

A Study of the Symbiotic Relationship between Prausnitzn Faecalibacterum and Other Colon Species

Amani Rasheed Mustafa

Assistant Lecturer, Diyala Education Directorate, Ministry of Education

amanirasheed63@gmail.com

Abstract

This study was conducted in Diyala province – Baqubah City in the period between 1/3/2018 to 1/9/2018. It is regarded as the first study in Iraq that includes detection of the symbiotic relationship between beneficial bacteria Faecalibacterum Prausnitzii and between other bacterial species present in the colon. All bacterial species of mucous lining of the colon were isolated by endoscopy and stool analysis. The study included (100) sample (50) of them were in the form of colon tissue biopsy taken from inpatients and outpatients aging from (18-80) years in Baqubah teaching hospital, section of internal medicine. endoscopy department. The other (50) sample were taken from the stool samples taken from inpatients and outpatients aging from (18-80) years in Baqubah teaching hospital, in Baqubah city. After planting the samples in specialized milieu like (*Nutrant Broth, Blood agar, Infusion agar, Macconk agar Brain & Heart ycFA*). When examining the extracts using microscope, it was found that the Faecalibacterum Prausnitzii in question cohabits with other types of bacteria like Escherichia coli, proteus and sulfur reducing bacteria SRB, which appeared in the patients with Inflammatory Bowel Disease IBD. As for the bacteria in question, it scattered most of the time and dominant in healthy people.

Keywords: symbiotic relationship, Faecalibacterum, colon species

Introduction

The importance of the interrelationships between organisms is of preventive importance within the body, which deserves to be examined and studied. In addition to the effective role of the immune system within an organism, there are friendly bacterial aggregates that play a role in reducing the pathological growth of the bacterial body if it invests the body. (Miyamoto-Shinohara et al., 2009)

The F. Prausnitzii constitutes (20.5%) of the bacterial aggregates in healthy adult intestines, whose importance lies in the production of butyrate through its metabolic efficacy and this in turn

represents a protective system for digestive cells of the gastrointestinal tract. (Muhammad Tanweer, et al., 2013).

Although further studies are needed to prove conclusively that the decline in *F.Prausnitzii* level is likely to cause Crohn's disease (CD), colon cancer, obesity, and asthma. Studies have also shown that *F. Prausnitzii* is the only type with anti-inflammatory effects in colon where it limits the growth of pathological bacterial species found in the form of colonies in the human guts.

On the other hand, recent studies have revealed that complex Gut microbes co-exist with a relatively limited number of dominant organisms, including verrucomicrobia, Fusobacteria, and Bacteroidetes, such as the sex of Clostridia Bacteroidetes, where their abundance constitutes (95%) followed by then produced in terms of Faecaliba Cterumo, Bifidobacterium, Alistipes, Rosebria.

(Arumugam et al., 2011).

1. Transplant samples

1.1 Implant of Endoscope samples.

1 mL of intermediate-conductive sample (Tryptic Soy Brooth) was taken into two nine (14) ml glass bottles with CO₂ gas and wax positioning on the seal. The farm contained (13) mL in the middle and completed in sample to (14) mL and placed in the anaerobic CO₂ incubator at 37°C for 24 hours. The specimen-planting milieus was in the middle of the YCFA of the bacteriaf.prunsnin and central Maconky Itzar to prove F.Prausnitzii, Ecoli & Brain Center Heart for *ProTous* and Blood Agar bacteria for *Clostridium*

2.1 Implant of stool samples

The fecal samples were planted according to (Annina Rintala, 2013) method by taking (1) mL of fecal sample and delivering them in sealed plastic bottles containing (9) mL of Physiological Saline solution and then placing the bottles in the centrifuge at a speed of 3000 rpm (15 minutes). Then, neglect the deposit, take 1 mL of the liquid product and plant it on the center of Brain, Blood agar, Maconky agar, YeFA, & Heart prepared in advance and then provide the anaerobic conditions of CO₂ existence and seal the bottles with wax and incubation in a anaerobic incubator at 37°C for 24 hours.

