Development of a Toothpaste Formulation Containing Endophytic Fungal Extracts Derived from *Clinacanthus nutans* for Periodontitis Prevention

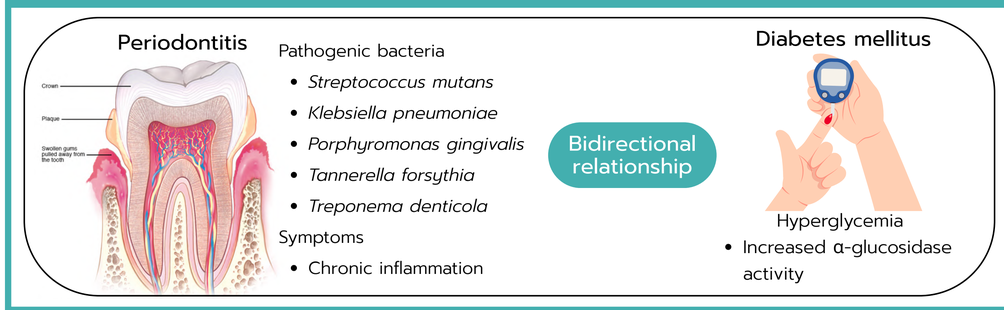
Kanyaphat Sakunna* and Itthayakorn Promputtha

Division of Microbiology, Department of Biology, Faculty of Science, Chiang Mai University, Thailand, *E-mail address : kanyaphat_sakunn@cmu.ac.th

ABSTRACT

Oral diseases resulting from bacterial accumulation are major contributors to periodontitis, particularly in patients with poorly controlled diabetes. Consequently, there is increasing interest in natural bioactive compounds capable of inhibiting both oral pathogens and glucose-metabolizing enzymes as alternatives to conventional antibiotics. This study evaluated the antibacterial, antibiofilm, and α-glucosidase inhibitory activities of endophytic fungal extracts isolated from the medicinal plant *Clinacanthus nutans* (phaya-yo), with the aim of developing a bioactive toothpaste formulation for the prevention of periodontitis. A total of 85 endophytic fungal isolates were obtained and screened. Among them, the extract from isolate S33 exhibited the strongest activity against key oral pathogens associated with periodontitis. It inhibited the growth of *Streptococcus mutans* and *Klebsiella pneumoniae* by 91.11% and 66.21%, respectively. The extract also demonstrated pronounced antibiofilm properties, inhibiting biofilm formation of *S. mutans* and *K. pneumoniae* by 74.29% and 76.22%, respectively, and degrading established biofilms by 90.95% and 71.43%. In addition, it showed substantial α-glucosidase inhibitory activity (78.75%), suggesting potential benefits for glycemic regulation in individuals at risk of diabetes-associated periodontal complications. Overall, these findings indicate that the endophytic fungal extract from isolate S33 is a promising candidate for incorporation into oral care formulations, offering dual functionality in periodontitis prevention and metabolic modulation.

