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The Relationship Between the Growth of *Fusobacterium nucleatum* ATCC 25586 in Glucose-Enriched Media and Protein Activity through Fourier Transform Infrared (FTIR)

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Abstract

Background: *Fusobacterium nucleatum* (*F. nucleatum*) is known to increase in number under hyperglycemic conditions, as it is thought to utilize glucose as a nutrient source. The process of glucose utilization in bacteria occurs with the assistance of enzymatic proteins such as glucokinase. This study aims to investigate the glucose utilization by *F. nucleatum* ATCC 25586 by examining its growth in glucose-enriched media and its relationship with protein activity through FTIR analysis.

Methods: *F. nucleatum* ATCC 25586 was cultured in media enriched with 2%, 1%, 0.75%, 0.5%, and 0.25% glucose. Its growth was measured using a spectrophotometer, and protein activity was assessed with FTIR at 24 and 48 hours of incubation.

Results: The results showed that *F. nucleatum* could utilize glucose as a nutrient source, indicated by growth and protein activity. The maximum growth of *F. nucleatum* occurred at a 0.75% glucose concentration at 24 hours. However, the Kruskal-Wallis test showed no significant differences in the growth and protein activity of *F. nucleatum* across the five glucose concentrations (growth, p=0.271 and protein, p=0.149). Spearman correlation analysis indicated no correlation between the growth and protein activity of *F. nucleatum* (p=0.323). The protein activity of *F. nucleatum* remained stable across various growth levels.

Conclusion: It can be concluded that glucose could influence the growth of *F. nucleatum*, although the growth and protein activity of the bacteria did not differ significantly based on glucose concentration. *F. nucleatum* grown in various glucose concentrations exhibits stable protein activity.

Keywords: ATCC 25586, *F. nucleatum*, Glucose-Enriched Media, Hyperglycemic.

Introduction

Fusobacterium nucleatum (*F. nucleatum*) is a Gram-negative anaerobic bacterium involved in the etiology of periodontal diseases. This bacterium is often isolated from dental plaque in healthy conditions, but its numbers increase in plaques associated with disease (1). *F. nucleatum* plays a crucial role in the effective colonization of bacteria in the oral cavity due to its extensive adhesion capabilities, functioning as a bridge between early and late colonizing bacterial members that cannot directly attach to each other (2,3). This support, particularly in subgingival biofilm formation, can trigger destructive periodontitis (4). Additionally, *F. nucleatum* has been linked to various diseases beyond the oral cavity, such as colorectal cancer, liver abscesses, and pregnancy implications (5-8).

According to Miranda (2017), poorly controlled hyperglycemic conditions in patients with type 2 diabetes mellitus and

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chronic periodontitis show an increase in periodontal bacteria, including *F. nucleatum (9)*. Bacterial growth in the oral cavity is influenced by various conditions, including nutrient availability (10). Under hyperglycemic conditions, increased glucose levels in gingival crevicular fluid are thought to affect nutrient sources for certain bacterial species (11). However, the glucose utilization by *F. nucleatum* is not well understood.

Glucose is a simple sugar that can be rapidly and efficiently used to generate energy for bacterial growth and replication (12, 13). The utilization of glucose as an energy source occurs through metabolic processes. The efficiency of nutrient metabolism by bacteria is influenced by the adjustment of enzyme levels within the cell (14). Enzymes are proteins that catalyze reactions in metabolic processes (15). Various enzymes are involved in metabolism, one of which is glucokinase (16). Analyzing the differential regulation of bacterial enzymatic proteins under various environmental conditions helps in study bacterial adaptation mechanisms, including nutrient utilization processes (17).

Fourier Transform Infrared (FTIR) is a method that can be used for protein analysis in bacteria (18, 19). This method has been used to study cellular responses to various conditions by observing the functional groups of protein, lipid, DNA, and polysaccharide components in cells. Any changes due to bacterial interaction with test substances can be observed by analyzing the position and intensity of infrared absorption peaks (20). FTIR can be directly applied to microorganisms in suspension, allowing for rapid and economical detection (21).

Based on the above description, this study aimed to investigate the glucose utilization by *F. nucleatum* by examining the relationship between the growth of *F. nucleatum* ATCC 25586 in glucose-enriched media and its protein activity based on FTIR spectrum peaks.

Materials and Methods

This study was experimental laboratory research with a post-test only control group design, conducted in June 2021. The research was conducted in several laboratories at Universitas Syiah Kuala: The Faculty of Dentistry laboratory for bacterial culturing, the Faculty of Veterinary Medicine laboratory for determining bacterial growth, and the Faculty of Engineering laboratory for protein activity testing using FTIR. *F. nucleatum* ATCC 25586 from the Oral Biology Laboratory, Faculty of Dentistry, Universitas Indonesia, was used as the research sample.

Preparation of Glucose Solution Concentrations

The glucose solution concentrations used in this study were 2%, 1%, 0.75%, 0.5%, and 0.25% (22). The glucose solutions were prepared by dissolving glucose in distilled water. To achieve each concentration with 20 ml of distilled water, the amount of glucose required was determined using the formula (23):

$$Concentration\ (\%) = \frac{Weight\ of\ glucose\ (g)}{Volume\ of\ glucose\ (ml)} \times 100 \quad (1)$$

The required weight of glucose for each concentration was calculated and then dissolved in 20 ml of distilled water to prepare the solutions.

Preparation of F. nucleatum ATCC 25586 Sample Suspension

Colonies of *F. nucleatum* ATCC 25586 cultured for 24 hours were taken from the culture using a loop (1-3 loops) and suspended in a test tube containing 0.9% NaCl solution, then homogenized using a vortex mixer. The suspension was then adjusted to match the McFarland standard 0.5 ($1.5x10^8$ CFU/mL) (24).

