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Research Article

Isolation and diagnosis new strain of fungi causes gingivitis

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Abstract

An inflammation of the gum tissue, gingivitis is frequently brought on by a bacterial infection. Using the ITS1 sequence pattern identification approach, this study planned to isolate and identify novel fungi from the gingival region. According to the findings of the present investigation, the fungal isolates that were identified are belonging to the genus *Cystobasidium*, namely the species *Cystobasidium minutum*, *Cystobasidium terricola*, and *Cystobasidium slooffiae*. These strains were accurately diagnosed at the the species levels, matched 96–99% of the reference sequences included within the Gen-Bank dataset. The three strains belong to *Cystobasidium minutum* were deposited under accession numbers **PX129019**, **PX129020**, and **PX129021**. Additionally, the study found polymorphic features that confirmed the presence of reducing mutations and other mutations that change amino acids. This suggests that there is intraspecific heterogeneity, which may indicate that these fungi can adapt to the oral environment in pathological sites like gingivitis. The documentation of the presence of *Cystobasidium* in the inflamed gingival area warrants future research into the colonization sites of this fungal genus, which has previously been documented in other environmental and clinical sources.

1. Introduction

One of the most common illnesses in the world, oral disorders have major negative effects on a person's health and finances, significantly lowering their quality of life. Gingivitis, loss of the tooth, cancer, and other oral disorders are the most common and serious conditions in the world [1]. Redness, inflammation, and bleeding of the gingiva are signs of gingivitis, but there is no disruption of connective tissue linkage. Most people have no knowledge of the illness or cannot identify it, and it is typically painful and rarely results in accidental bleeding [2] Plaque buildup and inadequate oral hygiene are well-known and significant warning signs for gingivitis. The likelihood of subsequently acquiring periodontal disease may be linked to plaque buildup during infancy and adolescence [3].

A number of unrelated pathologic lesions with plaque, can appear in human gingiva and other mouth tissues; in certain cases, these lesions may be signs of an illness or systemically problem. They might also be pathologic alterations that are specific to gum tissues. A buildup of plaque and the ensuing gingival inflammation may influence the clinical course of these lesions even though plaque is not the primary source of them [4]. Although they are rare, unrelated to plaque bacterial gingival diseases might result from a particular bacterial infection or from a breakdown in the balance between innate resistance of the host and unrelated to plaque microorganisms [5]. *Porphyromonas gingivalis* and other periodontal microorganisms have evolved a variety of tactics to compromise the gingival epithelium's functional and structural integrity. Human epithelial cells are the site of adhesion, invasion, and replication for *P. gingivalis* [6]. In oral cavity, they commonly reside or colonize tongue, palate, buccal mucosa and also subgingival plaque of adults with severe periodontitis. Apart from *C. albicans* , more candidal species, such as C. dubliniensis, *C. glabrata*, *C. tropicalis*, and *C. krusei* [7].

Several dimorphic basidiomycetes are only identified in their asexual stage, and the huge polyphyletic genus Rhodotorula is usually home to those that are tinted in various shades of red. There are several species of these common yeasts that have some clinical significance

[8].

It is thought that immunodeficient individuals are more susceptible to opportunistic illnesses due to the widespread presence of the *Cystobasidium* yeast. In clinical settings, detection employing molecular identification is crucial for accurately diagnosing and treating rare yeast infections since *Cystobasidium* shares phenotypic similarities with *Rhodotorula*, an emerging opportunistic pathogen [9].

The research aims to identify new fugal causes of gingivitis.

2. Methodology

2.1. Sample collection

Sample collection was performed at the Dental Center in Nasiriya city, the study group included 20 gingivitis adults, with an age range of 25 to 35 years.

The following technique was employed to gather samples from the plaque which contact with gums' afflicted area, following the methodology of [10]: Patients' samples were collected under the careful observation of dental professionals. and in a very aseptic environment. To get rid of the saliva in the afflicted area, Distilled water was used for several times washing the gums of the patient. Then utilized little blasts of air. For every patient, plaques were scraped beneath the gums. Samples were taken without coming into contact with any cavities, abscesses, or irritation that might be present.