INTRODUCTION

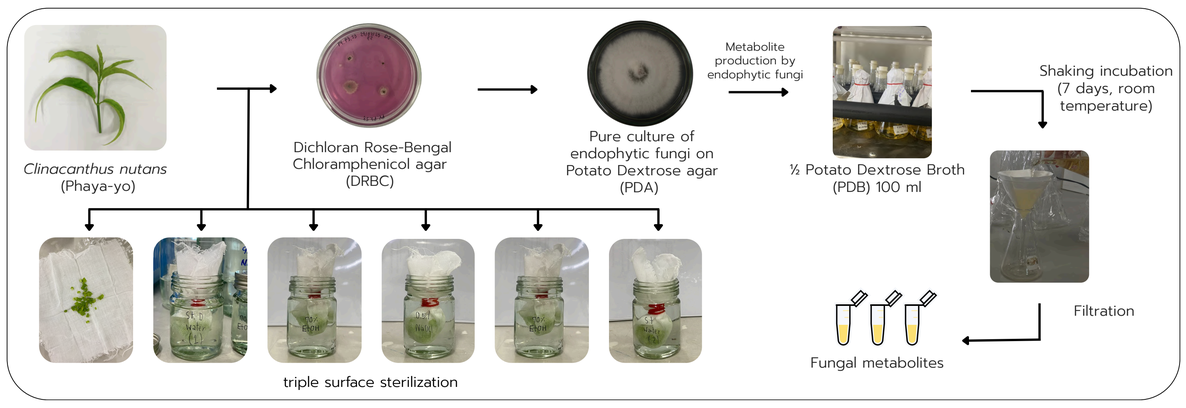


OBJECTIVES

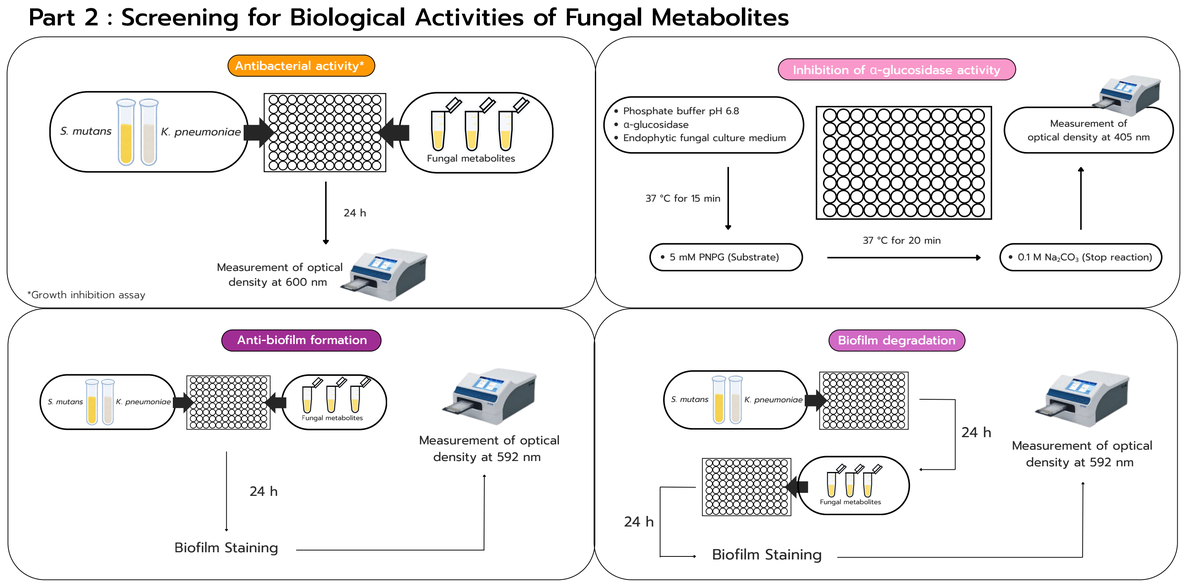
- To isolate endophytic fungi from *C. nutans* and prepare their bioactive compounds
- To evaluate the inhibitory activity of fungal bioactive compounds on periodontal-causing bacteria and α-glucosidase
- To develop an anti-periodontitis toothpaste formulation using selected fungal metabolites

METHODOLOGY

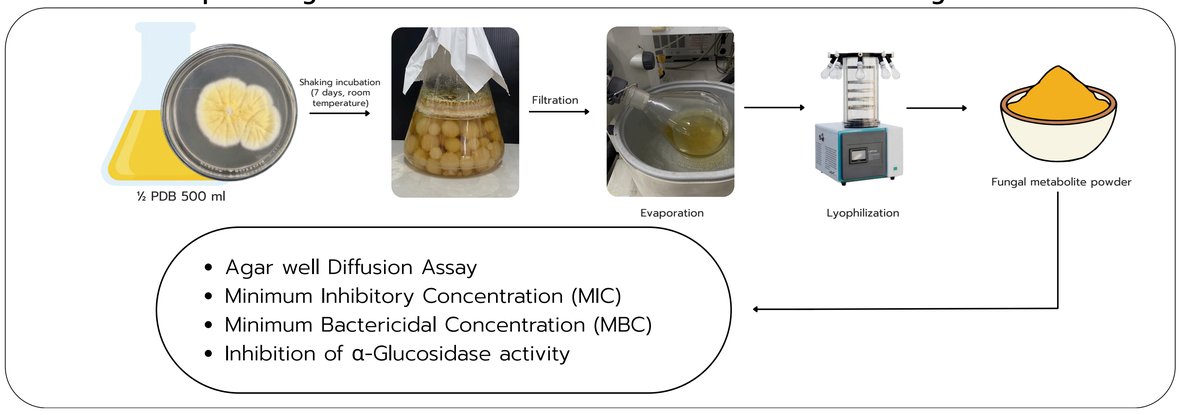
Part 1 : Isolation of Endophytic Fungi and Preparation of Fungal Metabolites