Inoculation of F. nucleatum ATCC 25586 in Glucose-Enriched Nutrient Broth

Inoculation was performed to grow bacteria in nutrient broth media enriched with different glucose concentrations: 2%, 1%, 0.75%, 0.5%, and 0.25% for 24 and 48 hours. For each glucose concentration (2%, 1%, 0.75%, 0.5%, and 0.25%), 0.5 ml of the glucose solution was taken and placed into a microtube. Then, 100 µl

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of the bacterial suspension was inoculated into the microtube using an Eppendorf micropipette. The samples were incubated for 20 minutes. Subsequently, 1 ml of nutrient broth media and 0.5 ml of PBS solution (pH 7) were added to the microtube. The microtube was vortexed to homogenize the components. After the bacterial cultivation was completed for all concentrations, the samples were incubated anaerobically at 37 ℃ (25).

Measurement of F. nucleatum ATCC 25586 Growth Using a Spectrophotometer

The growth level of *F. nucleatum* ATCC 25586 was determined after 24 and 48 hours of culture in media containing different glucose concentrations. The measurement was based on the assessment of turbidity or cloudiness (26). An ELISA reader spectrophotometer was used to measure the optical density (27). Bacterial samples from each concentration and untreated bacterial suspension were taken using an Eppendorf micropipette and placed into the wells of a microplate (200 µl per well) in triplicate. The measurement was then conducted using a microplate reader at a wavelength of 520 nm (Table 1) (25, 28).

Protein Testing of F. nucleatum ATCC 25586 Using FTIR

Protein testing of *F. nucleatum* ATCC 25586 was conducted using the FTIR method. After 24 and 48 hours of incubation, the bacterial suspension was centrifuged at 4472 RCF for 10 minutes. The supernatant was discarded, and the pellet was resuspended in distilled water. The sample was pipetted and dropped onto a KBr window crystal, then dried (29). The KBr window was placed in the holder of a Transmission FTIR (Shimadzu IR Prestige). Scanning was performed at wavenumbers ranging from 4000 to 400 cm^{-1} with a resolution of 41 cm^{-1}. The spectrum was processed using IR Solution Software Overview (Shimadzu), focusing on peak positions in the wavenumber regions of 1700-1600 cm^{-1}, 1600-1500 cm^{-1}, and 1350-1200 cm^{-1}, which correspond to the protein absorption areas in the bacterial samples (30, 31).

Data Analysis

The research results were analyzed using SPSS software. The Spearman correlation test was employed to determine if there was a relationship between the growth of *F. nucleatum* ATCC 25586 in glucose-enriched media and its protein activity. The Kruskal-Wallis test was used to determine whether glucose concentration influenced the growth and protein activity of *F. nucleatum* ATCC 25586. The Mann-Whitney test was used to assess whether incubation time affected growth, and the independent sample T-Test was used to determine its effect on protein activity. All statistical tests were considered significant if the p-value was ≤ 0.05.

Results

Results of F. nucleatum ATCC 25586 Growth Measurement

The growth of *F. nucleatum* ATCC 25586 was determined using an ELISA Reader spectrophotometer at a wavelength of 520 nm. The growth values of *F. nucleatum* were based on the optical density (OD) of each sample, subtracted by the OD value of the control bacteria, which was 0.045 for a 24-hour incubation period and 0.042 for the 48-hour incubation period. These values correspond to 0.5 McFarland standard. The growth levels of *F. nucleatum* at various glucose concentrations are shown in Figure 1.

Table 1. Calibration of Optical Density (OD) Values to Colony Forming Unit (CFU) values and McFarland Scale.

McFarland Scale	CFU(x10^4/mL)	OD
0.5	<300	0.05
1	300	0.1
2	600	0.2
3	900	0.3
4	1200	0.4
5	1500	0.5
6	1800	0.6
7	2100	0.7
8	2400	0.8
9	2700	0.9
10	3000	1.0

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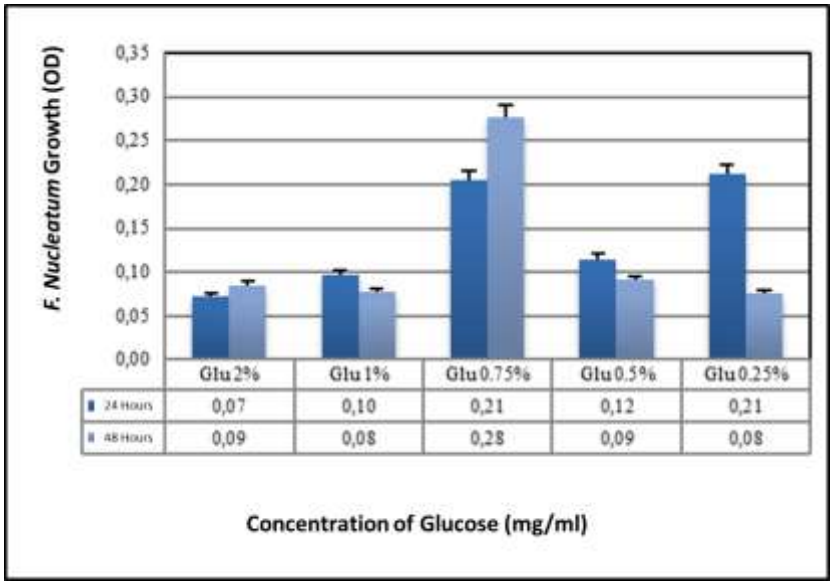

Fig. 1. The growth rate of *F. nucleatum* ATCC 25586 based on variations in glucose concentrations and incubation time, measured by optical density (OD) at 520 nm.

