Diyala Journal of Medicine

ORIGINAL RESEARCH
Published: 25 December 2022
Doi: 10.26505/DJM.23026860816

Molecular detection of static magnetic field forces on uropathogenic Escherichia coli number and fermentation activities

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Abstract

Background: The region that a magnetic force has an effect on is known as a magnetic field. Normally, two poles of this field are concentrated. Most magnetic objects are made up of a variety of tiny fields known as domains. There are many different techniques that have been published in the literature for using magnetic energy as a diagnostic tool and for treating illnesses in both humans and animals.

Objective: To examine the influence of varying amounts of static magnetic field on the viability of bacteria and the fermentation of carbohydrates.

Patients and Methods: Locally prepared dipolar static magnetic field of strength 400, 800, 1200 and 1600 Gauss were used in this study measured by Teslometer. Escherichia coli isolated from 75 isolates taken from the patient who had the UPE, 25 were identified as E. coli, and only (12) samples of identified E coli specifically detect changes numbers of bacteria and biochemical test, most of the sugars ferment that have be changed and the polymerase chain reaction was utilized to identify the 16SrRNA gene (PCR).

Results: The inhibitory impact of SMF is attributable, according to transcriptomic analysis, to differentially expressed genes (DEGs) predominantly included in carbon source consumption. The bacterial phenotype in SMF is consistently effectively restored by adding To the cultivation conditions, add glycolate or glyoxylate, and mutants losing glycolate oxidase are no longer susceptible to static magnetic field.

Conclusion: It was concluded that the magnetic field could notably obvious by exposing bacterial cells growth density will decrease and change bacterial biological activity on sugar fermentation and due to mutation.

Keywords: Magnetic field, 16SrRNA gene, *E.coli*, fermentation, PCR

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Received: 16 August 2022
Accepted: 5 October 2022

Introduction

Over a billion years ago, the Earth began to produce a weak static magnetic field (SMF), and known as the geomagnetic field (GMF), with an intensity at the ground of

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about 50 T [1] . There is growing information that GMF can work as signals to aid many microorganisms in adapting to environmental alterations or as energy to sporadically alter metabolic activities [2, 3].

The region that a magnetic force has an effect on is known as a magnetic field. Typically, two poles of this field are its focal points. Typically, north and south are assigned to these poles. The majority of magnetic objects are made up of numerous tiny fields known as domains; therefore these two dimensions are not the only ones a magnetic field can have. Since a century ago, people have been looking for evidence that magnetic fields have any kind of biological impact [4].There is a substantial amount of study on the impact of bio magnetic fields on the growth and development of diverse species, with both favorable and negative results. Powerful magnetic fields have been found to positively affect animal orientation, growth rate, enzyme activity, cellular metabolic, and DNA synthesis [5].

Extra intestinal E. coli causes a variety of disorders in humans, including newborn meningitis, persistent urinary tract infections, septicemia, and hemolytic uremic syndrome. Intestinal diarrheagenic E. coli, which causes diarrhea, may be additionally divided into two categories [6].

Many patients with an *E. coli* infection make a complete recovery, but consequences may be severe and sometimes fatal. Those with compromised immune systems, pregnant women, young children, and elderly persons are more likely to experience these problems [7].This research aims to examine the influence of varying amounts of static magnetic field on the viability of bacteria and the fermentation of carbohydrates.

Patients and Methods

In this investigation, a locally prepared, dipolar static magnetic field used, with strengths of 400, 800, 1200, and 1600 Gauss were prepared and quantify the field's strength by Teslometer at Salahddin University's science college.

Sample collected (75) samples from urinary tract infected patients in Hawler teaching hospital and Raparren hospital for child-Erbil during period from July to October 2022.Exactly (25) of samples E.coli isolated, then identified by using Vitek test method (BioMerieux Company) in Rezgary and Hawler teaching hospital, from which only 12 samples of identified E coli specifically detect changes in biochemical test. Most of the sugars ferment that has been changed and completely was utilized to perform polymerase chain reaction detection of the 16SrRNA gene (PCR).

Bacterial culture media in equal amounts of broth were subjected to the magnetic field for 24 hours. Additionally, the Vitek test of treated E. coli culture media was compared with untreated negative control samples in the bacterial growth subculture, which was checked for bacterial population using spectrophotometer and sugar fermentation with statically analysis by Graph Pad prism v.9 Wilcoxon t test [8].

