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**UTILIZATION OF SOYBEAN POWDER AS AN ALTERNATIVE MEDIA FOR
 THE GROWTH OF ANAEROBIC BACTERIA**

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ABSTRACT

Background: Culture media or microbial growth media is a material consisting of a mixture of nutrients used by microorganisms for growth and reproduction. Anaerobic bacteria can thrive by requiring little or no oxygen for growth and metabolism. **Purpose:** The purpose to determine the potential of soybean powder as an alternative nitrogen source in *Streptococcus mutans* bacterial growth media and determine the optimum concentration of soybean powder in alternative media for *Streptococcus mutans* bacterial growth. **Methods:** The research method is a laboratory experiment to test soybean powder as an alternative medium for the growth of *Streptococcus mutans* bacteria using the Candle jar method. The population used is soybean powder with the brand soy flour, while the sample used is *Streptococcus mutans* bacteria. **Results:** The results obtained can be known that the average value of colony growth of *Streptococcus mutans* bacteria in alternative media of soybean powder with a concentration variation of 2 grams was 30 colonies, a concentration variation of 3 grams was 54 colonies, a concentration variation of 4 grams was 40 colonies, and a concentration variation of 5 grams was 72 colonies. From the average value, it can be seen that the growth of *Streptococcus mutans* on alternative media of soybean powder can grow well, but the most optimum growth is in the concentration variation of 5 grams. **Conclusion:** Based on the observations obtained, it shows that soybean powder (*Glycine max* (L.) Merr) can be used as an alternative medium for the growth of *Streptococcus mutans* bacteria.

Keywords: Soybean Powder, *Streptococcus mutans*, Alternative Media

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INTRODUCTION

Streptococcus mutans is a facultative anaerobic Gram-positive bacterium that can produce lactic acid, which helps the bacteria survive as part of its metabolism. *Streptococcus mutans* bacteria are Gram-positive, non-motile (unmove), and are facultative anaerobic bacteria. *Streptococcus mutans* are round, form chains with a diameter of 0.5–0.7 µ during growth, and do not form spores. *Streptococcus mutans* bacteria grow optimally at temperatures between 18–40 °C. These bacteria are often found in the cavities of injured human teeth and most often cause cavities.³

Caries is a dental health problem triggered by multifactorial conditions. Four factors cause caries development, namely host, agent, diet, and time. Lesions can only develop under the interaction of all factors including the enamel surface and saliva as host factors,

bacteria as agents, fermentable carbohydrates as diet, and time. *Streptococcus mutans* is a bacterial agent commonly found in the oral cavity and contributes significantly to the occurrence of caries. This bacteria ferments carbohydrates into lactic acid which triggers demineralization of tooth enamel. Saliva acts as a buffer that can balance the remineralization process, but an imbalance between demineralization and remineralization will trigger the formation of dental caries.

Streptococcus mutans are naturally present as oral microbiota. This bacteria is mostly found in pits and crevices but least found on the buccal surface of the teeth. The presence of *Streptococcus mutans* in the oral cavity can turn into an opportunistic pathogen that initiates disease and damage to the host. *Streptococcus mutans* are associated with plaque formation and dental caries. This bacteria can metabolize

sucrose into lactic acid using the enzyme glucose $C_6H_{12}O_6 \rightarrow 2$ pyruvic acid $\rightarrow 2$ lactic acid + 2 ATP. The acidic environment in the mouth resulting from this process forges tooth enamel to become easily demineralized.²⁰

Selective media for the growth of *Streptococcus mutans* bacteria is *Mitis Salivarius Agar* which can inhibit the growth of oral bacteria other than *Streptococcus* bacteria. One important component in anaerobic bacterial growth media is a nitrogen source. In Indonesia there are rich natural resources, such as vegetable protein. This alternative protein source can be obtained from soybeans, this material is easy to obtain and does not require high costs. Soybean powder contains 35-40% protein, which is the highest protein content of all legumes. In terms of protein quality, soybeans are the best, their nutritional quality is close to meat protein. Soybean protein is the only grain with the most complete essential amino acid profile. Essential and non-essential protein compounds are composed of long amino acid chains consisting of the elements C, H, O, and N.⁵

Based on the background above, the formulation of the problem is whether soybean powder to replace nitrogen sources can be used as an alternative medium for the growth of anaerobic bacteria, especially *Streptococcus mutans* bacteria. The purpose of this study was to determine the growth of anaerobic bacteria, especially *Streptococcus mutans* bacteria in alternative soybean powder media, and to determine the optimum concentration of soybean powder in alternative media for the growth of anaerobic bacteria.