It was shown that glucose affected the growth of *F. nucleatum* ATCC 25586, with the bacterium being able to grow at all glucose concentrations but at varying levels. Generally, *F. nucleatum* exhibited higher growth at concentrations of 0.75%, 0.5%, and 0.25% compared to 2% and 1% glucose concentrations (Fig. 1). The 0.75% concentration demonstrated optimal growth of *F. nucleatum* at 48 hour (Table 2).

Table 2. Calibration of Optical Density (OD) Values to Colony Forming Units (CFU) and McFarland Scale (MF).

Concentration of glucose	Growth of *F. nucleatum* ATCC 25586					
	24 Hours			48 Hours		
	OD	CFU	MF Scale	OD	CFU	MF Scale
2%	0.07	<300	0.5	0.09	<300	0.5
1%	0.10	300	1	0.08	<300	0.5
0.75%	0.21	600	2	0.28	600	2
0.5%	0.12	300	1	0.09	<300	0.5
0.25%	0.21	600	2	0.08	<300	0.5

It was shown that based on CFU and McFarland scale values, the growth of *F. nucleatum* in 2% and 0.75% glucose concentrations remained the same between 24 and 48 hours. In contrast, at 1%, 0.5%, and 0.25% glucose concentrations, a decrease in growth was observed at 48 hours. The Growth of *F. nucleatum* at all concentrations and incubation times did not exceed 600 x 10^4 CFU/mL or 2 McFarland (Table 2).

Statistical testing was conducted to evaluate the differences in *F. nucleatum* growth among the five glucose concentrations tested. The Kruskal-Wallis test was used as an alternative to One-Way ANOVA due to non-normal distribution of the data. The results of the Kruskal-Wallis test indicate a p-value of 0.271, which means $p > 0.05$. This indicates that there is no significant difference in the growth of *F. nucleatum* ATCC 25586 based on the glucose concentrations tested (Table 3).

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To determine the difference in *F. nucleatum* growth between the 24-hour and 48-hour incubation periods, a Mann-Whitney test was conducted as the data was not normally distributed. The tests results, indicating a p-value of 0.465, which is greater than 0.05. This concluded that there was no significant difference in protein intensity of *F. nucleatum* ATCC 25586 between the 24-hour and 48-hour incubation periods (Table 4).

Table 3. Statistical Test Results of *F. nucleatum* ATCC 25586 Growth Based on Glucose Concentration and Incubation Time.

Variable	Type of analysis	*p* Value
Concentration of Glucose	Kruskal Wallis	0.271
Inquisition time	*Mann-Whitney*	0.465

Table 4. Statistical Test Results of *F. nucleatum* ATCC 25586 Protein Based on Glucose Concentration and Incubation Time.

Variable	Type of analysis	*p* Value
Concentration of Glucose	Kruskal Wallis	0.149
Inquisition time	*Independent Sample T-test*	0.087

FTIR Spectrum Results and Protein Analysis of F. nucleatum ATCC 25586

The FTIR spectra of *F. nucleatum* were scanned across the wavenumber range of 4000-400 cm^{-1}. The results displayed similar absorption patterns across all samples with several distinct peaks. The FTIR spectrum readings focused on the protein absorbance regions, specifically within the wavenumber ranges of 1700-1600 cm^{-1}, 1600-1500 cm^{-1}, and 1350-1200 cm^{-1} (Figs. 2 & 3).

Subsequently, an analysis was conducted on the intensity of protein functional groups based on the average intensity of all identified peaks of Amide I, Amide II, and Amide III. The protein intensity for these three Amide groups are illustrated in Figure 4. The analysis indicated that the intensity of protein, based on the concentrations of the Amide groups, did not show significant differences. Similarly, the intensity did not vary greatly based on incubation time, with an average increase or decrease of only 1% from 24 hours to 48 hours.

The protein intensity was further analyzed using the Kruskal-Wallis test. The results of the analysis indicated that there was no significant difference in protein intensity based on glucose concentration ($p > 0.05 = 0.149$). Additionally, based on incubation time, the analysis with an independent sample T-test also showed no significant difference ($p > 0.05 = 0.087$).

Relationship Between the Growth of F. nucleatum ATCC 25586 and Protein Activity

The growth and protein activity data of *F. nucleatum* were converted into percentage values. These values were then plotted on a single graph to illustrate the relationship between bacterial growth and protein activity (Fig. 5). At 2% glucose concentration after 24 hours, where bacterial growth was low, theprotein activity was almost the same as at other concentrations. In contrast, the growth of *F. nucleatum* appeared to align with its protein activity at 0.5% glucose concentration after 24 hours. For other concentrations, whether the growth of *F. nucleatum* was high or low, there was no consistent correlation between growth and protein activity.

The relationship between the growth of *F. nucleatum* and its protein activity was statistically analyzed using the Spearman correlation method, as the data did not follow a normal distribution (p value= 0.323)