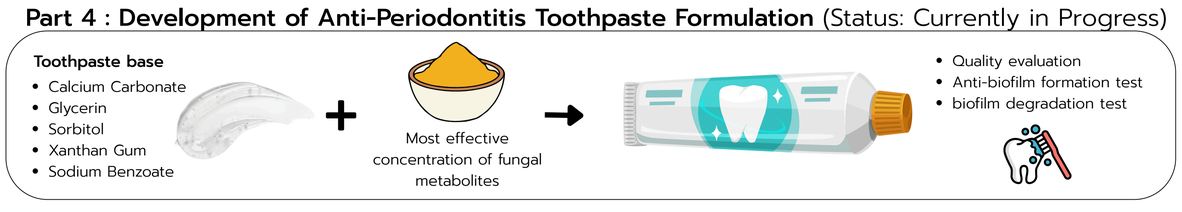
Part 2 : Screening for Biological Activities of Fungal Metabolites



Part 3 : Scale-up of Fungal Metabolite Production and Evaluation of Its Biological Activities



Part 4 : Development of Anti-Periodontitis Toothpaste Formulation (Status: Currently in Progress)



RESULTS

1. Endophytic Fungi Isolation

A total of 85 endophytic fungal isolates were obtained from the leaves and stems of *Clinacanthus nutans*. Some of them are shown in Fig 1.

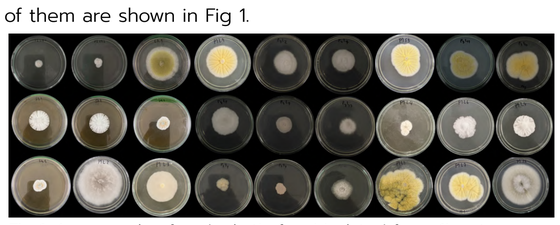


Fig 1. An example of endophytic fungi isolated from *C. nutans*.

2. Screening for Biological Activities of Fungal Metabolites

2.1 Antibacterial Activity

Six endophytic fungal isolates exhibited inhibitory activity against the two tested bacteria, *S. mutans* and *K. pneumoniae* (Fig 2).

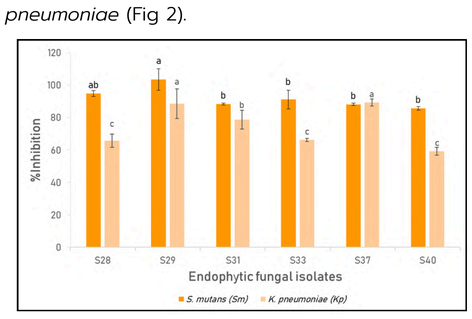


Fig 2. Growth inhibition (%) of *S. mutans* and *K. pneumoniae* by endophytic fungal isolates.

2.2 Anti-Biofilm Formation and Biofilm Degradation

The six endophytic fungal isolates that inhibited the growth of *S. mutans* and *K. pneumoniae* also exhibited biofilm formation inhibition and biofilm degradation activities (Fig 3-4).

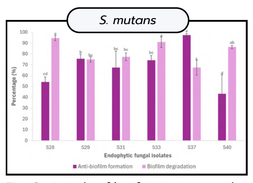


Fig 3. Anti-biofilm formation and biofilm degradation of *S. mutans*

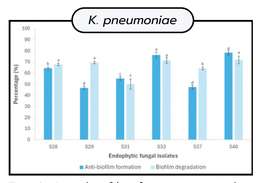


Fig 4. Anti-biofilm formation and biofilm degradation of *K. pneumoniae*

2.3 Inhibition of α-Glucosidase Activity

Among the six endophytic fungal isolates that inhibited both tested bacteria, several isolates also showed α-glucosidase inhibitory activity (Fig 5).