2- Isolation of bacterial aggregates

2-1 Isolation of the **F. Prausnitzii** bacteria

The results of the 50-sample transplant in YCFA milieu were shown to be from the colon smears

of people including 33 male and 17 female (18-80) years old. The male population was 17 patients and 16 healthy individuals; the female population was 12 female patients and 5 were healthy from the outpatients of Baquba public hospital, department of internal medicine, Endoscopy Division, table (1-2). the samples were transported by SoyaBroth, planted on the center of YCFA and incubated with the anaerobic incubator at a temperature of 37°C for 24 hours. The incidence of morbidity among males varied as they were distributed into the following form: 6 people with IBS, 3 people with Crohn's disease (CD), 2 people with Ulcerative Colitis (UC), 5 people with Internal piles (Ip), 1 person with external poly (PO) in the colon. As for female, (12) women were infected with one of the diseases as follows:

3 with undefined colitis, 1 with Crohn's disease (CD), 4 with Ulcerative Colitis (UC), 3 with colon cancer (CA), and one female suffering from external poly (PO) in the colon as in table (1-2)

Table (1-2)

Among the results of transplant samples of the Endoscopy and stool samples of both sexes, ranging from 80 to 18 years of age, the results show the pathological conditions and the number of injuries.

Sample type	Gender	Patients						Total patients	Healthy people	Total
		NSC	CD	UC	IP	PO	Ca			
Samples of Endoscopy	Males	6	3	2	5	1	–	17	16	33 66%
	Females	3	1	4	–	1	3	12	5	17 34%
	Grand total and percentage	9 18%	4 8%	6 12%	5 10%	2 4%	3 6%	29 58%	21 42%	50 100%
	Fecal smear	Males	7	4	1	3	2	–	17	3
	Females	10	6	5	4	1	–	26	4	30

samples	Grand total and percentage	17	10	6 12%	7	3 6%	– 0%	43	7	50 100%
---------	----------------------------------	----	----	----------	---	---------	---------	----	---	------------

The above table (1-2) also showed the results of the 50 transplant on the YCFA from the stool of healthy and sick persons between 80 and 18 years of age, from both sexes in Baquba public hospital, and from the inpatients of the residents of Baquba city, the districts, and its suburbs. The samples were transported in the center of the transporter (Physiological saline solution), after being planted and incubated in the anaerobic incubator at 37°C for 24 hours.

The group of 20 male samples was registered positive, including 17 sick persons and 3 healthy persons. The cases and numbers of the patients varied as follows:

(7) Men with IBS irritable Bowel syndrome and (4) people with Crohn's disease (CD), one person with (UC), and (3) three with internal IP and two with outer colon PO. As for females, 4 of them were physically healthy and 26 were female patients whose cases were distributed according to the following:

10 females with IBS, (6) females with (CD), (5) women with (UC), (4) with internal IP, and only one female suffering from outer colon PO.

2.2 isolation of **Proteus**

The results of the implantation of 20 samples on brain & heart infusion agar of colon smears of (12) ill and (8) healthy people, distributed among 10 males and 10 females, 5 patients and 5 apparently healthy females, the numbers were distributed among 3 females Seven sick women and seven sick women have been distributed as follows:

3 people have IBC, 1 person with (CD), and 1 has with (UC).

The male group, which had shown good condition, had positive implantation results and was found to be suffering slightly from urinary tract infection, while the female group was spread among the following diseases:

2 patients with IBC, 3 with CD, and 2 with UC. The results of the transplant of their samples have been shown to be *Proteus* positive are less likely to be present than the returning farms for the female patients.

Table (2-2)

The results of the transplantation of sample samples taken from the Endoscopy of healthy, sick persons between 80 and 18 for both sexes show positive results for *the presence of Proteus bacteria.*

Gender	Prepare patients based on their satisfactory condition			Total patients	Number of healthy	Grand total
	IBS	CD	UC			
Males	3	1	1	5	5	10
Females	2	3	2	7	3	10
Final total	5	4	3	12	8	20
Percent	25%	20%	15%	60%	40%	100%

3.2 isolating *E.Coli*

In this study, *E.Coli* was isolated from the intestinal lining by fluoroscopy and fecal analysis of samples that were for people of both sexes who had reached (26) ostensibly healthy people and (34) patients who spread male numbers to each of the diseases.