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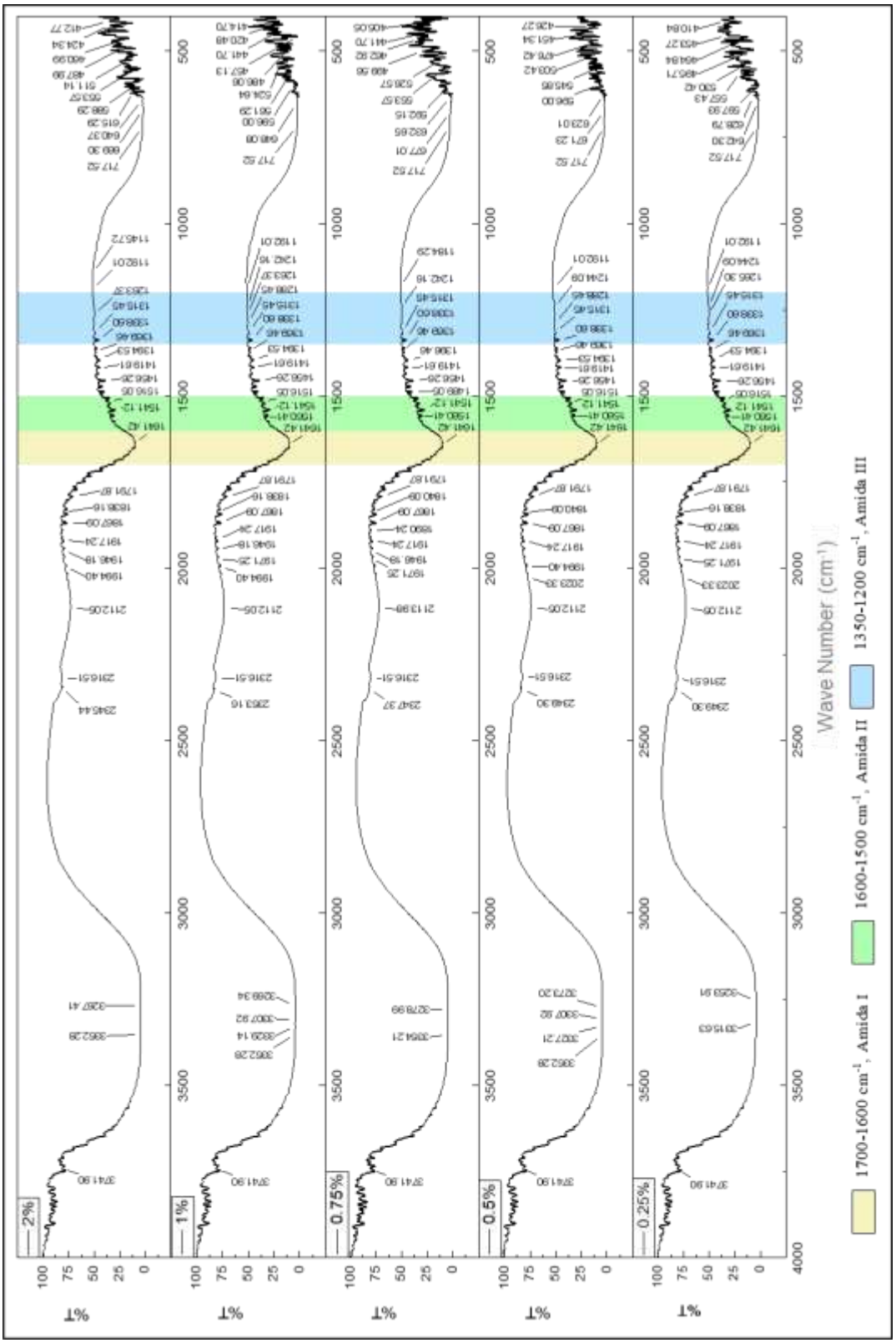

Fig. 2. FTIR spectrum of *F. nucleatum* ATCC 25586 at various sample concentrations (0.25%, 0.5%, 0.75%, 1%, 2%) with 24 hours incubation time. %T = % transmittance.

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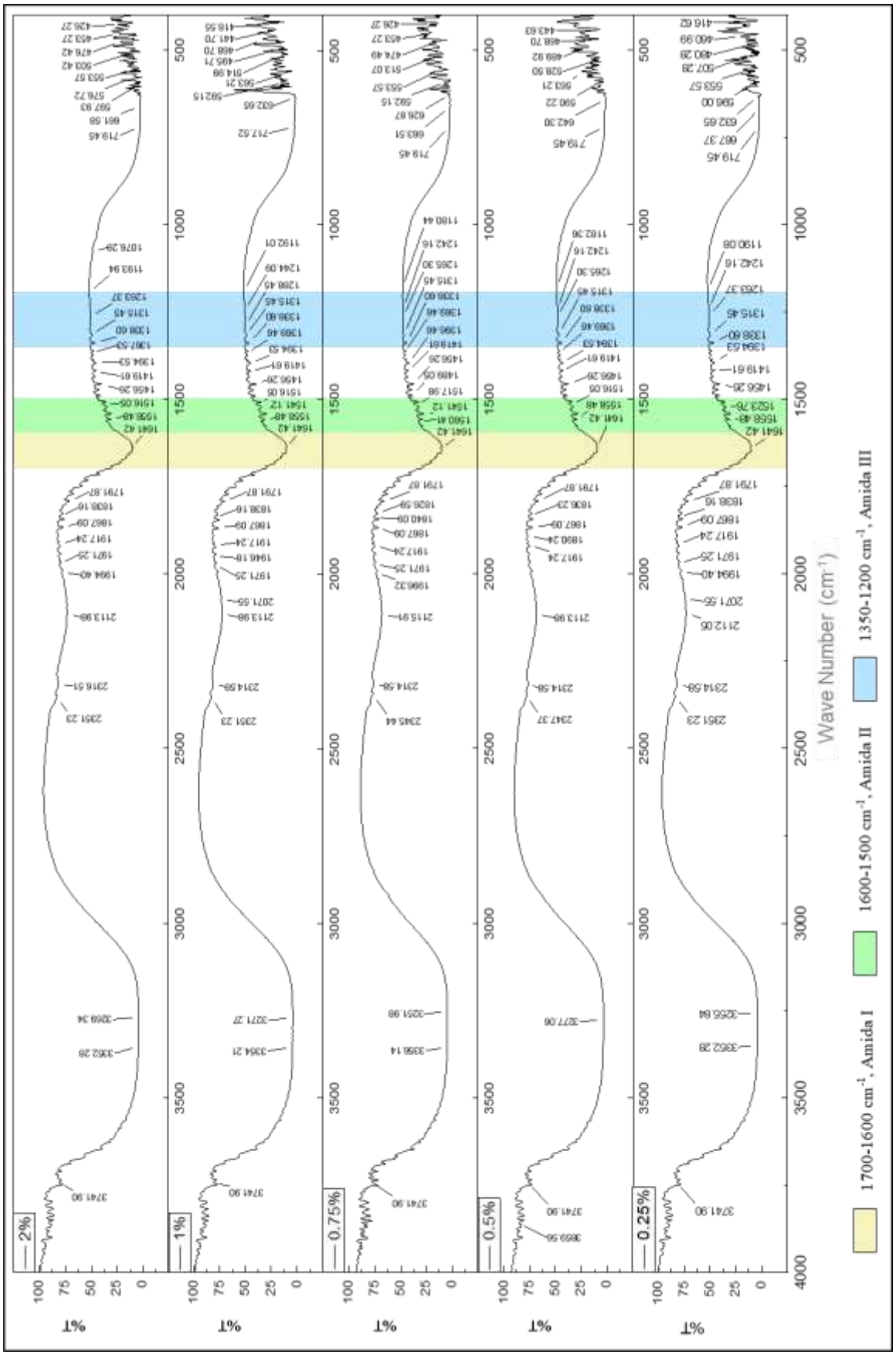