2.2. Samples activation

Samples were activated using B. H. I. Broth for 24 h. under 37°C after being delivered to the lab within two hours. Following that, each sample was diluted before being cultured in specific culture medium [11].

2.3. Diagnosis of fungi in the area of gingivitis

Potato dextrose agar and were used, this medium was made in accordance with the guidelines provided by the company that made it (Himedia), autoclaved, let cool down, and then 250 mg/L of chloramphenicol was used [12].

2.4. Molecular diagnosis

After cultured the conserved yeast samples on Sabouraud Dextrose Agar for 24 to 48 hours at 28°C, using saline suspension, the colonies were brought to a density of the McFarland standard of 0.5 at 530 nm. Microcentrifuge was used to centrifuge one ml of the cell solution for three minutes at 5,000. Following the manufacturer's instructions, The easy pure genomic DNA Kit was used for extracting the genome's DNA. [13]. The species-level identification of the yeast by employing the internal transcribed spacer (ITS) gene and yeast's D1/D2 domain sequence identification and polymerase chain reaction (PCR) amplified. To verify the identity of fungal isolates, the subsequent primers were employed for molecular diagnostics. The NCBI-Blast Alignment recognition tool (http://www.ncbi.nlm.nih.gov/BLAST /Blast.cgi) was used to examine and process the nucleotide sequences. Table 1

Table 1: Primer used in molecular diagnosis

The Primer's Name	Sequences of primers	Size
ITS86 (L)	5' GTGAATCATCGAATCTTTGAAC'3	310bp
ITS4	5' TCCTCCGCTTATTGATATGC'3	

3. Result

Only Five isolates of colored yeast were obtained from patients with gingivitis after inoculation with SDA medium. For 72 hours, the prepared plates had been incubating at 30°C, according to color and colony form. Initial identification of these yeasts indicated that they belonged to the *Cystobasidium* genus. The isolated sample was stored at 4°C for molecular diagnosis.

Five fungal isolates were subjected to forward and reverse primer sequencing analyses of the amplified Internal Transcribed Spacers (ITS) of the ribosomal DNA sequence. Accurate molecular identification at the genus and species levels was made possible by the results' strong similarity with reference sequences in the GenBank database. According to Figure 1,2,3,6,7,8 *Cystobasidium minutum* was detected in three isolates (Samples 1,2 and 3), with sequence identity varying from 98 to 99 percent using the reference sequence Accession No. LC4731261. While samples two and three each contained a single gap and multiple polymorphisms, involving silent mutations, missense mutations, and insertions, some of which may have an impact on the structure of proteins or function, sample one had the full alignment with no gaps and 284 bases. According to Figure 4,9, with 96–97% agreement to the reference sequence NR_1747811, sample 4 was determined to be *Cystobasidium terricola*. It displayed minimal sequence divergence, with four gaps in the forward alignment and five in the reverse alignment, ranging from 299 of 311 to 291 of 300 bases. According to Figure 6, *Cystobasidium slooffiae* was 96–97% identical with reference sequence MK3364611 in sample 5. Along with deletions at important locations, the alignment showed a number of silent and missense changes, most notably ATA to ATG and CAA to CAC. Table 2

