A Novel Design of Gas System and Develop Culture Medium for the Isolation and Sulfur Reducing Bacteria within Short Time

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Abstract

The current study has developed a proprietary system and a special device for the supply of oxygen-free nitrogen gas with a mixture of carbon dioxide and 80:20% according to the laboratory conditions required for the growth of sulfur-reducing bacteria. To find the best medium that fits the bacteria in those environments with shortening incubation period, the new medium developed is available in the specification of planting rich in energy sources, minerals and vitamins and high reduction ability promotes the early growth of sulfur-reducing bacteria .

Keywords: *Sulfur reducing bacteria, intestinal diseases, colitis, and inflammatory*

Introduction

Worldwide nearly, intestinal and intestinal diseases, colitis, and inflammatory bowel disease (IBD) are a group of chronic diseases that may be genetic, immunological, immunological or bacterial [1,2].

In the 19th century it was believed that the cause of IBD was bacterial and eventually leads to colon disease (CD). At the beginning of the 20th century, there was a link between ulcers Colon (UC) and Bacterial Infection [1].

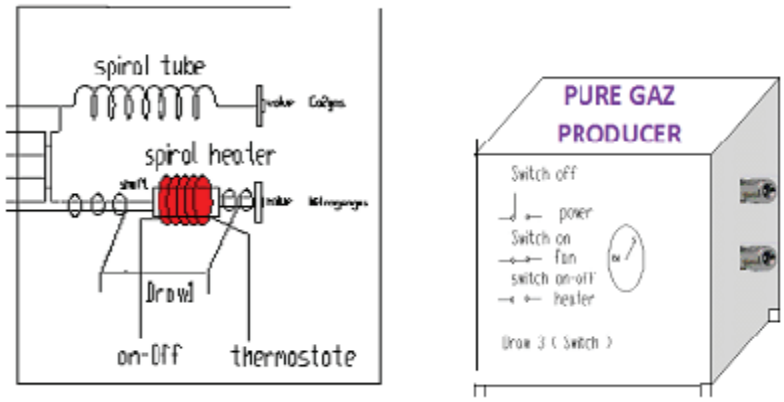
Scientific progress in cultivation-independent technologies and bioinformatics found that patients with colitis and Crohn's disease showed that *Bacteroidetes* and *Lachnospiraceae* were decreasing, while other groups were increasing, such as *Gammaproteobacter* and *Enterobacteriaceae* [3,4]. A significant increase was also observed in sulfate-reducing sulfate- reducing bacteria [5] and due to the activity of the sulfur-reducing bacteria producing the hydrogen sulfide that destroys the normal immune bodies as well as the natural fluorine produced by the vitamins present. Within the mucous membrane and encouraging the increase in the number of harmful bacteria and opportunistic, which causes dysfunction in the lining of the bowel and decrease the amount of mucus, it is called inflammation of the bowel [6].

Since the *Faecalibacterium prausnitzii* was found to be an indicator of the health status of people not infected with IBD because their anti-inflammatory agents secretion [7].

Current study aimed developing isolation diagnosis techniques of pathogenic bacteria and finding the best growth medium for the diagnosis of the laboratory.

Materials and Methods

1 – **Pure gas producer**: The device of the component is made of copper pipes wrapped in several rolls and then a part of a length of 10 centimeters with a cord electric heat around them to provide the required temperature degrees. The heating pipe is larger than the pipe before and after the pipe and are available in copper pipes that work with the help. The heat gained from the electric heater. The gas mixture is then passed through the syringe to a sterile syringe without exposure to contamination. All transplantation, purification and diagnosis are carried out using this method with the addition of the previously mentioned reduced agents. The device is designed to be able to obtain nitrogen gas or carbon dioxide oxide separately or together, as well as electrical control switches and the fan of thermal discharge from within the system manufactured [Figer 1].