METHODS

This research design was conducted using a laboratory experimental research method to test soybean powder as an alternative medium to replace nitrogen sources in anaerobic bacteria, one of which is *Streptococcus mutans* bacteria using the Candle jar method. The population in this study was soybean powder. The samples used were soybean powder and *Streptococcus mutans* bacteria. The tools used were BSC, desiccator, McFarland densitometer, incubator, oven, colony counter, and autoclave. The materials used were soybean powder, *Streptococcus mutans* bacteria, *Mitis Salivarius Agar* media, agar, crystal violet, dipotassium phosphate, sucrose, and 1% sterile potassium tellurite.

Making Alternative Media from Soybean Powder

Soybean powder is weighed with

different scales of 2 grams, 3 grams, 4 grams, and 5 grams. Add each agar, crystal violet, dextrose, dipotassium phosphate, sucrose, and add 100 ml of distilled water to the labeled Erlenmeyer. The media is heated and homogenized on a hotplate until dissolved. The top of the Erlenmeyer is closed using a cotton stopper and the Erlenmeyer flask is wrapped in coffee paper, then sterilized by autoclaving at a temperature of 121 °C for 15 minutes. The media is cooled and adds 0.1 ml of sterile 1% potassium tellurite solution, then the media is stirred until homogeneous. The media is poured into a petri dish with a volume of 15 ml and let the media cool and ready to use.

Making Mitis Salivarius Agar Media

The media powder was weighed according to the instructions on the packaging, which was 5.5 in 100 ml. The weighed media powder was dissolved with distilled water in an Erlenmeyer flask. The media solution was heated and homogenized on a hotplate. The media solution was sterilized in an autoclave at a temperature of 121 °C and a pressure of 1 atm for 15 minutes. The media was cooled and 0.1 ml of sterile 1% potassium tellurite solution was added, then the media was stirred until homogeneous. The media was poured into a suitable container. The media was poured into a 90 mm diameter petri dish.

Anaerobic Bacterial Growth Using the Candle Jar Method

Add 0.1 ml of *Streptococcus mutans* 0.3 McFarland bacterial suspension using a micropipette on the surface of the alternative soy powder media and *Mitis Salivarius Agar* media. The bacterial suspension is spread using a sterilized L rod onto the alternative media and *Mitis Salivarius Agar* media. The alternative soy powder media and control media are put into a desiccator. Light a white candle, put it in the desiccator, and close the desiccator tightly. After the candle goes out, the desiccator is put into an incubator at 37 °C for 48 hours. After 48 hours, bacterial growth is observed on the alternative media and control media.

Count Bacterial Colonies Using a Colony Counter

Streptococcus mutans colonies growing on each petri dish in alternative media of soybean powder and *Mitis Salivarius Agar* were counted using a colony counter. The results obtained were recorded.

RESULTS

The data analysis used is qualitative and quantitative descriptive data. Qualitative descriptive analysis was conducted to determine

the colony shape, colony color, colony edge, and colony elevation of the growth of *Streptococcus mutans* bacteria on alternative soybean powder media based on concentration variations. Quantitative descriptive analysis aims to identify the number of *Streptococcus mutans* bacterial colonies to determine the optimum concentration of soybean powder on alternative media.

General description obtained after conducting research on the growth of *Streptococcus mutans* bacteria on alternative media of soybean powder with different concentrations (2 grams, 3 grams, 4 grams, and 5 grams). The technique used is a spread plate, with an incubation duration of 2x24 hours at a temperature of 37 °C. The growth results are presented in the table below.