Score	Expect	Identities	Gaps	Strand
508 bits(275)	2e-139	281/284(99%)	0/284(0%)	/Plus

```
Query 10  TTTGGGATTCCGAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTATTTT 69
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 246 TTTGGTATTCCGAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTATTTT 305

Query 70  GTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCTCAGC 129
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 306 GTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCTCAGC 365

Query 130 TGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTATTTCG 189
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 366 TGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTATTTCG 425

Query 190 CTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTTAAAGCACTTTAAG 249
          |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct 426 CTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTTAAAGCAACTTAAG 485

Query 250 CTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTTAAGCA 293
          |||||||||||||||||||||||||||||||||||||||||||||
Sbjct 486 CTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTTAAGCA 529
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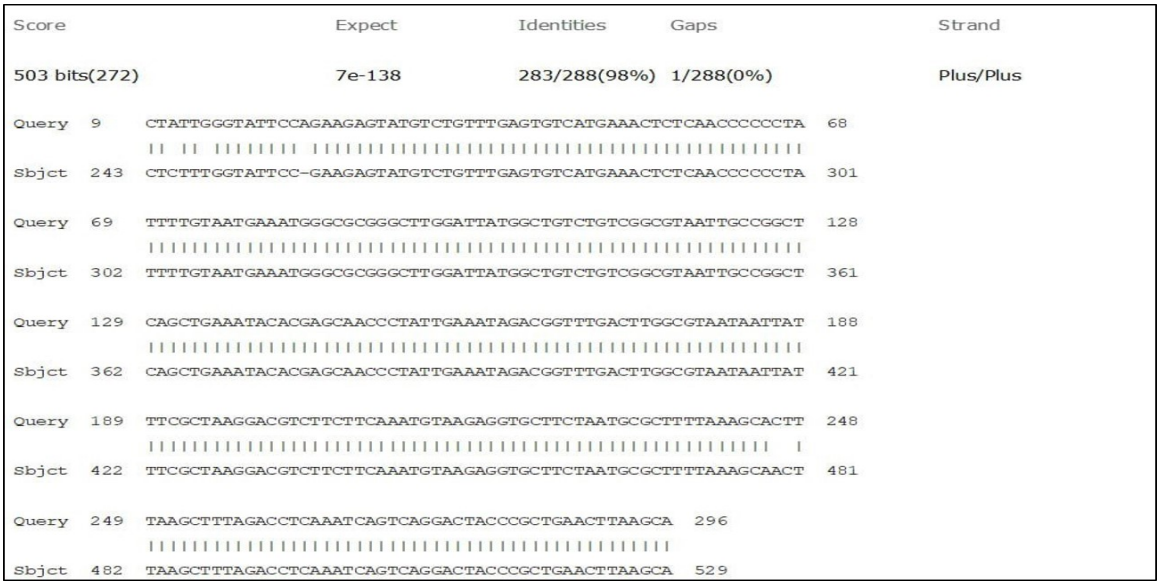
Figure 1: Alignment statistics for Cystobasidium minutum (ITS1) gene for sample (1)



Score	Expect	Identities	Gaps	Strand
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            || || |||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  243  CTCTTTGGTATTCC-GAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTA  301

Query  69   TTTTGTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCT  128
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  302  TTTTGTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCT  361

Query  129  CAGCTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTAT  188
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  362  CAGCTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTAT  421

Query  189  TTCGCTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTTAAAGCACTT  248
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  |
Sbjct  422  TTCGCTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTTAAAGCAACT  481

Query  249  TAAGCTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTTAAGCA  296
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  482  TAAGCTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTTAAGCA  529
```
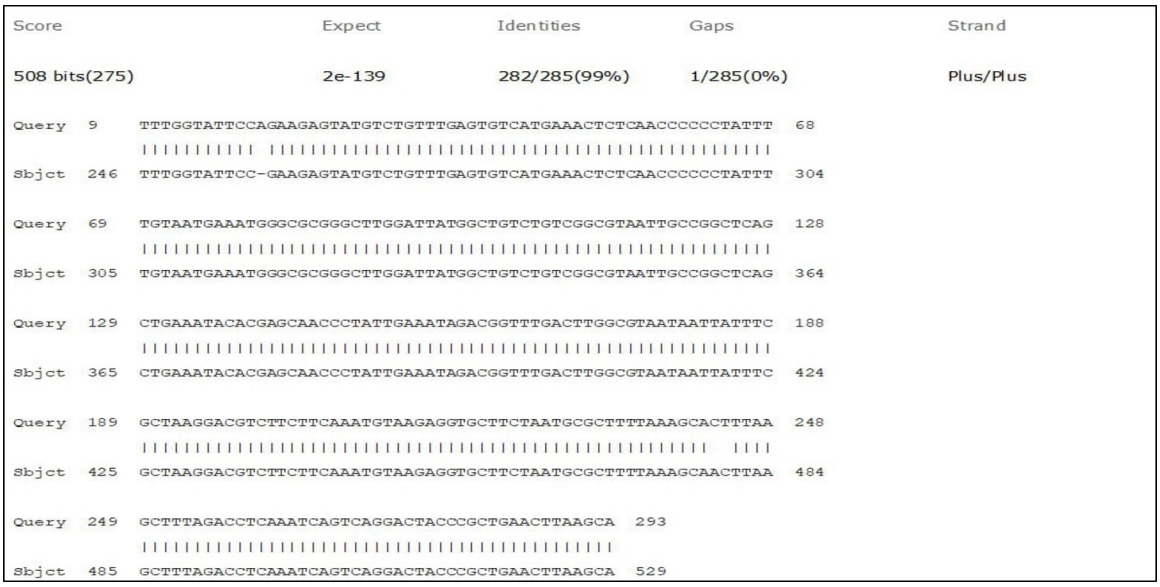