Figer (1): The content plan of the gas purification plant

2 - Preparation of converted culture media:

The agricultural communities were used as

As shown in the table (1) below various culture media tested to the best one for faster isolation sulfur reducing bacteria from samples under study

Table (1) various culture media tested for sulfur reducing bacteria

Type of culture media for isolation of sulfur reducing bacteria				
المتركيب الكيميائي Chemical ingredient	Kushkevych, 2013	Postgate&Campbell ,1965	API,1975	Feio,et all.1998
KH2PO4	+	+	+	+
NH4CL	+	+	-	+
Na2so4	+	+	-	+
NaCl	+	+	+	-
C acl2.6H2o	+	+	+	+
MgSo4.H2o	+	+	+	+
Sodium acetate	+	+	-	-
Sodium citrate	+	-	+	+
Casamino-acid	+	-	-	-
Tryptone	+	-	-	-
Thio-glycolic acid	+	-	+	-
FeSo4.7H20	+	-	-	+
Mineral	+	-	-	-
Vitamin	+	+	+	+
Ascorbic acid	+	+	+	-

Cont... **Table (1) various culture media tested for sulfur reducing bacteria**

NH4)2 FeSO4)	+	-	-	-
Cestine	+	-	-	-
Na bicarbonate	+	-	-	-
Yeast extract	–	+	+	+
Ca So4	–	-	-	-
PH	7.5	7-7.5	7-7.2	7-7.5

3- Progression in media by adding one of the following substances per-liter basis and releasing the KM2015 axis: KH_2PO_4 0.3 g, Na_2SO4 0.5 g, K_2HPO_4 0.5g, (NH_4) SO_4 0.2 g, NH_4Cl 1g, $CaCl_2.6H_2O$ 0.06 g , $MgSO_4.7H_2O$ 0.1 g, $C_3H_5O_3Na$ 2ml, Yeast extract 1 g, $FeSO_4.7H_2O$ 0.004 g, Sodium citrate.2 H_2O 0.3 g.

With addition of Mohr's salt solution 1 ml / L, which consisting of the following materials: copper sulphate 0.1 g, boric acid 0.1 g, zinc sulphate 0.1 g, nickel chloride 0.2 g, cobalt 1 g, manganese chloride 1 g, salts of these minerals were present in one liter of distilled water Deodorizer. The following substances were added for each liter: Casamine 2 mg, Vitamin solution 10 ml / l, cestine 3%, Sodium bicarbonate 30 ml from 8%, Sodium thioglycolate 10ml from 1%, NH4) 2Fe (SO4) $2.6H_2O$ 10 ml from 10%, Na_2S $9.H_2O$ 0.05 ml from 1%. [7] . Add 5 grams per liter of acryl to get a semi-solid medium. In the case of steel medium, add 15 grams of salt.

Cultivation of the samples:

Immediately transfer the samples to the refrigerator and store them briefly to speed up the hardening of the food medium. During the incubation period, growth and the observation of developing colonies are observed after the appearance of the colonies that take an alternate shape in the center of growth as shown in Figure 2. The single colony is withdrawn by a pipette Pasteur is transferred to the center of the developer's spindle for the purpose of activation, incubating at a temperature of 37 ° C and for 48 hours. After that, the activated colony was transferred to bottles filled with the center of the steel axis and incubation at the same degree for three days for the purpose of studying their phenotypes and dyeing and conducting biochemical and genetic tests on them. The colonies were isolated in the form with Gram stain and malachite green to separate spore forming isolates from non spore forming in individual colonies.

Results and Discussion

The results show that the best developed medium suitable for bacteria in these environments with shortening the incubation period, the new developed center is available in the form of a rich implantation of energy sources, minerals and vitamins and a high reduction ability promotes the early growth of sulfur reduced bacteria.

Anaerobic conditions preparation machine:

Previous studies have shown the adoption of simplified methods to obtain the gases required for the development of bacteria[8,9] . However, the present study developed a special system for the supply of oxygen-free nitrogen gas with carbon dioxide (80 : 20%), and laboratory conditions required for the growth of sulfur-reducing bacteria. Carbon dioxide and nitrogen are passed from cylinders available in local markets. The device or system enters a copper tube wrapped in several rolls to increase surface area exposed to heat. The copper-assisted copper tubes are available to reduce oxygen and obtain oxygen-free nitrogen gas and facilitate their transfer to the irrigated areas. This is the gateway to the gas supply to the plantations from the other end of the

copper tube. In addition to facilitating the process was of mixing carbon dioxide and required percentage and then passes the mixture of gases to the bottles of the implant by a sterile injection without exposure to pollution, as all the transplantation, purification and diagnosis using this method with the addition of the previously mentioned reduced factors. To ensure the success of the appropriate conditions in the food medium, add drops of blue Rezozarin solution. In the absence of a change in the color of the center towards the blue indicates that the circumstance in the middle is lacking oxygen (conditions of reduction) and vice versa [10]. The system can also be adopted to obtain nitrogen-free gas without mixing with carbon dioxide.