Table 1. Macroscopic images of *Streptococcus mutans* bacteria

Consentration	Observation	
	Description	Macroscopic Images
2 grams	Colony shape: small round Colony color: black Colony edge: smooth Colony elevation: convex	
		
3 grams	Colony shape: small round Colony color: black Colony edge: smooth Colony elevation: convex	
		

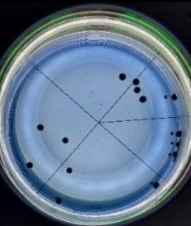
4 grams	Colony shape: small round Colony color: black Colony edge: smooth Colony elevation: convex	
5 grams	Colony shape: small round Colony color: black Colony edge: smooth Colony elevation: convex	
Mitis Salivarius Agar	Colony shape: medium round Colony color: dark blue Colony edge: smooth Colony elevation: convex	

Table 2. Comparison of average growth of *S. mutans* colonies on alternative media and control media

Concentration	Alternative media soy powder (0.3 McFarland)	Mitis Salivarius Agar control medium (0.3 McFarland)
2 grams	30	8
3 grams	54	173
4 grams	40	14
5 grams	72	201

Based on Table 2, it can be seen that the average growth value of *Streptococcus mutans* bacterial colonies on alternative soybean powder media with a concentration variation of 2 grams was 30 colonies, a concentration variation of 3 grams was 54 colonies, a concentration variation of 4 grams was 40

colonies, and a concentration variation of 5 grams was 72 colonies. From the average value, it can be seen that the growth of *Streptococcus mutans* on alternative soybean powder media can grow well, but the most optimum growth is found in the concentration variation of 5 grams. It is concluded that soybean powder can be used as an alternative source of nitrogen for the growth of *Streptococcus mutans*.

DISCUSSION

Based on the results of observations obtained, it shows that soybean powder (*Glycine max* (L.) Merr) can be used as an alternative medium for the growth of *Streptococcus mutans* bacteria. The results of the number of colonies that grow have different numbers in each concentration variation. This study was conducted using soybean powder as an alternative medium for the growth of *Streptococcus mutans* bacteria with the same treatment on Mitis Salivarius Agar media. The bacterial suspension used was 0.3 McFarland and incubated in an anaerobic atmosphere at a temperature of 37 °C for 48 hours, the growth was observed which was marked by the formation of colonies on the surface of the media.

The control media used in the growth of *Streptococcus mutans* was Mitis Salivarius Agar media. The results showed that the average number of colonies growing on Mitis Salivarius Agar media was 99 colonies. The average number of *Streptococcus mutans* colonies growing on alternative media of 2 gram concentration of soybean powder was 30 colonies, 3 gram concentration was 54 colonies, 4 gram concentration was 40 colonies, and 5 gram concentration was 72 colonies. Alternative media of 5 grams concentration of soybean powder was the best concentration because it had a higher nutrient content compared to other concentrations. The higher the concentration, the higher the nutrient content.

The difference in the number of colonies between the alternative media of soybean powder and Mitis Salivarius Agar media can be caused by several factors such as oxygen and bacterial cultivation using spread plates. Anaerobic bacteria require conditions without oxygen in the environment, the presence of oxygen will cause anaerobic bacteria to die or inhibit growth. In an oxygen atmosphere, it will form H₂O₂ (Hydrogen peroxide) and Reactive Oxygen Species (ROS) which are toxic to anaerobic bacteria, because the bacteria have no defense against aerobic organisms and so cannot survive.²⁵ Spread plate is a technique used to

grow colonies on agar media. The advantage of using the spread plate technique is that bacteria can spread evenly, but the disadvantage is that it is quite difficult to spread the suspension with a curved rod so that it can cause contamination of the bacterial culture. It is important to pay attention to the selection of tools used in leveling the suspension, this will affect the number of bacterial colonies that grow.¹²

Soybeans are a source of protein, fat, and various sources of vitamins A, E, and K, several types of vitamin B, and minerals K, Fe, Zn, and P. Beans have a protein content of 20 to 25%, but in soybeans it reaches 40%. The factor that causes an increase in the number of *Streptococcus mutans* bacterial colonies is the difference in the nutritional content of each soybean powder media and Mitis Salivarius Agar media. The nutritional composition of Mitis Salivarius Agar media is adjusted to the characteristics of bacterial metabolism, so that the nutrients in Mitis Salivarius Agar media are processed faster and easier, allowing bacterial metabolic activity that can produce optimal bacterial growth. This is different from the alternative media of soybean powder which has a more complex nutritional composition so that it must first be changed through an enzymatic process into simpler compounds so that it can be used for bacterial metabolic activity.¹¹