Figure 2: Alignment statistics for Cystobasidium minutum (ITS1) gene for sample(2)



Score	Expect	Identities	Gaps	Strand
508 bits(275)	2e-139	282/285(99%)	1/285(0%)	Plus/Plus

```
Query  9    TTTGGTATTCCAGAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTATTT  68
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  TTTGGTATTCC-GAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTATTT  304

Query  69   TGTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCTCAG  128
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  305  TGTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCTCAG  364

Query  129  CTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTATTTC  188
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  365  CTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTATTTC  424

Query  189  GCTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTTAAAGCACTTTAA  248
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||  ||||
Sbjct  425  GCTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTTAAAGCAACTTAA  484

Query  249  GCTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTTAAGCA  293
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  485  GCTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTTAAGCA  529
```

Figure 3: Alignment statistics for Cystobasidium minutum (ITS1) gene for sample (3)

Score	Expect	Identities	Gaps	Strand
505 bits(273)	2e-138	299/311(96%)	4/311(1%)	Plus/Plus

```
Query  3    ACTCTTTGGTATTCCGAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTA  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  353  ACTCTTTGGTATTCCGAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTA  412

Query  63   TTTTGTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCT  122
            |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  413  TTTTGTAATGAAATGGGCGTGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCT  472

Query  123  CAGCTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTAT  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  473  CAGCTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTAT  532

Query  183  TTCGCTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTT---AAAGCA  239
            |||||||||||||||||||||||| ||||||||||||||||||||||||   ||||||
Sbjct  533  TTCGCTAAGGACGTCTTCTTCAAATATAAGAGGTGCTTCTAATGCGCTTTTTCGAAAGCA  592
```

Figure 4: Alignment statistics for *Cystobasidium terricola* (ITS1) gene for sample (4)

Score	Expect	Identities	Gaps	Strand
473 bits(256)	6e-129	275/284(97%)	2/284(0%)	Plus/Plus

```
Query  9    CTATTGGGTATTCCAGAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTA  68
            || || |||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  302  CTCTTTGGTATTCC-GAAGAGTATGTCTGTTTGAGTGTCATGAAACTCTCAACCCCCCTA  360

Query  69   TTTTGTAATGAAATGGGCGCGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCT  128
            ||||||||||| ||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TTTTGTAATGAGATGGGCGTGGGCTTGGATTATGGCTGTCTGTCGGCGTAATTGCCGGCT  420

Query  129  CAGCTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTAT  188
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CAGCTGAAATACACGAGCAACCCTATTGAAATAGACGGTTTGACTTGGCGTAATAATTAT  480

Query  189  TTCGCTAAGGACGTCTTCTTCAAATGTAAGAGGTGCTTCTAATGCGCTTTT-AAAGCACT  247
            |||||||||||||||||||||||| |||||||||||||||||||||||| ||||||
Sbjct  481  TTCGCTAAGGACGTCTTCTTCAAATATAAGAGGTGCTTCTAATGCGCTTTTTAAAGCAAC  540

Query  248  TTAAGCTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTT  291
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  TTAAGCTTTAGACCTCAAATCAGTCAGGACTACCCGCTGAACTT  584
```

