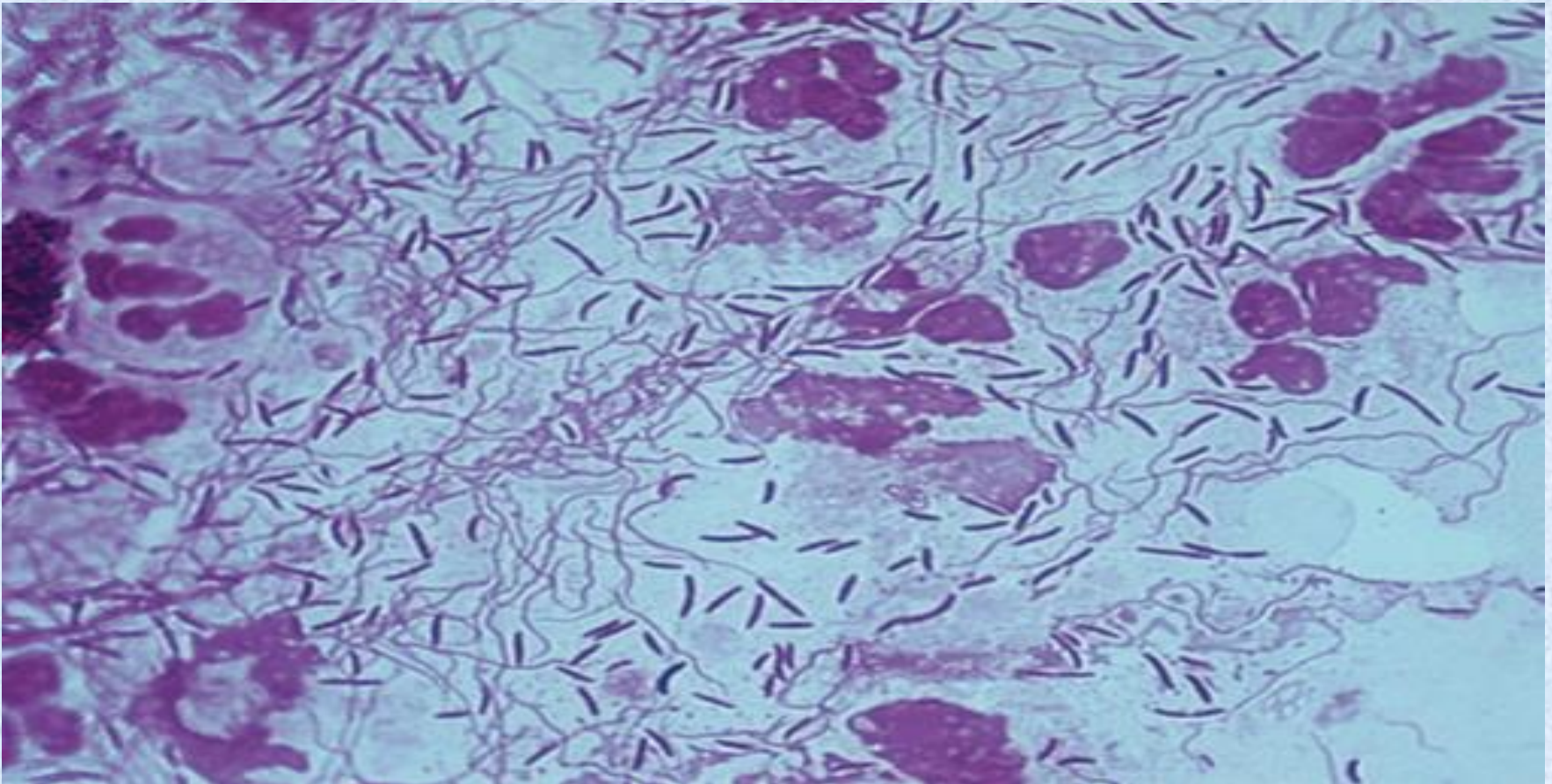
❖ **Culture and identification:** Grows on **blood agar** as dull, granular colonies with an irregular, rhizoid edge.