Factors that affect the growth of bacterial colonies include physical factors (abiotic) and chemical factors (biotic). Abiotic factors can include pH, temperature, light or radiation, and osmotic pressure. Biotic factors include organic growth factors including oxygen, carbon, and nutrients contained in the growth medium. Bacteria require nutrients with a certain composition to grow and divide. The nutrients needed by bacteria must consist of water, a nitrogen source, a carbon source, vitamins, minerals, and gasses. Minerals used by the bacterial body for certain functions include Mg, K, Fe, Cu, Co, and Zn.³⁰ Nitrogen is very important in the process of bacterial growth, so it can maximize bacterial activity during growth. Nitrogen is a component of protein, nucleic acid, and coenzyme that has physiological functions for bacteria. The nitrogen content in soybeans is 20-35%.² Soybeans contain 35-40% protein which is the highest protein content of nuts.⁵

Identification of *Streptococcus mutans* on alternative soy powder media and Mitis Salivarius Agar media was carried out microscopically with gram staining. The results of Gram staining were examined at 100x magnification using a microscope. In this study,

Gram-positive bacteria were found which were characterized by a purple color, round shape, and forming chains. The results of the study showed the morphological characteristics of the colonies that grew on *Mitis Salivarius Agar* media, namely round shape, medium colony size, convex elevation, and dark blue color. Based on Gram staining and the characteristics of the colony morphology, it can be concluded that the bacteria are *Streptococcus mutans*. The morphological characteristics of the colonies formed on alternative soy powder media are round, small in size, convex elevation, and black in color. Based on the results of the study, the growth of *Streptococcus mutans* bacteria obtained almost the same macroscopic and microscopic images on alternative soy powder media and *Mitis Salivarius Agar* media. In this study, *Streptococcus mutans* bacteria provided an image of the colony shape, colony color, colony edges, and elevation that were similar to *Mitis Salivarius Agar* as a control medium.

Based on the results of research on the modification of *Mitis Salivarius Agar* media using soybean powder on the growth of *Streptococcus mutans* bacteria, it can be concluded that soybean powder used as a substitute for nitrogen sources can be used as an alternative material in making *Mitis Salivarius Agar* media on the growth of *Streptococcus mutans* bacteria. The growth of bacterial colonies using the most optimum soybean powder alternative media occurred at a concentration variation of 5 grams, because the bacteria that grew had a higher average value compared to the concentration variations of 2 grams, 3 grams, and 4 grams.