Figure 5: Alignment statistics for *Cystobasidium slooffiae* (ITS1) gene for sample (5)

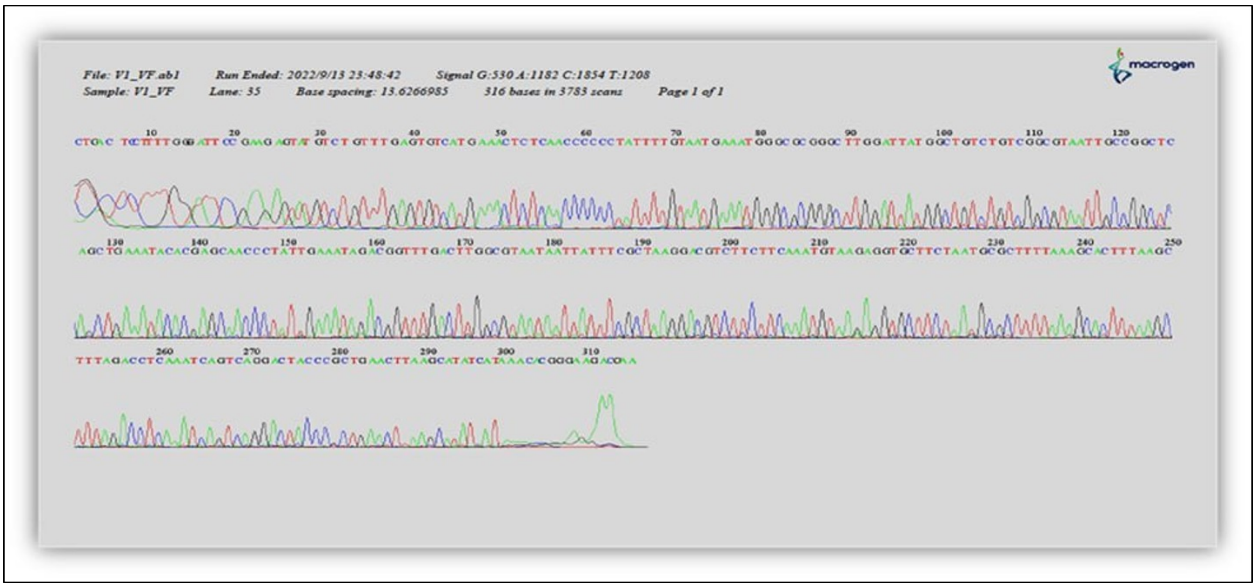


Figure 6: Forward primer sequencer-generated data for *Cystobasidium minutum* (ITS1) gene