3 suffer from (UC), 2 with (CD), (3) neurocolon syndrome (IBS), 2 external PO in colon, 3 people with (IP), and the ages of both healthy and sick people have varied between (22 and 60) years. As for females, their numbers were distributed as follows:

3 females with UC, 2 with IBS, 1 female with external colon PO.

Table (2-3) showing the results of transplant samples of the Endoscope and positive presence *E.Coli*

Gender	Prepare patients based on their satisfactory condition					Total patients	Number of healthy	Grand total
	US	CD	IBS	PO	IP			

Males	3	2	3	2	3	13	10	23
Females	7	8	4	2	–	21	16	37
Final total	10	10	7	4	3	34	26	60
Percent	17%	17%	12%	7%	5%	57%	43%	100%

2.4 isolation of sulfur-reduced bacteria from the colon binoculars (SRB) **The Isolation of Sulfur Reducing Bacteria**

Farms with growth of sulfur-reductive bacteria have been completely masculine because of the deposit of iron from the sulphide compounds (Romond, Beerens, 1977) the results of the transplant of 20 sample of Endoscope smears for patients arriving at the Baquba Public Hospital (Endoscopy Division) on the liquid medium (KM 2015). (2000, beech, Zinkerich) after incubating the milieus of bacterial samples in anaerobic conditions under (37) °C.

The samples were taken from healthy and ill people with (IP, UC, CD) and at different ages for both sexes.

Table (4-2)

Shows the results of transplant samples taken from colon smears for the presence of bacteria (S.R.B.).

Gender	Transplant positive		Age (year)
	+	–	
Males	0	2	30-18
	1	4	60-31
	1	2	80-61
Females	0	4	30-18
	0	4	60-31
	2	0	80-61
Total	4	16	20
Percent	20%	80%	100%

3- Diagnosis of the Bacterial Aggregates

The Bacterial Aggregates that appeared on the farms were diagnosed according to their shape The apparent degree of its response to Gram stain and according to the approved growth medium and sources Energy, temperature, pH, and its ability to form the species As shown in a table (3-1)

Table (3-1)

Shows the apparent characteristics of the diagnosis of bacterial detoxins in the disease that come to the Baquba General Hospital (Al-Endoscopy Division).

Bacterial sex	Virtual form	The formula as a gram	The middle of growth	Power sources	Temperature	PH degree	Species composition
F.Prausnitzii	Rod CCI	+	YCFA	Propion and stellin	37	7-6.88	-
Proteus	ROD Cocci	-	Braien & heart	Protein	37	7	-
E.Coli	ROD	-	Maconky Agar	East extract	37	7	-
S.R.B	ROD	-	Nutrunt Agar	Carbohydrat	37	7	+

4- Results and discussion

After the transplantation of specimens from the Endoscope implanted in YcFA, the contrast of the colors of the YcFA was observed 24 hours after the injection of the samples with the bacterial samples, where the intensity of the color and turbidity varied.

It was noted that the herd milieus that had been injected with the samples of healthy people amounting (12) appeared in a very white color and high turbidity, which showed that the farm flourished in high numbers of bacculaureate fluoras *F.Prausnitzii*, and the rest of the cannabis (29) were coniums of which were inoculated in samples of persons with inflammatory bowel disease (IBD) its color was closer to light pink and medium turbidity. evidence of low bacterial growth compared to healthy person farms.

The study found that there is a clear relationship between the number of these bacteria and the incidence of colon diseases of various kinds and these findings are somewhat consistent with findings referred to in previous studies such as those found (Louis, et al., 2009). The finding that *the presence of F.Prausnitzii* reduces intestinal inflammation (IBD) in healthy people and that the imbalance between the preventive and harmful types of bacteria is one of the most important

causes of the acute Respiratory Syndrome (IBD).

The remaining three remaining cannabis were from three people with a cancerous mass in the Colon. with no bacterial growth showing that *their F.Prausnitzii bacteria did not exist*, and previous studies found that acute colon infections, if developed, became cancerous growth, caused by the lack of a number of *F.Prausnitzii* (other Khan, 2010). This is clear to the effect that the presence of this type of bacterial type in colon constitutes a protective shield from cancer diseases because it produces the most important metabolic substance (butyrate), the energy source of the colon mucous membranes and the superior ability to combat cancer cells, as it inhibits the histon of tumorous cells. It also prevents DNA synthesis of these cells (Huebner et al., 2006). The results of the current study also agreed with what the researcher (Emmanuelet al., 2013) found that there was a sharp decline in *the F.Prausnitzii bacteria*, accompanied by an increase in acute colonic inflammation and that the shift caused by chemotherapy in the intestine could significantly affect the immune level of the person with cancer.