REFERENCES

- Adriana, A. N. I. (2022). Uji Aktivitas Ekstrak Etil Asetat Daun Sirih Hijau (*Piper Betlel* L) terhadap *Streptococcus mutans*. *Pharmacology And Pharmacy Scientific Journals*, 1(1), 20–28. <https://doi.org/10.51577/papsjournals.v1i1.305>
- Alfarisi, C. D., Yelmida, Zahrina, I., dan Mutamima, A. (2021). Pembuatan nata de cassava dari limbah cair tapioka dengan menggunakan sumber nitrogen alami yang berbeda. *Jurnal Ilmiah Pertanian*, 17(2), 93–100. <https://doi.org/10.31849/jip.v17i2.6208>
- Andriyani. (2020). Uji Daya Hambat Getah Jarak Pagar (*Jatropha curcas* Linn.) terhadap *Staphylococcus aureus* dan *Streptococcus mutans* secara In vitro.
- Atmanto, Y. K. A. A., Asri, L. A., dan Kadir, N. Abd. (2022). Media Pertumbuhan Kuman. *Jurnal Medika Utama*, 04(01), 3069–3075.
- Basalamah, N. A., Nurlaelah, I., dan Handayani, H. (2018). Pengaruh Substitusi Ekstrak Kedelai Terhadap Karakteristik Selulosa Bakteri *Acetobacter xylinum* Dalam Pembuatan Nata De Sweet Potato. *Quagga: Jurnal Pendidikan Dan Biologi*, 10(01), 24. <https://doi.org/10.25134/quagga.v10i01.805>
- Dwijastuti, N. M. S., dan Dewi, I. G. A. A. S. (2023). Perbedaan Hasil Kultur Anaerob Dengan Candle Jar dan Gas Generating Sachet Untuk Bakteri Asam Laktat *Lactobacillus Plantarum* E12.1. *Jurnal Biologi Makassar*, 8(1), 92–100.
- Garone, S. (2022). Soybean Nutrition Facts and Health Benefits.
- Gms, I. (n.d.). Tm 221–Mitis Salivarius Agar Base.
- Hasanuddin, A. R. P., dan Salnus, S. (2020). Uji Bioaktivitas Minyak Cengkeh (*Syzygium aromaticum*) Terhadap Pertumbuhan Bakteri *Streptococcus mutans* Penyebab Karier Gigi. *Jurnal Biologi Makassar*, 5(2), 241–250.
- Ifmaily. (2019). Uji Daya Hambat Ekstrak Daun Cherry (*Muntingia calabura* L) Sebagai Antibakteri Terhadap Bakteri *Streptococcus mutans*. *Prosiding Pokjanas*, 75–78.
- Indrayati, S., Utami, P. R., dan Oktaviani, I. R. (2021). Pemanfaatan Serbuk Kacang Kedelai (*Glycine max* L. Merr) sebagai Bahan Pengganti Beef Extract pada Media Nutrien Agar (NA) untuk Pertumbuhan Bakteri *Staphylococcus aureus*. *Prosiding Seminar Kesehatan Perintis*, 74–79.
- Kurniawan, S. Y., Ariami, P., dan Rohmi. (2023). Si Pinter Sebagai Alat Penghitung Koloni Bakteri Penunjang Laboratorium Mikrobiologi. *Jurnal Biotek*, 11(1), 87–97.
- Lacerda Rangel Esper, M. Â., Junqueira, J. C., Uchoa, A. F., Bresciani, E., Nara de Souza Rastelli, A., Navarro, R. S., dan de Paiva Gonçalves, S. E. (2019). Photodynamic inactivation of planktonic cultures and *Streptococcus mutans* biofilms for prevention of white spot lesions during orthodontic treatment: An in vitro investigation. *American Journal of Orthodontics and Dentofacial Orthopedics*, 155(2), 243–253. <https://doi.org/10.1016/j.jajodo.2018.03.027>
- Lagiman, Suryawati, A., dan Widayanto, B. (2022). Budidaya Tanaman Kedelai Di Lahan Pasir Pantai. *LPPM UPN Veteran Yogyakarta*.
- Lukaraja, W., Lessy, W., Seumahu, C. A., dan Pesik, A. (2020). Aktivitas Antibakteri Dan Penghambatan Biofilm Ekstrak Metanol Kulit Batang *Hibiscus tiliaceus* Terhadap Pertumbuhan Bakteri *Streptococcus mutans*. *Rumphius Pattimura Biological Journal*, 2(2), 37–43.
- Matsumoto-Nakano, M. (2014). Dental Caries. In Reference Module in Biomedical Sciences. Elsevier. <https://doi.org/10.1016/B978-0-12-801238-3.00001-5>
- Mayasari, E., Ayuningsih, B., dan Hidayat, R. (2015). Pengaruh Penambahan Nitrogen dan Sulfur pada Ensilase Jerami Jagung Terhadap Kecernaan Bahan Kering dan Bahan Organik pada Sapi Potong (In Vitro).