Fig. 3. FTIR spectrum of F. nucleatum ATCC 25586 at various sample concentrations (0.25%, 0.5%, 0.75%, 1%, 2%) with 48 hours incubation time. %T = % transmittance.

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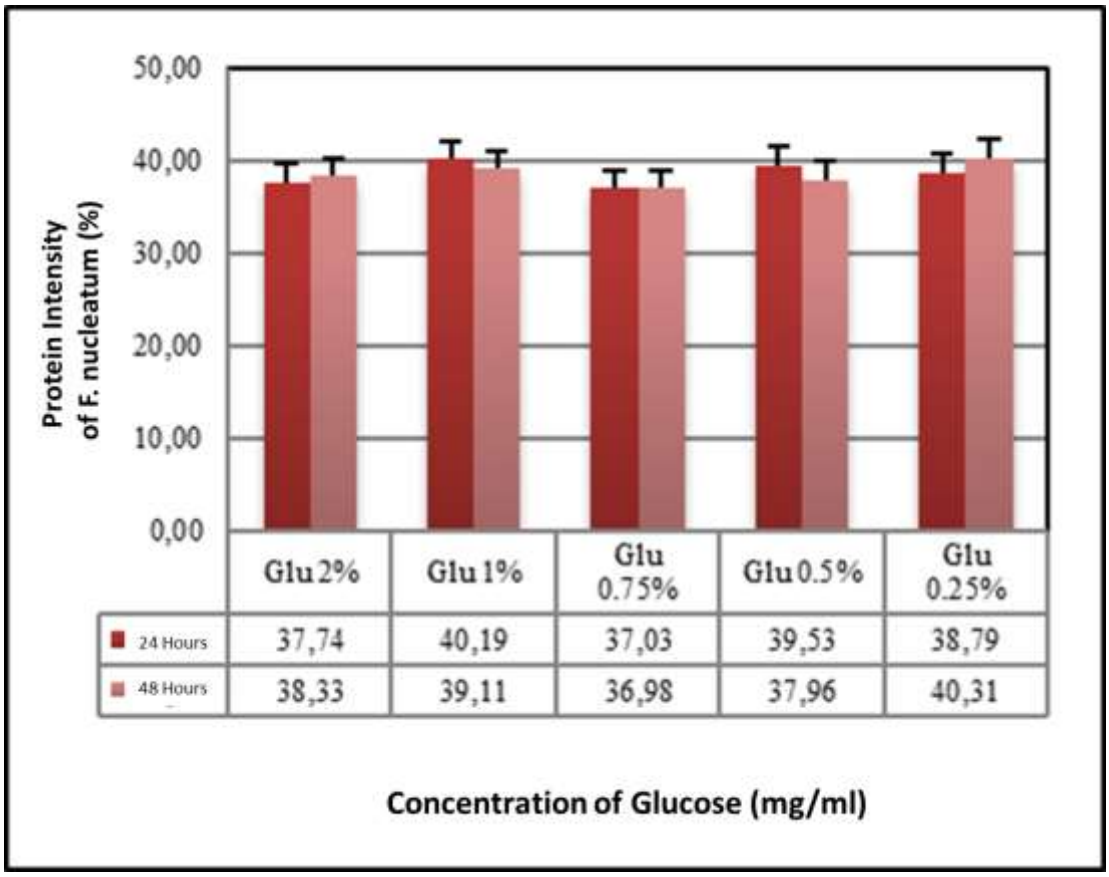

Fig. 4. Protein Intensity of *F. nucleatum* Based on Concentration and Incubation Time.