* Development of the growth medium for the growth of the Sulfur reduced bacteria in culture media and the reduction of incubation period :

In the current study, four stem cells were studied, as shown in table (1), and in a preliminary series of experiments to reach the best plant medium for the growth of the sulfur-reducing bacteria in the stomach, intestines and colon environment and shortening the period of the lap, which causes negative effects on human health after vaccination. These circles are characterized by stool, Endoscope and under anaerobic conditions that provide nitrogen gas, carbon dioxide and incubation at a temperature of 37 ° C and for 2 to 3 days. It was found that the center of [12] was the best of the vegetative circles, with the emergence of growth (medium to black color change) as a maximum of three days compared with the rest of the other circles as mean [12,13,14], as shown in Table (2). Preliminary experiments in these circles found that some of them were dedicated to sulfur-reducing bacteria in their natural soil and water environment [11]. No environment was prepared to isolate the bacteria that reduced sulfur from the stomach and intestines and to obtain an intestinal environment closer to the bowel environment. The center was stimulated by carbonic sources such as organic acid [15] and additional energy sources. The incubation period was reduced to 48 hours instead of 72 hours. These results were enhanced by studying bacterial growth density using UV-SPECTROPHOTOMETER, and encouraging the growth of more bacterial species of sulfur-reducing bacteria than other growth-specific species.

Table (2): A better test medium for the development of sulfur-reducing bacteria in stool samples and Endoscope swabs

Culture media	Source of sample	Growth	Number of Healthy	Number of Patient	Incubation time(days)
Kushkevych,2013 (KM2015)developed	Stool Endoscope swab	+ +	15 –	25 10	2-3 2-3
Postgate &Campbell	Stool Endoscope swab	+ +	15 –	25 10	7- 14 7-21
API	Stool Endoscope swab	+ +	–	25 15	7-14 7-21
Feio,et.al.1998	Stool Endoscope swab	+ +	15	25 10	7-21 7-21

The appearance of black color in the growth environment is due to the addition of sulfuric substances to the nutrient medium and their reduction to hydrogen sulfide due to metabolic reactions during the anaerobic respiration process. The reduction of the diastolic condition accelerates the bacterial divisions. [12] This was observed in stool samples and Endoscope scabs under study - Such as sodium thioglycolate and Cystine compounds in the center axis reduced the incubation period to 2-4 days as well as the addition of growth promoters for the same medium of vitamins, minerals of trace minerals and Casamine, and reinforced that assumption as shown in Table (1).

4: Description colony of sulfur reducing bacteria and Cultural Characteristics

The results of the sample implantation on the KM2015 liquid medium showed that the positive growth of the sulfur-reducing bacteria was characterized by homogeneity by the deposition of the iron which exported the sulfite compounds [16]. The members of this group are described as members of the chemo heterotrophic diet. [17] Development and purification of the colonies on the KM2015 semi-steel medium under anaerobic conditions and incubation at a temperature of 37 ° C and for three days, the colonies appear black in the depth of the acre and diameter 1-2 mm, After a week of farm life, it is shrinking, and when exposed to the air, its black color quickly disappears into yellow, meaning cell death [11]. The colonies do not grow except in specialized circles where the optimal conditions for growth are found, in the circles, when developing them in their specialized circles without adding the sources of sulfur to show them growth. These results were reinforced [13], because sulphite compounds are the ultimate future of electrons during aerobic respiration [7]. When the isolates were examined under the microscope, most of them were found to be negative for gram, macrophage, other coliform, staphylococcus and nematode. As for the components of spores a small percentage compared to other groups and groups this is consistent with the novelty of Rey [19]. When examining its ability to produce Catalase, the colonies appeared negative to that test by being a mandatory anaerobic enzyme producing the hydrogenase.