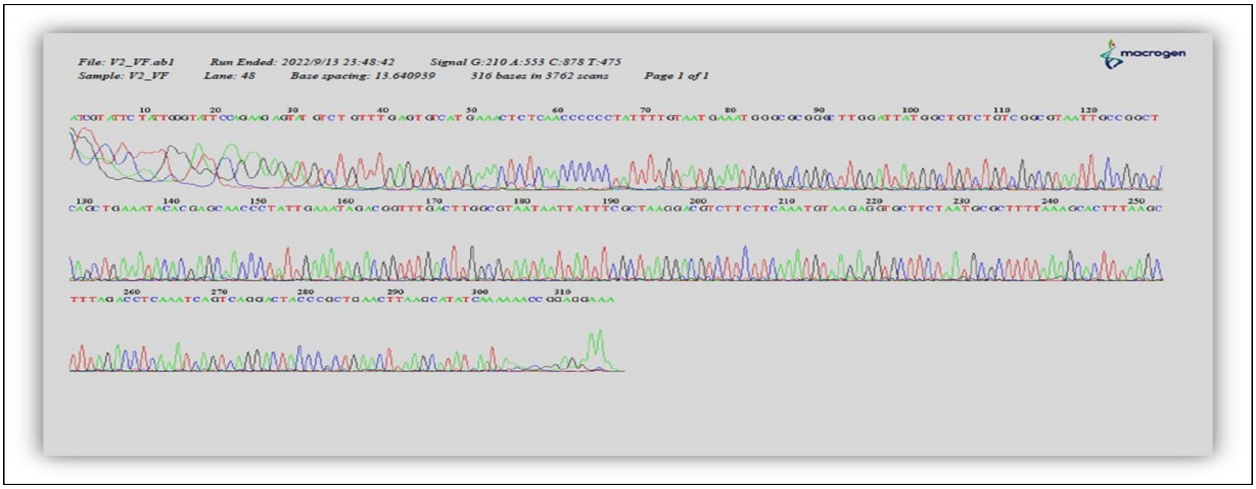


Figure 7: Forward primer sequencer-generated data for *Cystobasidium minutum* (ITS1) gene

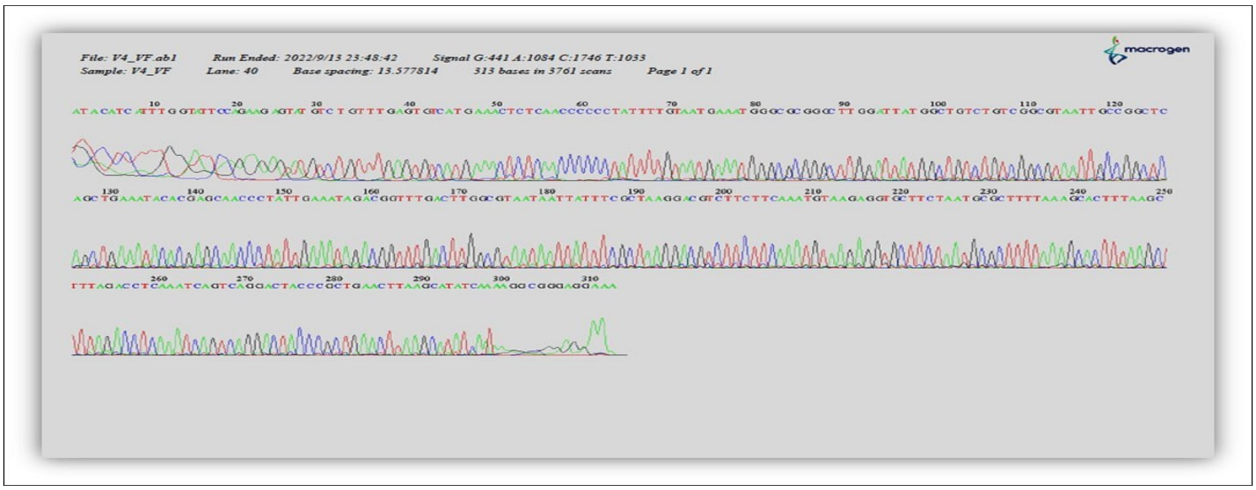


Figure 8: Forward primer sequencer-generated data for *Cystobasidium minutum* (ITS1) gene