❖ As fusobacteria can remove sulphur from cysteine and methionine to produce odoriferous hydrogen sulphide and methylmercaptan, they are thought to be associated with **halitosis**.

- **acute (necrotizing)** ulcerative gingivitis or trench mouth (**Vincent's angina**, an ulcerative tonsillitis causing tissue necrosis, often due to **extension** of acute ulcerative gingivitis).



❖ **Diagnosis** : A Gram-stained smear obtained from deep gingival plaque of a patient with acute ulcerative gingivitis showing the fusospirochaetal complex.



Leptotrichia spp

- Leptotrichia spp are oral commensals **previously** thought to belong to the genus Fusobacterium. They are Gram-negative, strictly anaerobic, slender, filamentous bacilli, usually with one pointed end.
- **Leptotrichia buccalis**, present in low proportions in dental plaque, is the sole representative of this genus.
- **Leptotrichia buccalis** It is a constituent of normal oral flora.

❖ **Morphology:** *Leptotrichia*

species are typically large, fusiform-shaped, non-sporulating, and non-motile rods.

• **Pathology:** Almost every case of severe infection with *Leptotrichia buccalis* reported in medical literature occurred in patients with **neutropenia** .

Culture characteristics :

- Obligate anaerobic / AEROTOLERANT
- **Brucella blood agar (BBA):** The colonies are 0.5-3.0 mm, convex and with a convoluted surface. They are sparsely filamentous to irregular and **grayish brown** in color, with a dark central spot in old colonies. Some colonies are opaque and dry in consistency.



- ***Aggregatibacter actinomycetemcomitans***

- It is one of the bacteria that might be implicated in **destructive periodontal disease**.
- it has been found more frequently in **localized aggressive periodontitis**

• Culture

- Culture: **tryptone soy serum bacitracin vancomycin agar (TSBV agar)**
- The colonies are translucent/transparent, with irregular edges, smooth, circular, convex in shape. Fresh isolates have a “**star shaped**” (or) “**crossed cigar**” morphology form, embedding in the agar.



Figure 1. *Aggregatibacter actinomycetemcomitans* colonies develop a star-shaped structure after prolonged incubation.

- **Anaerobic Bacteria: Bacteroides, Porphyromonas, Prevotella & Tannerella**

- Short Gram-negative rods or coccobacilli.

- **Tannerella, Porphyromonas and Prevotella.**

Together they comprise a substantial proportion of the microflora of the **dental plaque, intestine and the female genital tract.**

- **Collectively, *Tannerella*, *Porphyromonas* and *Prevotella* species** are referred to as **black-pigmented anaerobes**, as some organisms from these genera form a characteristic brown or black pigment on blood agar.

Bacteroides species

☐ Bacteroides is a genus of Gram-negative, obligate anaerobic bacteria.

Bacteroides species are **non endospore-forming bacilli**, and may be either motile or nonmotile, depending on the species.

☐ **Bacteroides fragilis is the main pathogen.**



❖ **Culture :**

- ❖ *Bacteroides fragilis*: they demonstrate **slow growth on blood agar and appear as grey to opaque, translucent colonies.**
- They grow well in **Robertson's cooked meat medium** supplemented with yeast extract.

• *Porphyromonas* spp

- *Porphyromonas* spp. are a saccharolytic pigmented species and form part of the normal oral flora. They are agents of periodontal disease and hence considered as **periodontopathic** organisms.
- *P. gingivalis* is sometimes recovered from the tongue and tonsils.