18. Pamujiningtyas, K. (2018). Cegah Obesitas dengan Konsumsi Kacang Kedelai Secara Teratur. <https://kumparan.com/kumparanfood/cegah-obesitas-dengan-konsumsi-kacang-kedelai-secara-teratur/full>
19. Putri, C. A. J., dan Maulana, I. T. (2020). Potensi Aktivitas Antibakteri Ekstrak Tetraselmis chui Buther terhadap Pertumbuhan Bakteri Streptococcus mutans. 146–151.
20. Putri, W. L., Siregar, E., & Anggani, H. S. (2021). Antibacterial Effect of Titanium Dioxide Nanoparticles and Chitosan Nanoparticles in Orthodontic Adhesive Resin on Streptococcus mutans Colony. Dentino Jurnal Kedokteran Gigi, 6(1), 111–116.
21. Rahmawati, I., dan Anliza, S. (2024). Pemanfaatan Limbah Cair Tahu Sebagai Media Alternatif Pertumbuhan Bakteri Escherichia coli. Jurnal Ilmiah Kesehatan, 16(1), 117–123.
22. Sari, R. S. (2020). Uji Aktivitas Antibakteri Obat Kumur Minyak Atsiri Kulit Kayu Manis (Cinnamomum burmannii L.) Terhadap Pertumbuhan Bakteri Streptococcus mutans.
23. Sayaka, B., Swastika, D. K. S., dan Saputra, Y. H. (2021). Challenges of soybean self-sufficiency policy in Indonesia. IOP Conference Series: Earth and Environmental Science, 648(1), 012035.
24. Suhartati, R., Sulistiani, dan Nuraini, A. (2018). Pemanfaatan Serbuk Kacang Kedelai (Glycine Max) sebagai Bahan Pembuatan Media Manitol Salt Agar (MSA) untuk Pertumbuhan Bakteri Staphylococcus. Prosiding Seminar Nasional Dan Diseminasi Penelitian Kesehatan, 163–167.
25. Tjampakasari, C. R., dan Hanifah, N. (2023). Kultivasi dan Identifikasi Bakteri Anaerob Bacteroides Fragilis. MAHESA: Malahayati Health Student Journal, 3(11), 3717–3729. <https://doi.org/10.33024/mahesa.v3i11.11537>
26. Wahyuningsih, N., dan Zulaika, E. (2018). Perbandingan Pertumbuhan Bakteri Selulolitik Pada Media Nutrient Broth dan Carboxy Methyl Cellulose. Jurnal Sains Dan Seni ITS, 7(2), 36–38.
27. Wahyuningsih, S., Auliah, N., dan Salwi, S. (2020). Mouthwash Jus Buah Nanas (Ananas comosus L. Merr) Terhadap Bakteri Streptococcus mutans. Jurnal Kesehatan, 13(2), 171. <https://doi.org/10.24252/kesehatan.v13i2.16423>
28. Wakhidatul Kiromah, N. Z., dan Rahmatulloh, W. (2020). Uji Aktivitas Antibakteri Ekstrak Metanol dan Akuades Daun Ganitri (Elaeocarpus ganitrus Roxb.) Terhadap Bakteri Streptococcus mutans. Acta Pharmaciae Indonesia: Acta Pharm Indo, 8(2), 89. <https://doi.org/10.20884/1.api.2020.8.2.3237>
29. Widyarman, A. S., Erfan, E., Binarta, C. T. O., dan Sunoto, R. I. (2019). Buku Praktikum Mikrobiologi.
30. Wulandari, Kurniati, I., Dermawan, A., dan Nurhayati, D. (2019). Pemanfaatan Tepung Sayuran Sebagai Media Alternatif Pertumbuhan Staphylococcus aureus dan Escherichia coli. Jurnal Riset Kesehatan Poltekkes Depkes Bandung, 11(1), 285–292. <https://doi.org/10.34011/juriskesbdg.v11i1.796>
31. Yevani, F., Yasinta Moi, M., dan Ernaningsih, D. (2023). Daya Antibakteri Ekstrak Etil Asetat Daun Kligong (Crassocephalum Crepidiodes) terhadap Pertumbuhan Bakteri Escherichia Coli dan Staphylococcus Aureus. Jurnal Syntax Admiration, 4(1), 1–16. <https://doi.org/10.46799/jsa.v4i1.544>
32. Zaenap, Syuhriatin, dan Andini, A. S. (2021). Tingkat Pencemaran Air Sungai Oloh Bawi Abian Tubuh Kota Mataram yang disebabkan Limbah Perusahaan Tahu Menggunakan Metode Alt (Angka Lempeng Total). Lombok Journal of Science, 3(3), 17–26.