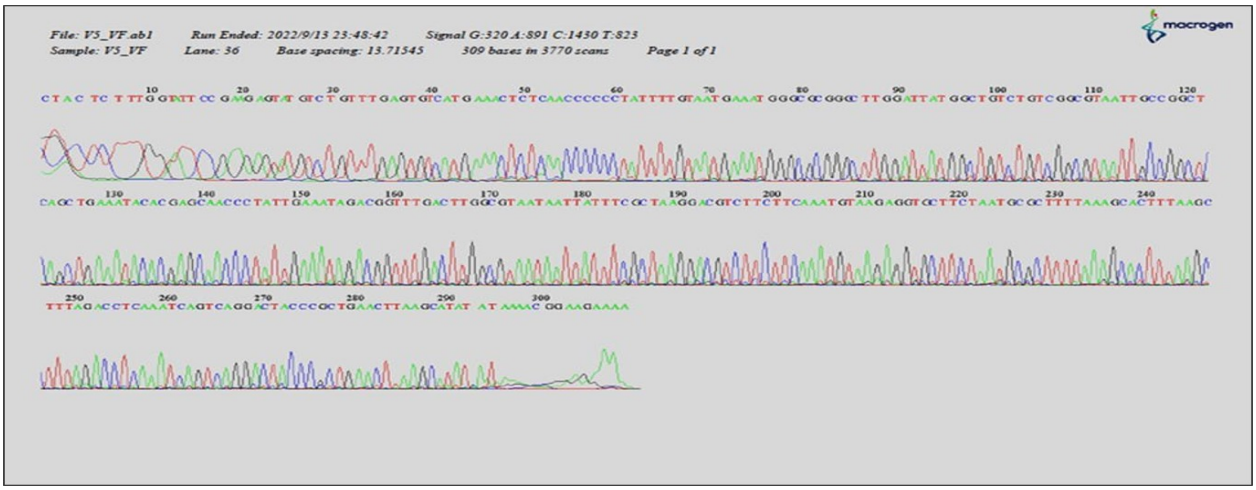


Figure 9: Forward primer sequencer-generated data for *Cystobasidium terricola*(ITS1) gene

Table 2: Excision numbers of yeast isolates

	Micro.	Excision numbers
Sample 1	***Cystobasidium minutum***	**PX129019**
Sample 2	***Cystobasidium minutum***	**PX129020**
Sample 3	***Cystobasidium minutum***	**PX129021**

4. Discussion

Oral microflora is a rich and varied type of microbiota found in the oral cavity. Typically, oral microflora is found in the biofilms that are affixed to the mouth cavity's many soft as well as hard surfaces [14]. Because of this diversity, any inflammation that may occur in any of the oral tissues may be susceptible to bacterial or fungal attack In this investigation,fungal isolates from the species *Cystobasidium minutum*, *Cystobasidium terricola*, and *Cystobasidium slooffiae* were isolated and identified from dental plaque close to gingivitis-affected sites for the first time . Nonetheless, the current study has similarities to the research conducted by [15], In which, oral samples from a 60-year-old patient with angular cheilitis were used to isolate and identify the uncommon yeast species *Cystobasidium calyptogenae* for the first time. This yeast species has never before been isolated from oral human samples. Also, the study by [16] which showed that *Cystobasidium* was present in e-cigarette mouthpieces, this microorganism is extremely dangerous. In their lab tests, they demonstrated that long-term exposure to this particular fungus caused obstructive lung illness and excessive mucus secretion in lab mice. Comparing the current study's findings with those of [17], who demonstrated that *Cystobasidium slooffiae* has certain virulence mechanisms (biofilm production and related genes) as a possible pathogen in women's cervical and vaginal infections in Erbil. The isolation and identification of these fungal species from plaque in contact with the gingivitis area suggests that these organisms may be involved in the development of this disease. The coexistence of fungi and bacteria in the tooth's dentinal tubules, particularly in carious teeth, supports the idea that both species play a similar role in disorders of the oral cavity. [18], used electron microscopy to show that different dentinal tubules contain monomorphic bacterial and fungal biofilms. We present a hitherto unseen phenomena in which bacteria and fungus have different habitats within carious dentin. The current study's findings are crucial for demonstrating the extent to which fungus can induce and develop other oral diseases, such gingivitis. Because of the buildup of metabolic end products, the pH at which ATPase functions efficiently drops, increasing the microorganism's competitiveness within the biofilm structure [19]. The aforementioned supports the results of the current investigation, which isolated novel fungal species and that they are important contributors to gingivitis development.

5. Conclusion

This study showed that gingivitis may be linked to a diversity of fungi in the affected area. The results of the isolation of three species of *Cystobasidium* point to the possibility that fungi contribute to the development of gingivitis, leading the way for a deeper comprehension of the fungal microbiome linked to oral disorders. This information also provides a scientific basis for developing more effective therapeutic and preventive strategies to reduce the complications of gingivitis and improve overall oral health.

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