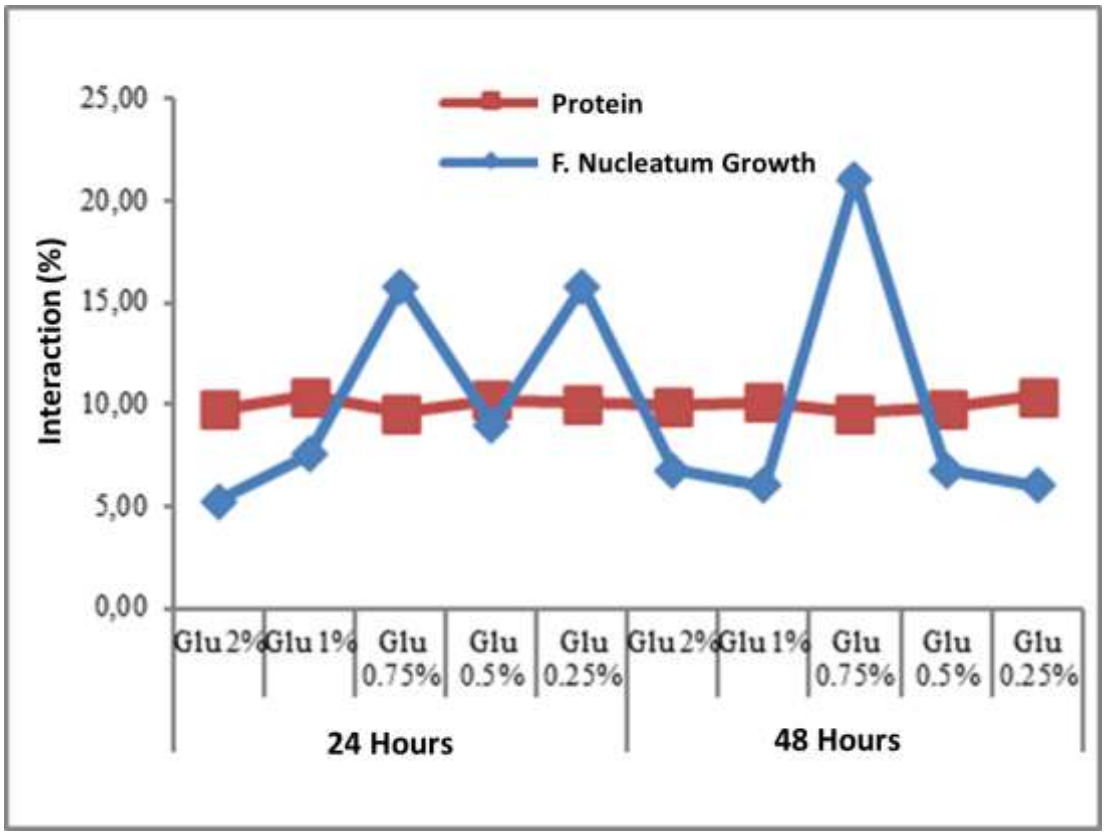

Fig. 5. Graph of *F. nucleatum* Growth Relationship with its Protein Activity.

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Discussion

This study demonstrated that glucose influences the growth of *F. nucleatum*, as indicated by its utilization of glucose as a nutrient source. Specifically, lower glucose concentrations (0.75%, 0.5%, and 0.25%) were found to better support the growth of *F. nucleatum* compared to higher concentrations (2% and 1%), with maximal growth observed at 0.75% after 24 hours. Edward (1970) explained that an increase in nutrient concentration can enhance the physiological parameters of bacterial cells, leading to growth due to metabolic stimulation. However, further increased in concentration may not improve these parameters because, at this point, other environmental factors limit supply, or the cells themselves have reached their capacity under existing culture conditions. Eventually, increasing nutrient concentration further could decrease the physiological parameters of bacteria, and severe inhibition might even cause simultaneous bacterial cell death (32). Mizzi et al. (2020) reported that low glucose concentrations could be utilized by bacteria as a nutrient source, while high glucose concentrations might inhibit bacterial growth, with one of the contributing factors being reduced water activity, leading to osmotic stress in bacterial cells (33, 34). However, statistical analysis in this study indicated that the growth of *F. nucleatum* did not differ significantly based on the glucose concentrations tested, suggesting that both high and low glucose concentrations generally had a similar effect on *F. nucleatum* growth.

Regarding the incubation periods used, *F. nucleatum* appeared to be in a stationary phase or entering the death phase at 48 hours. The stationary phase was characterized by a lack of increase or decrease in bacterial growth, indicating that growth had peaked (27). Statistical analysis showed no significant difference in the growth of *F. nucleatum* between 24 and 48 hours, suggesting that glucose continued to support nutrient availability for *F. nucleatum* growth up to 48 hours, but might not enhance growth beyond this point. Moreover, *F. nucleatum* growth across all glucose concentrations, at both 24 and 48 hours, remained below 600 x 10^4 CFU/mL or 2 McFarland, which is within the range observed in healthy gingival tissues (5.7 x 10^6). In contrast, in chronic periodontal disease and acute ulcerative gingivitis, *F. nucleatum* counts range from 3.3 x 10^7 to 9.3 x 10^7 (35).

In utilizing glucose as a nutrient source for growth, *F. nucleatum* must produce specific proteins, including enzymes that degrade polysaccharides, transport proteins that accumulate sugars within the cell, and enzymes involved in metabolism. A central pathway for glucose utilization is the Embden-Meyerhof glycolysis pathway (Bacteria-Bacterial Metabolism | Britannica). The FTIR test demonstrated protein activity during the interaction between the bacteria and glucose, based on observations of the FTIR spectrum in the wave number regions of 1700-1500 cm^{-1} and 1350-1200 cm^{-1}, which are specific for proteins. The results showed peaks in the absorption bands corresponding to protein functional groups: Amide I at 1641.42 cm^{-1}, Amide II at 1560.41-1516.05 cm^{-1}, and Amide III at 1338.6-1242.16 cm^{-1}. Other protein absorption bands were also observed in the 1500-1350 cm^{-1} region, but they were not specific due to overlapping with lipid absorption (31). According to Consumi et al. (2020), proteins observed with FTIR in bacteria represent the total cellular proteins, including exoproteins and endoproteins such as membrane, cytoplasmic, and ribosomal proteins (36). The enzymes involved in glucose glycolysis are primarily cytoplasmic (37), including enzymes like glucokinase, phosphoglucose isomerase, phosphofructokinase, fructose 1,6-bisphosphate aldolase, phosphoglycerate kinase, and others (38). While the FTIR method cannot specifically identify the proteins involved in glucose metabolism, it does indicate general protein activity during

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the interaction of *F. nucleatum* with glucose, suggesting that the bacterium utilizes glucose for growth.