Identification process using VITEK 2 GN

Utilizing the VITEK 2 method, it was possible to determine the isolates' identities [9]. Using VITEK 2 Dens check equipment (bioMérieux), a suspension of bacteria

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prepared in 0.45% aqueous NaCl was calibrated to a McFarland standard of 0.5.We used the 47 substrate card for biochemical assays for Gram negative bacterial strains Table(2). An unexplained biochemical pattern was compared to the database of reactions for each taxon in order to interpret the results, and a statistical likelihood was calculated. Based on probability estimates, degrees of identification were assigned as Good (89-92), Very Good (93-95), Excellent (96-99), and Acceptable (85-88) [9].

Molecular detection of uropathogenic *E coli*

Preparation of Bacterial DNA

Genomic DNA Kit (Jena Bioscience, Germany) was used to extract the *E. coli* chromosomal DNA from all *E. coli* isolates.

Detection of 16srRNA gene of Uropathogenic E.coli Isolates by PCR

The particular primer pairs of the 16SrRNA gene that were employed to amplify this gene were as follows:

Forward 27F – 5'-AGAGTTTGATCMTGGCTCAG-3'

Reverse 1492R– 5'-CGGTTACCTTGTTACGACTT-3'

16SrRNA gene PCR cycling conditions: initial denaturation at 96°C for four minutes, followed by 35 cycles of denaturation at 94°C for thirty seconds, annealing at 57°C for thirty seconds, extension at 72°C for one minute, and a final extension at 72°C for seven minutes [10].Using agarose-gel electrophoresis, the PCR result was analyzed maximum volume of reactions 25 µL containing 12.5 µl of Master Mix(USA), 8.5 µl PCR water, 1 µL from both of them forward and reverse prime, 2µL of the DNA extraction from positive sample as a template, the tubes were closed mixed by vortex and centrifuged at maximum speed for a few seconds [11].

Statistical Analysis

Statistical analysis was done by using Graph Pad prism v.9 Wilcoxon T test.

Results

E. coli was subjected to various MF forces (400, 800, 1200 and 1600 Gausses). Exposure to these magnetic forces dramatically slowed E. coli cell development, especially after 24 hours of incubation, and significantly reduced the number of cells in the exposed bacteria as compared to the control Table (1).

Table (1): Spectrophotometer is used to figure out how fast *E. coli* grows in each group

Magnetic force	OD 620 nm at 24 hours	Rate of bacterial cell count CFU ($x10^6$/ml)
Control	1.19	6.08
400 G	1.10	3.52
800 G	1.09	3.488
1200 G	1.06	3.392
1600 G	1.01	3.232

Additionally, the magnetic field enhanced the logarithmic phase within the first four to six hours of treatment, but it reduced in comparison to the control after 16-24 hours. With more exposure time and/or induction, the viability declines. The strength of magnetic force must be understood, though. In this investigation, *E. coli* showed the

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greatest reduction in viability employing the strongest magnetic field as compared to growth of unexposed bacteria just after the magnetic field was turned on [12].

In the present study, isolated strains of E coli are SUCCINATE alkalinization (SUCT) Negative. In Table (2) except in sample number (9) but after exposed to different power of magnetic field converted to positive. Also L-LACTATE alkalinization (ILATk) an apparent recognized characteristic to distinguish among samples and after treatment with (400, 800, 1200 and 1600 Gausses) powers of magnetics converted if compared with untreated sample, however, the Tyrosine Arylamidase (TyrA), alpha-galactosidase (AGAL), beta-glucuronidase (BGUR), L-Proline A (ProA), and O/129 Resistance (O129R) fermentation converted after treated to magnetic field if compared to first state.

Table (2): presented the result of sugars fermentation metabolism in *E.coli*

Sample number	bio number	TEST	Mnemonic	Before treatment	After treatment (1) 400 G	After treatment (2)800G	After treatment (3)1200 G	After treatment (4)1600G
1	0405610440004610	L-LACTATE alkalinization	ILATk	-	+	+	+	+
		Tyrosine ARYLAMIDASE	TyrA	-	+	-	-	-
		ALPHA-GALACTOSIDASE	AGAL	-	+	-	+	+
		SUCCINATE alkalinization	SUCT	-	+	+	+	+
2	0405610440006610	L-Proline ARYLAMIDASE	ProA	-	-	+	+	+
		SACCHAROSE/SUCROSE	SAC	-	+	+	+	+
		L-LACTATE alkalinization	ILATk	-	-	+	+	+
		SUCCINATE alkalinization	SUCT	-	-	+	+	+
		Tyrosine ARYLAMIDASE	TyrA	-	-	+	+	+
		ALPHA-GALACTOSIDASE	AGAL	-	-	+	+	+
		BETA-GLUCURONIDASE	BGUR	+	+	-	-	+
3	2405610440004610	L-LACTATE alkalinization	ILATk	-	+	+	+	+
		SUCCINATE alkalinization	SUCT	-	+	+	+	+
		Tyrosine ARYLAMIDASE	TyrA	-	+	+	+	+
		ALPHA-GALACTOSIDASE	AGAL	-	+	+	+	+
4	0405610440006610	L-LACTATE	ILATk	-	+	+	-	+