Table (4-1)

A positive distribution shows that the *F.Prausnitzii has been found in smear samples Colon and feces of both sexes*

Sample type	The total number of samples for males and females	Number of samples by sex Male Female	Positive results of screening by sex Male Female
Binoculars	50	33 17 (66%) (34%)	33 14 (66%) (28)%
Stool samples	50	20 30 40% 60%	20 30 (36%) (64%)
Total	100	53 47 (53%) (47%)	51 46 (51%) (46%)
Sample ratio			97%

The current study, as shown in the table (4-1), found that the positive samples for males and females are 97 of them (51) for males and (40) for females, i.e. 51% and 46% for males (80-18) and for females (70-30), and three of the specimens were found to be age-

old A negative screening result for bacterial non-appearance was recorded at 6%, with women with colon cancer aged 30-64-73, compared their farms with those for healthy people showing significant growth of 97%. As shown in Table 4.1, this indicates the presence of this type of bacteria and its existence constitutes a protective shield against cancerous diseases for the production of the most important substance of the disease (butyrate). The results were largely in line with Emanuel, who saw a sharp fall in *F.Prausnitzii* in return for an increase in the size of the cancerous mass due to the availability of energy, as these bacterial fluorocarcinoma supports high energy and protects against cancer. He pointed out that their lack of colon cancer in the disease is due to chemotherapy that reduces the immune level in the body and kills useful nuts in colon (Emmanuel et al., 2013) (Sokol et al., 2008) these findings are also consistent with the findings of the researcher (Mireia et al., 2012), where she found 13 isolates from *F.Prausnitzii* in the human fecal samples at a very high rate as the isolates were for healthy volunteers

In addition, samples taken from the feces of healthy and sick people were planted in other media (YCFA), and other bacterial races emerged when they were mastered on selective media, showing *Proteus*, *E.Coli* and *S.R.B* sulfur-decomposing bacteria. In comparing the transplant results with those of other researchers who showed when analyzing fecal samples that bacterial communities in corn patients differ from the bacterial communities in healthy feces, this difference is due to the nature of bacterial formation where *F.Prausnitzii* belongs to the female species (Chow et al., 2010) this corresponds to the results of the current study during the analysis of the feces of both the healthy and the sick.

We also note that bacterial diversity has been significantly reduced in patients with CCD. By contrast, the increasing bacterial species of *Clostridium* include *F.Prausnitzii*, whose anti-inflammatory efficacy has been mediated in the membrane. This means that the low number of coats is not resistant to antibiotics found in the colon mucous membranes. (Honda et al., 2012).

The current study also found that *Proteus* bacteria can be found in animal/decomposed substances, soil, human highlight, and wastewater (Guentzel 1996), which is a natural pest in the human intestine (Candela et al., 2010) and its existence is associated with the occurrence of colon infections (Misra et al., 2009).

It is the main agent of up to 90% acquired diseases as it can be isolated in hospital intensive care

lounches, particularly in patients with frequent injuries and people with urinary tract infections (Ahmad, 2008)

The presence of Proteus has also proved to be incompatible with the presence of the bacteria The bacterium F.Prausnitzii coexists with this bacterial type and not The metabolism of F.Prausnitzii is an antiagent of Proteus where it was isolated Of the same samples as patients with F.Prausnitzii bacteria But, in varying degrees, there is a reverse relationship on the rise Decrease in the percentage of bacteria observed a table (4-2)

In Table 3-2, we note that *E.Coli* has been isolated from healthy and sick people and these findings explain what it found (Nester et al., 2004) If a study finds that *E.Coli* is a common cause of stomach and intestinal diseases and that *E.Coli*'s virulence and pathological prescriptions are attributable to its possession of the enzyme aluryriz and proteins (Sharif, et al., 2007)

I found *E.Coli* in ostensibly healthy people who were isolated from the *F.Prausnitzii bacteria*. This conclusion leads us to the fact that it is satisfactory in all cases and that its existence is not an indicator of the disease, and that its disease is not a risk to the digestive system. It is also considered a natural pent in the intestine but is becoming more dangerous as it becomes more severe if it enters the body from an external environment, as it can cause serious injury in the host (Dabbagh, 2000).