The increase in FTIR peak intensity suggested an increase in the number or concentration of protein functional groups, which implied increased protein activity (39,40). Since this study found no significant differences in intensity, the protein activity or cellular response of *F. nucleatum* during glucose metabolism remained stable across various glucose concentrations and incubation times. This aligned with the findings of Noack et al. (2017), who studied the regulation of enzyme proteins in *Corynebacterium glutamicum* and found that the bacterium maintained stable enzyme concentrations when grown in glucose as the primary carbon and energy source (41). Chubukov et al. (2013) explained that most metabolic enzymes are present in excess, so changes in central metabolic flow, such as due to variations in substrate concentration, did not lead to modifications in enzyme concentration unless the substrate concentration was extremely low or high, necessitating re-regulation of metabolic enzymes (41, 42). Therefore, it was likely that the enzyme proteins in *F. nucleatum* were sufficient to meet the bacterium's needs for glucose metabolism across all tested concentrations, eliminating the need for re-regulation of metabolic proteins that would alter their concentrations.

Overall, the protein activity in this study remained consistent across different growth levels, suggesting that *F. nucleatum* may had similar enzyme activity in glucose metabolism at various growth stages. The statistical analysis further showed no correlation between growth rates and protein activity. Noack et al. (2017) also reported that in *Corynebacterium glutamicum*, the growth rate was only weakly correlated with metabolic enzyme concentration, as enzyme levels remained stable even at different growth rates. To maintain constant enzyme concentrations across different growth rates, bacteria must synthesize enzymes at proportionally varying rates. The availability of excess enzymes enables bacteria to rapidly adapt their metabolic processes when environmental conditions change (41).

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Conflicts of Interest
The authors approved this study in Ethical Number: Ref: 263/KE/FKG/2021. The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely witnessed by the authors.

References
1. Chew J, Zilm PS, Fuss JM, Gully NJ. A proteomic investigation of *Fusobacterium nucleatum* alkaline-induced biofilms. BMC Microbiol. 2012;12(1):1.
2. Lima BP, Shi W, Lux R. Identification and characterization of a novel *Fusobacterium nucleatum* adhesin involved in physical interaction and biofilm formation with *Streptococcus gordonii*. Microbiologyopen. 2017;6(3):1–10.
3. Thurnheer T, Karygianni L, Flury M, Belibasakis GN. *Fusobacterium* species and subspecies differentially affect the composition and architecture of supra- and subgingival biofilm models. Front Microbiol. 2019;10:1–11.

[DOI: 10.61186/rbmb.13.3.310]

4. Lagha A Ben, Haas B, Grenier D. Tea polyphenols inhibit the growth and virulence properties of *Fusobacterium nucleatum*. Sci Rep. 2017;7:1–10.
5. Sun CH, Li BB, Wang B, Zhao J, Zhang XY, Li TT, et al. The role of *Fusobacterium nucleatum* in colorectal cancer: from carcinogenesis to clinical management. Chronic Dis Transl Med. 2019;5(3):178–87.
6. Nagpal SJS, Mukhija D, Patel P. *Fusobacterium nucleatum*: a rare cause of pyogenic liver abscess. Springerplus. 2015;4(1):0–4.
7. Vander Haar EL, So J, Gyamfi-Bannerman C, Han YW. *Fusobacterium nucleatum* and adverse pregnancy outcomes: epidemiological and mechanistic evidence. Anaerobe. 2018;50:55–9.
8. Han YW. *Fusobacterium nucleatum*: A commensal-turned pathogen. Curr Opin Microbiol. 2015;23:141–7.
9. Miranda TS, Feres M, Retamal-Valdes B, Perez-Chaparro PJ, Maciel SS, Duarte PM. Influence of glycemic control on the levels of subgingival periodontal pathogens in patients with generalized chronic periodontitis and type 2 diabetes. J Appl Oral Sci. 2017;25(1):82–9.
10. Willis JR, Gabaldón T. The human oral microbiome in health and disease: From sequences to ecosystems. Microorganisms. 2020;8(2):1–28.
11. Zhou M, Rong R, Munro D, Zhu C, Gao X, Zhang Q, Dong Q. Investigation of the effect of type 2 diabetes mellitus on subgingival plaque microbiota by high-throughput 16S rDNA pyrosequencing. PLoS One. 2013;8(4):e58044.
12. Passalacqua KD, Charbonneau ME, O'Riordan MXD. Bacterial metabolism shapes the host-pathogen interface. Microbiol Spectr. 2016;4(3):1–21.
13. Rohmer L, Hocquet D, Miller SI. Are pathogenic bacteria just looking for food? Metabolism and microbial pathogenesis. Trends Microbiol. 2011;19(7):341–8.
14. Chen Y, Nielsen J. Energy metabolism controls phenotypes by protein efficiency and allocation. Proc Natl Acad Sci U S A. 2019;116(35):17592–7.
15. Rocha D, Romero A, Ruiz-Villafán B, Sanchez S. Significance of microbial glucokinase. In: Brachmachari G, Demain AL, Adrio JL, editors. Biotechnology of microbial enzymes. United Kingdom: Elsevier; 2012. p. 299–323.
16. Romero-Rodríguez A, Ruiz-Villafán B, Rocha-Mendoza D, Manzo-Ruiz M, Sánchez S. Biochemistry and regulatory functions of bacterial glucose kinases. Arch Biochem Biophys. 2015;577:80–9.
17. Yang Y, Hu M, Yu K, Zeng X, Liu X. Mass spectrometry-based proteomic approaches to study pathogenic bacteria-host interactions. Protein Cell. 2015;6(4):265–74.
18. Sjahfirdi L, Mayangsari, Nasikin M. Protein identification using Fourier transform infrared. Int J Recent Res Appl Stud. 2012;10(3):418–21.
19. Kosa G, Shapaval V, Kohler A, Zimmermann B. FTIR spectroscopy as a unified method for simultaneous analysis of intra- and extracellular metabolites in high-throughput screening of microbial bioprocesses. Microb Cell Fact. 2017;16(1):1–11.
20. Walker JM. Methods in Molecular Biology. New York: Humana Press; 2012: 195–197.
21. Forfang K, Zimmermann B, Kosa G, Kohler A, Shapaval V. FTIR spectroscopy for evaluation and monitoring of lipid extraction efficiency for oleaginous fungi. PLoS One. 2017;12(1):1–17.
22. Goodson JM, Hartman ML, Shi P, Hasturk H, Yaskell T, Vargas J, et al. The salivary microbiome is altered in the presence of a high salivary glucose concentration. PLoS One. 2017;12(3):e0170437.
23. Mubarak Z, Chismirina S, Daulay HH. Aktivitas antibakteri ekstrak propolis alami dari sarang lebah terhadap pertumbuhan *Enterococcus faecalis*. J Syiah Kuala Dent Soc. 2016;1(2):175–86.
24. Romadhani DF, Fahmy AH, Alam IP, Salim HM. Bactericidal effects of extract basil leaves in in-vitro study of *Pseudomonas aeruginosa*. Biomol Health Sci J. 2020;3(2):105.
25. Soraya C, Mubarak Z, Gani BA. The growth and biofilm formation of *Enterococcus faecalis* in ethanol extract of *Citrus aurantiifolia* Indonesian species. J Pharm Pharmacogn Res. 2020;8(6):558–68.
26. Kralik P, Beran V, Pavlik I. Enumeration of *Mycobacterium avium* subsp. *paratuberculosis* by quantitative real-time PCR, culture on solid