Figure (2) Growth of colonies of sulfur-reducing bacteria on central KM2015 liquid, semi-alkaline and steel

Most of the colonies are produced by hydrogen sulfide gas. This is observed by deposition of iron around the colonies under study and the undesirable odor when opening the implant bottles.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad Alrafidain University College and all experiments were carried out in accordance with approved guidelines.

References

1. Tannock G W. The BowelMicrobiota and Inflammatory Bowel Diseases.International Journal of Inflammation 2010;10:1-10.
2. Sushila R, Dalal, Eugene B. Chang. The microbial basis of inflammatory bowel diseases.The Journal of Clinical Investigation. 2015;124:(10)4190-4196.
3. Gophna U, Sommerfeld K, Gophna S, Doolittle WF, and Veldhuyzen van Zanten SJO. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. J. Clin. Microbiol. 2006;44(11):4136–4141.
4. Frank D N, St Amand A L, Feldman R A, Boedeker E C, Harpaz N, Pace N R. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. 2007 ;104(34):13780–13785 .
5. Roediger W E, Moore J, Babidge W. Colonic sulfide in pathogenesis and treatment of ulcerative colitis. 1997;42(8):1571–1579.
6. Kushkevych I V. Identification of sulfate-reducing bacteria strains of human large intestine. StudiaBiologica • 2013;3:115–132.
7. Sokol H, Seksik P. The intestinal microbiota in inflammatory bowel diseases: time to connect with the host. CurrOpin Gastroenterol. 2010;26(4):327–331.
8. .Zinkevich V, Beech I B. Screening of sulfur reducing bacteria in colonoscopy samples from healthy and colitic human gut mucosa.FEMS Microbiology Ecology . 2000;34:147-155.
9. John A Hardy, W Allan. The oxygen tolerance of sulfate-reducing bacteria isolated from North Sea waters. 1981;(6)5: 259–262.
10. Werner B, Rudolf K. Gregory eikus .Isolation and characterization of Desulfovibrio growing on hydrogen plus sulfate as the sole energy source . Archives of Microbiology, 1978;116(1):41-9 .
11. Ghazy E A, Mohmond M G, Asker M S, Mahmoud M N, Abo Elsoud M M. Cultivation Of Sulfate Reduacing Bacteria (SRB) In Sea Wuter. Journal Of American science, 2011;7(2):604-608.
12. Kushkevych I V. IDENTIFICATION OF SULFATE-REDUCING BACTERIA STRAINS OF HUMAN LARGE INTESTINE. StudiaBiologica • 2013;3:115–132.
13. Postgate J R, Campbell L L. ClassifiCation Of The Sporeforming Sulfate – Reducing Bacteria. Bacteriological Reviews , 1965;29(3):359-363.
14. Ivan K, Peter K, Pavel S, Aleš I. Activity of selected salicylamides against intestinal sulfate-reducing bacteria.Neuro endocrinology letters 36, 2016.
15. Kushkevych I, Bolis M, Bartos M. Model-based Characterization of the Parameters of Dissimilatory Sulfate Reduction Under the Effect of Different Initial Density of DesulfovibriopigerVib-7-bcterial Cells. The Open Microbiology Journal. 2015; 9: 55-69.
16. Beerens H, Romond C. Sulfate-reducing anaerobic bacteria in human feces. The American Journ. of Clin. Nutr,; 1977; 30: 1770–1776.
17. John A Hardy, W Allan. The oxygen tolerance of sulfate-reducing bacteria isolated from North Sea waters. Microbiology, 1981; (6)5:259–262
18. Holt J G, Krieg N R, Sneth P H, Staley J T, Williams S T. Bergeys Manual of determinative Bacteriology. 9th ed. Williams and Wikins. 1994.
19. Rey F E, Mark D G, Jiye C, Meng Wu, Philip P A, Jeffrey I G. Metabolic nich of a prominent sulfate reducing human gut bacterium.PNAS. 2013; 110:13587-13587.