The results of the transplant (20) samples of nazure smears of patients who come to the Baquba Educational Hospital (DOC) on the liquid mean (km 2015) axis, (Beech, Zinkevich, 2000) have shown that the incubation of the milieus with bacterial samples is considered to be anaerobic and subseptic conditions (37) degrees Celsius. It was observed that the results were a series of (18) samples for an (SRB) presence at the same time as *F.Prausnitzii bacteria* and two samples showing a positive result for female patients who were aged (65-73) where, after the farm incubation, a black color in the farm showed evidence of reduced sulfur bacteria (SRB). The two patients who showed a positive test result were suffering from colon cancer, and they showed a negative test result in culturing their samples on the medium (YcFA) of *F.prausnitzii*, and this is a clear relationship between the possibility of the absence of bacterial flora in patients with colon cancer.

In terms of the (18) people who did not have SRB bacteria, they showed a positive result for *F.prausnitzii* when planting samples taken from them on YcFA medium and in varying degrees

despite their disease (IP, UC, CD) and this is consistent with what was found Mechanism (Loubinoux and others, 2000) that the presence of sulfur-reducing bacteria is a common characteristic in the digestive system of humans and animals, especially the intestine. Many researchers have strengthened their studies that have reached the relationship between the presence of this bacteria in the digestive tract and between inflammatory colitis (IBD) and ulcerative colitis (US). (Elson, et al., 2000).

Table(2-4)

Demonstrates the relationship between the presence of beneficial bacteria *F. prausnitzii* and the bacterial species in the colon that were under study

Bacterial type	Total implanted samples	Positive for <i>F.praw</i>	Negative for <i>F.praw</i>
Proteus	20	20	0
E.coli	60	60	0
S.R.B	20	18	2

From the above, we conclude that the bacterial groups coexist in the human gastrointestinal tract, especially what belongs to the intestinal family, and that the bacterial type *F.*

Conclusion

1. The bacterial type *Fecalibacterum prausnitzii* is a beneficial species of great importance for the health of the human gastrointestinal tract, especially the colon, and its presence in high levels is a clear evidence of the integrity of the gut.
2. There is a close relationship between the presence of *F.prausnitzii* and the probability of developing IBD, and it is an inverse relationship, as its presence limits the bacterial growth of patients and reduces the increase of the pathological bacterial numbers such as *E.coli*, *Proteus*.
3. Lack of the beneficial bacterium *F.prausnitzii* is associated with colon cancer.
4. The presence of *F.prausnitzii* bacteria is absent with the presence of (S.R.B) (Sulfat redusing bacteria) inside the digestive canal. This is a sufficient reason to increase the risk to the gastrointestinal tract in the absence of this type, which is a protective shield for the digestive

channel.

Arabic Sources:

- 1- Ahmed, Selda Saeed Yassin (2008) Isolation and diagnosis of causes of wound infection and its sensitivity to antibiotics and antiseptics at Kirkuk City General Hospital Master Thesis Tikrit University / College of Education.
- 2- Al-Dabbagh, Ayman Ghanem Amin. (2000). Urinary tract infections in children under five years of age and its relationship to poor nutrition and some vital variables in Nineveh Governorate
□ Master Thesis University of Mosul, College of Science.
- 3- Sharif, Adiba Younes, Omar Ghiyath (2007). Isolation and diagnosis of some intestinal bacteria from cases of diarrhea in children in the city of Mosul and a study of their ability to produce proteinase enzyme, Journal of Research of the College of Basic Education. 179-165: (3) 5.