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		alkalinization						
		SUCCINATE alkalinization	SUCT	-	+	+	-	-
		L-LACTATE alkalinization	ILATk	-	-	+	-	-
5	0405610554526610	L-LACTATE alkalinization	ILATk	+	-	-	-	-
		L-Proline ARYLAMIDASE	ProA	-	-	+	-	-
		SUCCINATE alkalinization	SUCT	-	-	+	+	+
		L-LACTATE alkalinization	ILATk	-	-	+	-	-
7	2401610440424610	O/129 RESISTANCE (comp.vibrio.)	O129R	+	-	-	+	+
		SUCCINATE alkalinization	SUCT	+	-	-	-	+
		ALPHA-GALACTOSIDASE	AGAL	+	-	-	-	+
		FERMENTATION/ GLUCOSE	OFF	-	-	-	-	+
8	0405611550426600	L-LACTATE alkalinization	ILATk	-	+	+	+	+
		O/129 RESISTANCE (comp.vibrio.)	O129R	-	+	+	+	+
		BETA-GLUCURONIDASE	BGUR	+	-	-	-	-
9	0407610540506210	L-LACTATE alkalinization	ILATk	+	-	+	+	+
		O/129 RESISTANCE (comp.vibrio.)	O129R	+	-	-	-	-
		D-MALTOSE	dMAL	+	-	+	+	+
		ORNITHINE DECARBOXYLASE	ODC	+	-	+	+	+
		D-MANNITOL	dMAN	+	-	+	+	+
		D-TREHALOSE	dTRE	+	-	+	+	+
		SUCCINATE alkalinization	SUCT	+	-	+	+	+
		LYSINE DECARBOXYLASE	LDC	+	-	+	+	+
		D-GLUCOSE	dGLU	+	-	+	+	+
		D-MANNOSE	dMNE	+	-	+	+	+
		Tyrosine ARYLAMIDASE	TyrA	+	-	+	+	+
		COUMARATE	CMT	+	-	+	+	+
		FERMENTATION/ GLUCOSE	OFF	+	-	+	+	+
		BETA-	BGUR	-	+	+	+	+

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		GLUCURONIDASE						
		GAMMA-GLUTAMYL-TRANSFERASE	GGT	+	-	+	+	-
		D-SORBITOL	dSOR	+	-	+	+	+
		L-Proline ARYLAMIDASE	ProA	-	-	+	+	+
		ALPHA-GALACTOSIDASE	AGAL	-	-	+	+	+
		PHOSPHATASE	PHOS	-	-	+	-	-
10	0405610540424210	O/129 RESISTANCE (comp.vibrio.)	O129R	+	-	+	+	-
		SUCCINATE alkalinization	SUCT	+	-	+	+	+
		LYSINE DECARBOXYLASE	LDC	+	-	+	+	+
		FERMENTATION/ GLUCOSE	OFF	+	+	+	+	-
11	0405610444124611	ADONITOL	ADO	-	+	+	+	+
		SUCCINATE alkalinization	SUCT	-	+	+	+	+
		Tyrosine ARYLAMIDASE	TyrA	-	+	-	-	+
		ELLMAN	ELLM	+	-	-	-	-
		5-KETO-D-GLUCONATE	5KG	+	-	-	-	-
12	0405610544520252	L-Proline ARYLAMIDASE	ProA	-	+	+	+	+
		L-LACTATE assimilation	ILATa	+	-	+	+	-
		BETA-GLUCURONIDASE	BGUR	-	-	-	+	+

Molecular Findings

The 16srRNAgene in *E. coli* was studied using the Polymerase Chain Reaction found that all specimens included the current gene; however, in group I until VI represent sample1 till sample7 respectively all specimens of *E. coli* contained 16srRNA gene. Also sub groups of samples(0)represent samples of E coli as control without exposed to magnetic field and other sub groups (1,2,3,4)samples of e coli after exposed to different magnetic field(400G,800G,1200G,1600 G.) respectively (positive finding) harbored16srRNAgene and other samples of *E coli* were isolated (positive finding) harbored the current gene.