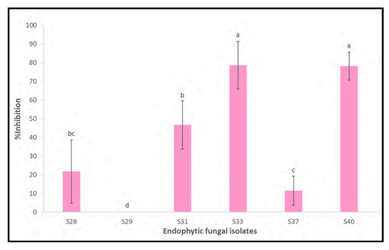


Fig 5. α-Glucosidase inhibitory activity of endophytic fungal isolates.

3. Summary of Overall Biological Activities for Periodontitis Prevention

Among the evaluated isolates, endophytic fungal isolate S33 showed the highest potential in most biological assays (Fig 7).

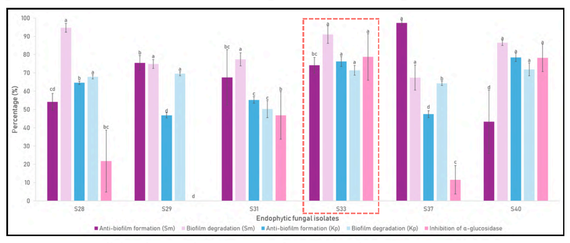


Fig 6. Overall biological activities for periodontitis prevention among six endophytic fungal isolates.

4. Scale-up of S33 Metabolite Production and Evaluation of Its Biological Activities

4.1 Physical Appearance of the Extract Powder

The S33 extract powder was golden yellow in color, with a fine texture and light weight (Fig 7).



Fig 7. Physical appearance of the S33 extract powder

4.2 Antibacterial Activity

4.2.1 Agar Well Diffusion Assay

The extract exhibited antibacterial activity as indicated by the formation of clear inhibition zones, as shown in Fig 8 and Table 1.

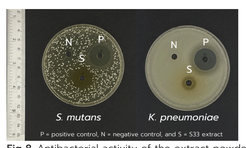


Fig 8. Antibacterial activity of the extract powder.

Table 1 Inhibition zone diameters of the S33 extract against test bacteria.

Bacteria	Inhibition Zone Diameter (mm)
S. mutans	23.67 ± 1.15
K. pneumoniae	12.33 ± 0.58

4.2.2 MIC and MBC

The S33 extract exhibited MIC and MBC values against *S. mutans* and *K. pneumoniae* of 125 mg/mL and 500 mg/mL, respectively (Table 1).

Table 2 MIC and MBC values of the S33 extract against *S. mutans* and *K. pneumoniae*.

Bacteria	Concentration (mg/ml)	
	MIC	MBC
S. mutans	125	125
K. pneumoniae	500	500

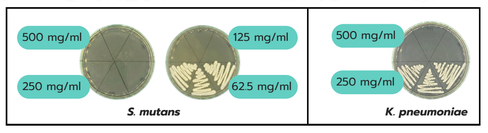


Fig 9. Bactericidal activity of S33 extract against test bacteria

4.3 Inhibition of α-Glucosidase Activity

The S33 extract powder showed the highest α-glucosidase inhibitory activity at 500 mg/mL, with an average inhibition of 66.96% (Fig 10). The IC_{50} value was 377.36 mg/mL.

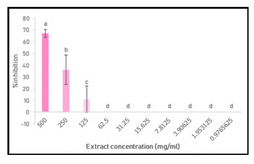
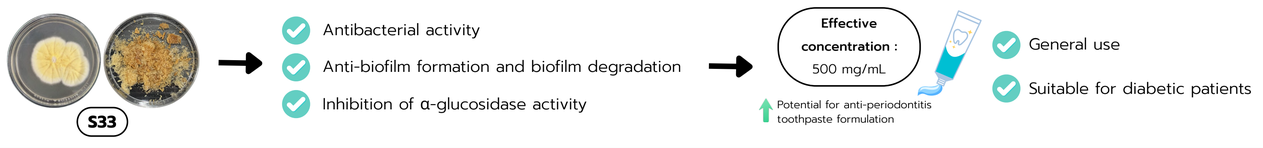


Fig 10. α-Glucosidase inhibitory activity of the S33 extract at different concentrations.

5. Development of Anti-Periodontitis Toothpaste Formulation (in progress)

CONCLUSION



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