[DOI: 10.61186/rbmb.13.3.310]

media and optical densitometry. BMC Res Notes. 2012;5(1):114.
27. Madigan MT, Martinko JM, Bender KS, Buckley DH, Stahl DA. Brock Biology of Microorganisms. 14th ed. USA: Pearson; 2015.
28. Sutton S. Measurement of microbial cells by optical density. J Valid Technol. 2011;17(1):46–9.
29. Kamnev AA, Dyatlova YA, Kenzhegulov OA, Vladimirova AA, Mamchenkova PV, Tugarova AV. Fourier transform infrared (FTIR) spectroscopic analyses of microbiological samples and biogenic selenium nanoparticles of microbial origin: Sample preparation effects. Molecules. 2021;26(4):1146.
30. Gieroba B, Krysa M, Wojtowicz K, Wiater A, Tomczyk M. The FT-IR and Raman spectroscopies as tools for biofilm characterization created by cariogenic streptococci. Int J Mol Sci. 2020;21(11):1–20.
31. Naumann D. Infrared spectroscopy in microbiology / Encyclopedia of Analytical Chemistry / Ed. Meyers R.A. Wiley: Chichester, UK, 2000.
32. Edwards VH. The influence of high substrate concentrations on microbial kinetics. Biotechnol Bioeng. 1970;12(5):679–712.
33. Mizzi L, Maniscalco D, Gaspari S, Chatzitzika C, Gatt R, Valdramidis VP. Assessing the individual microbial inhibitory capacity of different sugars against pathogens commonly found in food systems. Lett Appl Microbiol. 2020;71(3):251–8.
34. Chismirina S, Sungkar S, Adlim M, Darmawi D. *Streptococcus mutans* serotype analysis from dental plaque of caries patients in Banda Aceh based on the GTF gene. Rep Biochem Mol Biol. 2023;12(1):205–10.
35. Hofstad T. The Genus *Fusobacterium*. In: Prokaryotes. 3rd ed. Springer; 2006.
36.Consumi M, Jankowska K, Leone G, Rossi C, Pardini A, Robles E, et al. Non-destructive monitoring of *Pseudomonas fluorescens* and *Staphylococcus epidermidis* biofilm under different media by Fourier transform infrared spectroscopy and other corroborative techniques. Coatings. 2020;10(10):1–15.
37. Didiasova M, Schaefer L, Wygrecka M. When place matters: Shuttling of enolase-1 across cellular compartments. Front Cell Dev Biol. 2019;7:61.
38. Jojima T, Inui M. Engineering the glycolytic pathway: A potential approach for improvement of biocatalyst performance. Bioengineered. 2015;6(6):328–34.
39. Sheng M, Gorzsás A, Tuck S. Fourier transform infrared microspectroscopy for the analysis of the biochemical composition of *Caenorhabditis elegans* worms. Worm. 2016;6(1):1–14.
40. Brandes A, Lun DS, Ip K, Zucker J, Colijn C, Weiner B, et al. Inferring carbon sources from gene expression profiles using metabolic flux models. PLoS One. 2012;7(5):e34559.
41. Noack S, Voges R, Gätgens J, Wiechert W. The linkage between nutrient supply, intracellular enzyme abundances and bacterial growth: New evidences from the central carbon metabolism of *Corynebacterium glutamicum*. J Biotechnol. 2017;258:13–24.
42. Chubukov V, Uhr M, Le Chat L, Kleijn RJ, Jules M, Link H, et al. Transcriptional regulation is insufficient to explain substrate-induced flux changes in *Bacillus subtilis*. Mol Syst Biol. 2013;9:709.

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