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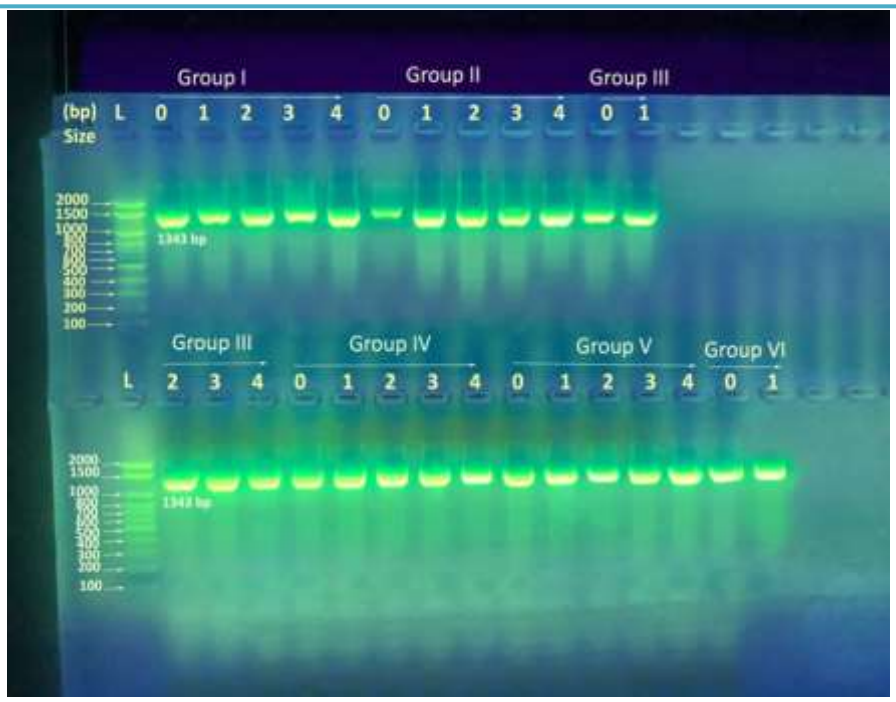

Figure (1): Agarose gel electrophoresis of 16SrRNA gene amplification, L: ladder Group I- VI: positive results

Discussion

The data in Table (2) show that the growth rate has significantly changed for exposure times of 24 hours. These data also show that the lag phase was brief and that the exposure groups' active growth periods were shorter than those of the unexposed cells in all times [13].

It has been studied in bacteria how magnetic fields of varied flux densities affect the viability of germs. These findings show that the physical features of the magnetic signal, notably the wave forces, which were connected to damage to cell membranes, may have a considerable impact on the biological repercussions that magnetic fields have.

It has been documented that exposure to a static magnetic field alters the composition and typical behavior of bacteria. These findings could be very significant for assessing the advantages and risks of direct exposure to the static magnetic field [4].

Pyruvic acid and all other derivatives of mixed-acid fermentation are demonstrated to have chemical components. Fermentative reactions; under hazardous conditions, the TCA cycle, which primarily has anabolic effects, is fully functional. MalEFG (maltose ABC transporter), galP (galactose:H+ symporter), ptsG (fused glucose-specific PTS enzyme: IIB and IIC component), manXYZ (mannose PTS permease), glk (glucokinase), pgi (glucose-6-phosphate isomerase), pfk (6-phosphofructokinase), fba (malate dehydrogenase)[14].

Transcriptomic investigation showed that the down regulated expression of the glcoperon, which is important in central carbon consumption, was closely associated to the poor growth of SMF-treated bacteria. Two additional operons, glp FKX (involved in glycerol breakdown) and ast CADBE (involved in Arginine catabolism), were also shown to be highly down-regulated in the

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SMF-treated bacteria in addition to the glc operon. Since succinct, an intermediary of the TCA cycle, and nitrogen are both provided by the AST(Arginine succinyl transferase) route, nitrogen and carbon starvation function in concert to dynamically control the ast operon's expression [14].

It's significant to note that when Arginine was employed as a nitrogen source, glycerol substantially stimulated the ast operon promoter in continuously expanding cells [14], the glp and ast operons are expressed in a tightly controlled manner. Future research will need to focus on the mechanism through which SMF coordinately regulates the glyoxylate, glycerate, and AST mechanisms [1].

These findings suggest that alternatively expressed genes (DEGs) predominantly engaged in carbon source consumption are responsible for the inhibitory action of SMF [15].