Culture and identification

Grows anaerobically, **with dark pigmentation**, on media containing lysed blood,

• *Prevotella*

- ❖ *Prevotella* spp. include saccharolytic oral and genitourinary species; some species are periodontopathic.
- ❖ *Prevotella* spp. are members of the **oral, vaginal, and gut microbiota** and are often recovered from anaerobic infections of the respiratory tract.
- ❖ *Prevotella* spp. predominate in **periodontal disease and periodontal abscesses**

- Strains of *Prevotella intermedia* are associated more with periodontal disease.
- *Prevotella nigrescens* is isolated more often from healthy gingival sites

❖ **Prevotella Culture :**

Non-motile, short, round-ended, Gram-negative rods; **brownblack** colonies on blood agar (when pigmented). Molecular techniques are required to differentiate some species.

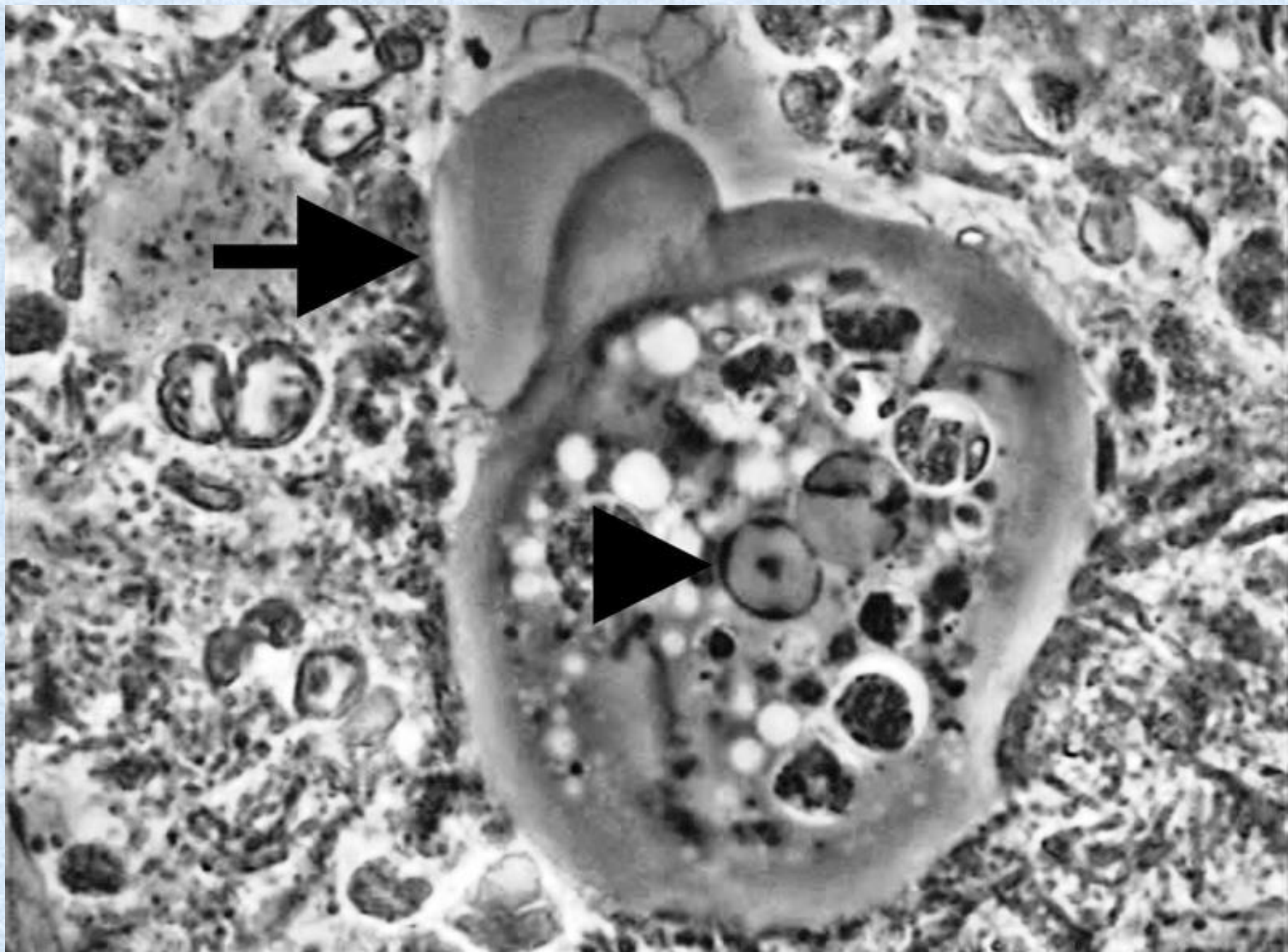
- **Tannerella** (*T.forsythia*)
- **Tannerella spp.** are black pigmented, anaerobic rods, strongly implicated as a major pathogen of periodontal disease.
- *T. forsythia* is frequently isolated with *P. gingivalis* indicating an ecological relationship between them.