Foreign sources:

- 1- Anniina Rintala. (2013) Diagnostics and clinical significance of Faecalibacterium prausnitzii University of Jyrakyla
- 2- Arumugam, M., Rase J, pelletier E., Le Paslier, D. T. Yamada, D. R. Mende, G. R. Fernandes, J. Tap, T. Bruls, J- M. Batto, M. Bertalan, N. Borruel, F. Levenez, C. Manichanh, H. B.Nielsen E., Nitelsen, N. Pons, J. Poulain, J. Qin, T. Sicherilz – ponten, S. Tims, D. Torrents, E. Ugarte, E. G. Zoendall, J – Wang, F. Gurner, O. Pedersen, W. M. De vos, S. Brunak, J. Dore, Meta HIT Consortium, J. Weissnbach S. D, Ehrlich and p. Bork (2011). Enterotypes of the human gut microbiome. Nature. 473 :174- 180.
- 3- Beerens, H., Romond, C.(1977). Sulfate – reducing anaerobic bacteria in human feces. The American Journ. Of Clin. Nutr. ;30:1770-1776.
- 4- Cho W.J., Lee, S.M., Shen, Y., Khosravi, A., and. Mazmanian, S.K., (2010). Host – Bacterial Symbiosis in Health and Disease. Adv. Immunol. 107:243-274.
- 5- Elson, C. O. (2000) Commensal bacteria as targets in Crohn's disease Gastroenterology. 119, 254-257.
- 6- Emmanuel, M., Eric., Bi., Se'bastien, M., Thomas, C., Jocelyne, C., Sophie, L.F., Nathalie C., Jean, B.H., Philippe M., Cilles P., Françoise L. V. And Marie F.C. (2013). 16s rRNA Gene Pyrosequencing Reveals Shift in Patient Faecal Microbiota During High –Dose Chemotherapy as Condition in Rejection for Bone Marrow Transplantation. Microb Eco;

67(3) : 690-9.

- 7- Honda K, and Littman. D. R. (2012).The Microbiome in Infectious Disease and Inflammation. *Annu Rev Immunol*.30:759-95>
- 8- Khan, M.T., (2013B).Novel physiological and metabolic insights into the beneficial gut microbe *Faecalibacterium prausnitzii* from carbohydrates to current.
- 9- Loubinoux, J., F. Mory, I.A. C. Pereira, and Faou, A. E. Le. (2000). Bacteremia caused by a strain of *Desulfovibrio* related to the provisionally named *Desulfovibrio fairfieldensis*. *J. Clin. Microbiol.* 38: 931-934
- 10- Louis P, Flint HJ. Diversity. (2009). metabolism and microbial ecology of butyrate – producing bacteria from the human large intestine *FEMS Microbiol Lett*; 294:1-8.
- 11- Mireia, L. S., Tanweer. M. K., Sylvia H.D., Hermie J. M., Jes'us.G., and Harry J. F. (2012). Cultured Representatives of Two Major Phylogroups of Human Colonic *Faecalibacterium Prausnitzii* can Utilize Pectin, Uronic Acids and Host – Derved Substrates for Growth *Applied and Environmental Microbiology* Vol. 78 no. 2:420-428.
- 12- Misra, V.,Misra,S.P., Singh, P. A., Dwivedi, M, Verma, K., Narayan, U. (2009) Significance of cytomorphological and microbiogcal examination of bile collected by endoscopic cannulation of the papilla of vater. *Indian. J. Pathol. Microbiol* 52(3) : 328-331.
- 13- Miyamota – Shinohara Y, Imaizumi T, Sukenobe J, Murakami Y, Kawamura S, Komatus Y. (2000). Survival rate of microbes after freezdrnging and long – term storage *crybiology* 41:251-255
- 14- Nester, E. W., Anderson, D. G., Roberts, C. E.,Pearsall, N. N., Nestor, M. T. (2004). *Microbiology, A human Prespective* 4th ed.
- 15- Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez- humaran L. G, Gratadoux JJ, Blugeon S, Bridonneau CC, FuretJP, Corthier G, Grangette C,Vasquez N, Pochartp, Trugnan G, Thomas G, Blotiere HM, Dore J, Marteau P, Seksik P, Langella P. (2008A). *Faecalibacterium prausnitzii* is an anti – inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patient. *Proc Natl Acad Sci USA*, 105: 16731-16736
- 16- Znikevich, V., and Beech, I. B, (2000). Screening of Sulfur reducing bacteria in colonoscopy samples from healthy and colitic human gut mvcosa. *FEMS Mivrobiology Ecology.* 34: 147-155.