The Polymerase Chain Reaction findings for 16srRNAgene in *E. coli* demonstrated that all samples contained the gene; Moreover, Himi et al., 2015 [16] reported that 54% of E. coli had this gene. 15 of 20 *E. coli* specimens were found to have the 16srRNA gene [16]. The PCR approach was the most accurate way for identifying *E. coli* bacteria by the detection of the16srRNA gene; this technique was employed after conventional methods such as culture, morphological characterization, staining procedure, and biochemical experimenting [17, 18].

Conclusions

It was concluded that the magnetic field could notably obvious by exposing bacterial cells growth density will decrease and change bacterial biological activity on sugar fermentation and due to mutation.

Recommendations

According to the study's findings, It was advised using magnetic force in addition to genetic control to prevent bacteria from multiplying too much because it alters their genes and their ability to respond to different kinds of antibiotics.

Source of funding: The current study was funded by our charges with no any other funding sources elsewhere.

Ethical clearance: Ethical approval was obtained from the College of Medicine / University of Diyala ethical committee for this study.

Conflict of interest: Nil

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Diyala Journal of Medicine

ORIGINAL RESEARCH
Published: 25 December 2022
Doi: 10.26505/DJM.23026860816

الكشف الجزيئي عن قوى المجال المغناطيسي الساكن على عدد الإشريكية القولونية المسببة للأمراض البولية وأنشطة التخمير

امانج جمال عزيز [١] ، د. فؤاد حسين كامل [٢]

الملخص

خلفية الدراسة: عرف المنطقة التي تؤثر فيها القوة المغناطيسية بالمجال المغناطيسي. عادة ، يتركز قطبان من هذا المجال. تتكون معظم الأجسام المغناطيسية من مجموعة متنوعة من المجالات الصغيرة المعروفة باسم المجالات. هناك العديد من التقنيات المختلفة التي تم نشرها في الأدبيات لاستخدام الطاقة المغناطيسية كأداة تشخيصية ولعلاج الأمراض في كل من البشر والحيوانات.

اهداف الدراسة: لدراسة تأثير كميات متفاوتة من المجال المغناطيسي الساكن على حيوية البكتيريا وتخمير الكربوهيدرات.

المرضى والطرائق: مجال مغناطيسي ثنائي القطب محضر محليًا بقوة ٤٠٠ ، ٨٠٠ ، تم استخدام ١٢٠٠ و ١٦٠٠ Gauss في هذه الدراسة التي تم قياسها بواسطة Teslometer. تم عزل *Escherichia coli* من ١٠٠ عزلة مأخوذة من المريض الذي كان مصابًا بـ UPE ، وتم التعرف على ٢٥ عزلة على أنها *E. coli* ، وفقط (١٢) عينة من E coli التي تم تحديدها تكتشف على وجه التحديد التغيرات في الاختبار البيوكيميائي ، ومعظم السكريات التي تخمر والتي تم تغييرها و تم استخدام تفاعل البلمرة المتسلسل(PCR) لتحديد جين١٦ SrRNA .

النتائج: يُعزى التأثير المثبط لـ SMF ، وفقًا لتحليل الترانسكريبتوميك ، إلى الجينات المعبر عنها تفاضليًا (DEGs) المدرجة في الغالب في استهلاك مصدر الكربون. يتم استعادة النمط الظاهري للبكتيريا في SMF بشكل فعال باستمرار عن طريق إضافة إلى ظروف الزراعة ، وإضافة الجليكولات أو الجليوكسيلات ، ولم تعد الطفرات التي تفقد أوكسيديز الجليكولات عرضة للحقل المغناطيسي الثابت. تُظهر هذه النتائج أن السمات الفيزيائية للإشارة المغناطيسية ، ولا سيما قوى الموجة ، التي كانت مرتبطة بتلف أغشية الخلايا ، قد يكون لها تأثير كبير على التداعيات البيولوجية التي تحدثها المجالات المغناطيسية.واستهلاك مصدر الكربون هي المسؤولة عن العمل المثبط . وطريقة تفاعل البوليميراز المتسلسل هي الطريقة الأكثر دقة لتحديد بكتيريا الإشريكية القولونية من خلال الكشف عن جين.

الاستنتاجات: أن المجال المغناطيسي يمكن أن يكون واضحًا بشكل ملحوظ من خلال تعريض كثافة نمو الخلايا البكتيرية سيقلل ويغير النشاط البيولوجي البكتيري على تخمير السكر وبسبب الطفرة.

الكلمات المفتاحية: المجال المغناطيسي ، ١٦ SrRNA ، الإشريكية القولونية ، التخمير ، تفاعل البوليميراز المتسلسل.

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تاريخ استلام البحث: ١٦ آب ٢٠٢٢

تاريخ قبول البحث: ٥ تشرين الاول ٢٠٢٢

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