❖ **Characteristics**

- Non-motile, pleomorphic, spindle-shaped
Gram-negative rods, Anaerobic Rod

Parasite

- *Entamoeba gingivalis* is a non-pathogenic ameba that inhabits the human oral cavity and occasionally other sites.
- Although it is often found in conjunction with periodontal disease.



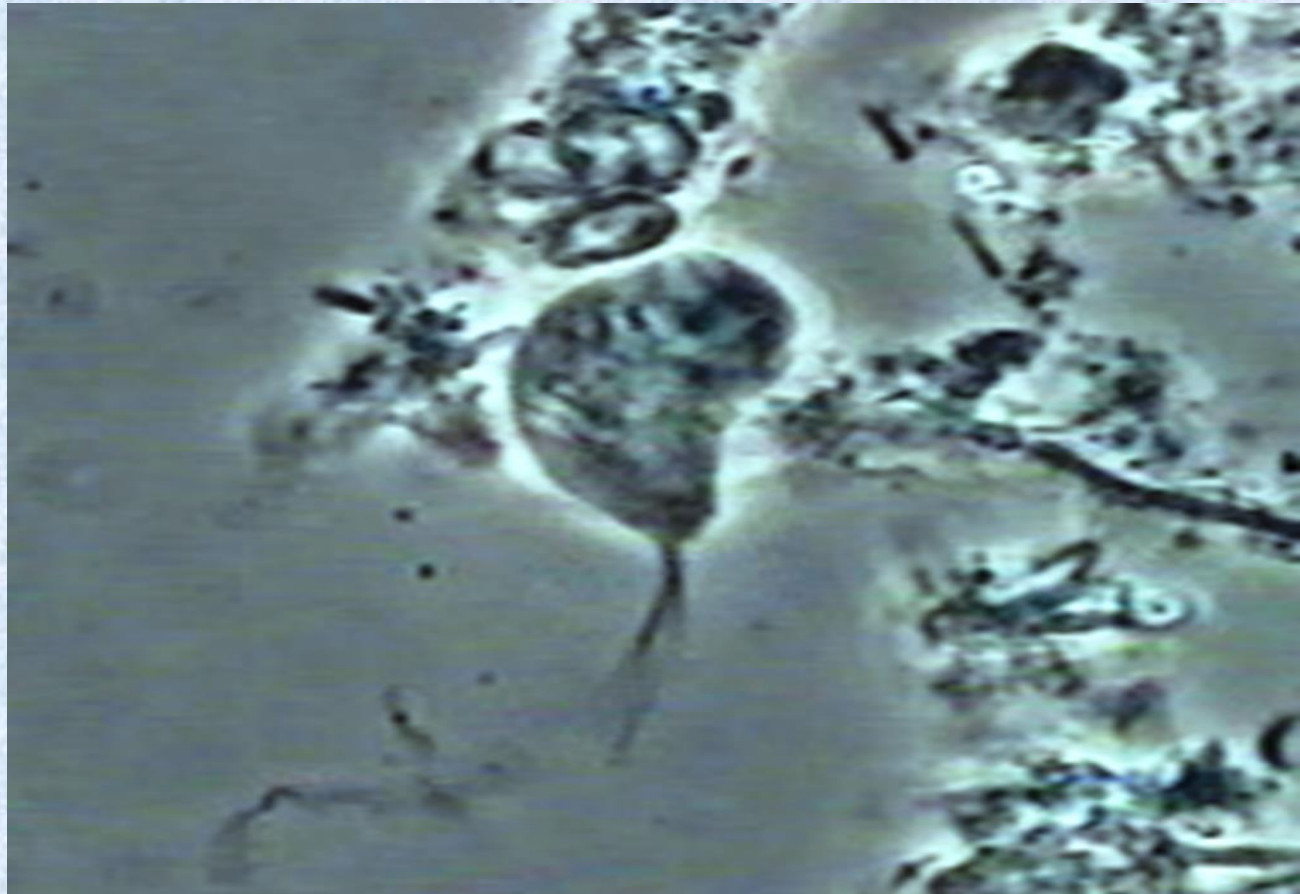
- **Clinical Presentation**
- *E. gingivalis* is common in individuals with poor oral hygiene or periodontal disease.
- It appears that diseased periodontal tissue and associated Actinomyces bacteria simply provide a favorable environment for the amoeba to develop.
- There is no known cyst stage for Entamoeba gingivalis; trophozoites live in the oral cavity of humans, residing in the gingival pockets near the base of the teeth.

- **laboratory diagnosis**

Diagnosis of *E. gingivalis* infection is made by microscopic examination of tooth and gum scrapings stained with routine microbiologic stains (e.g. Wheatley's trichrome stain)

- **Trichomonas tenax**

T. tenax, also known as *T. buccalis*, is a harmless commensal which lives in mouth, in the **periodontal pockets, carious tooth cavities and, less often, in tonsillar crypts** .



- **Diagnosis**
- The specimen of choice for diagnosing *Trichomonas tenax* trophozoite is mouth scrapings.
- Microscopic examination of tonsillar crypts and pyorrheal pockets of patients suffering from *T. tenax* infections often yields the typical trophozoites.
- *T. tenax* can easily be detected through the use of **phase-contrast microscopy**.

Mycology

- **Candida albicans** is a **diploid fungus** that grows both as **yeast** and **filamentous cells** and a causal agent of opportunistic oral and genital infections in humans.
- **Candida spp. as commensal are carried in the mouths of about 90% of the population as a normal component of the oral microbiota. Overgrowth is prevented by other microorganisms**

- **SPECIES**

- The causative organism is usually **Candida albicans** or less commonly other Candida species such as
- (C. tropicalis ,C. glabrata , C. parapsilosis ,C. krusei)

Symptoms

- Creamy white lesions on your tongue, inner cheeks, and sometimes on the roof of your mouth, gums and tonsils
- Slightly raised lesions with a cottage cheese-like appearance
- Redness, burning or soreness that may be severe enough to cause difficulty eating or swallowing
- Redness, irritation and pain under dentures (denture stomatitis)
- In Infants infection may have trouble feeding .They can pass the infection to their mothers during breast-feeding and causes red, sensitive, cracked or itchy painful nipples



- **Pseudomembraneous candidiasis** can involve any part of the mouth, but usually it appears on the tongue, buccal mucosae or palate.
- It is classically an acute condition, appearing in **infants**, **people taking antibiotics or immunosuppressant medications**, or **immunocompromising diseases**.



Angular cheilitis

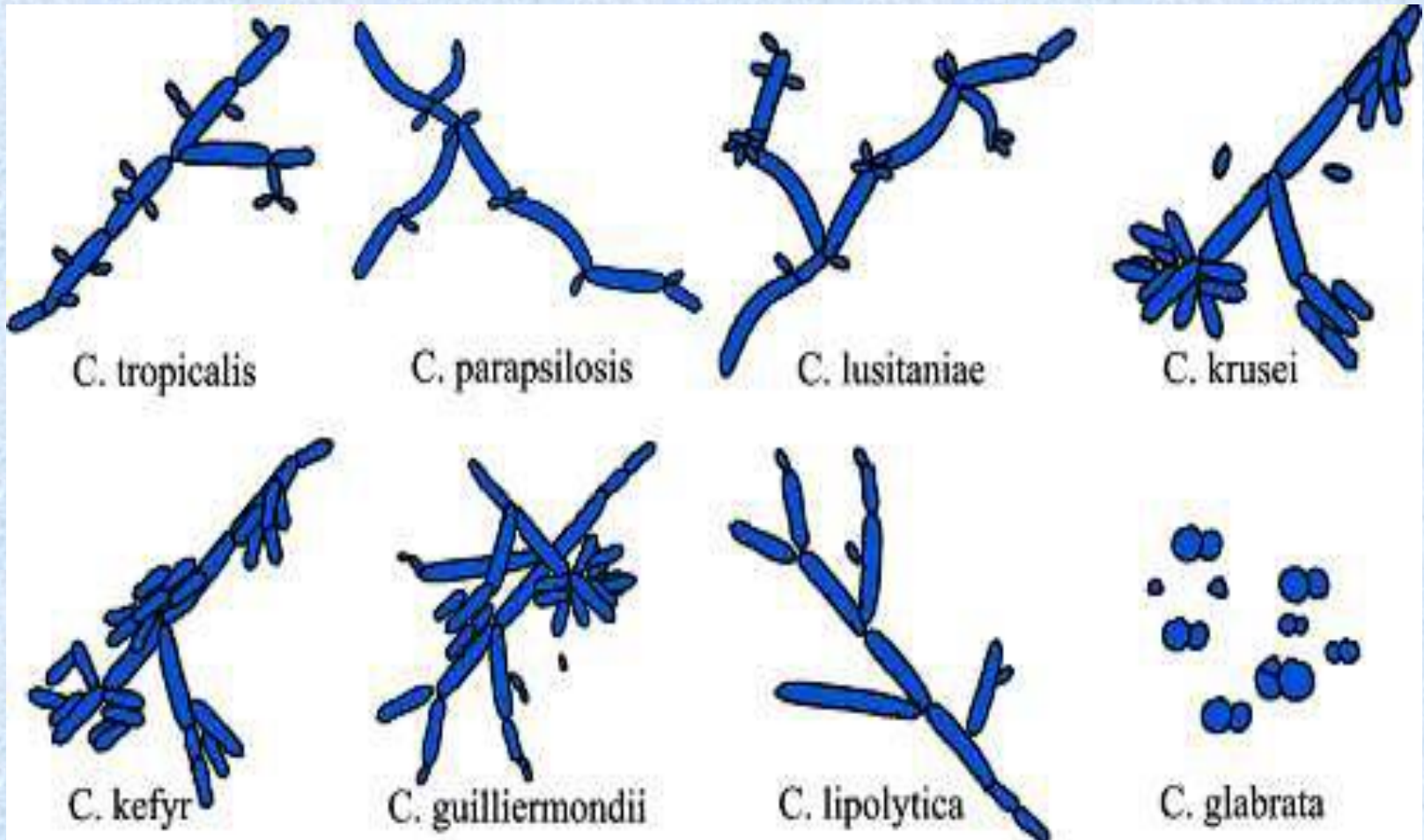
- Angular cheilitis is inflammation at the corners (angles) of the mouth, very commonly involving *Candida* species
- *Candida* spp alone are responsible for about 20% of cases, and a mixed infection of *C. albicans* and *Staphylococcus aureus* for about 60% of cases
- Signs and symptoms include soreness, erythema (redness), and fissuring of one, or more commonly both the angles of the mouth




- **Culture :**
- *Candida albicans* growing on Sabouraud agar *Candida* appears as large, round, white or cream colonies with a yeasty odor on agar plates at room temperature or 37C.



Candida spp – DIRECT MICROSCOPY



A close-up photograph of a bouquet of white daisies with bright yellow centers, interspersed with green foliage. A small, rectangular, cream-colored card is pinned to the bouquet with a red and black ladybug-shaped fastener. The card has the words "Thank you!" written in a black, cursive script.

Thank
you!