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Biocontrol of Clinical Bacteria Infecting Urinogenital System by Probiotic Bacteria

T.I. El-Sayed, Soheir S. Abdel Salam[#], G. Enan[*], A. A. Shahin[**] and H. M. Ahmed

Botany Department, Faculty of Science, Benha University, Benha; [*]Botany Department, Faculty of Science, Zagazig University and [**]Microbiology Department, Faculty of Medicine, Zagazig University, El-Sharkia, Egypt.

BACTERIA are considered the dominant microorganisms, which cause urinary tract infection (UTI) of both male and female. The development of multi-drug resistance phenomenon in bacteria leads to the necessity of finding an alternative remedy. Probiotics are suggested as a suitable and appropriate solution. In this study, cell free supernatant CFS of *Enterococcus faeciumNM$_2$* is examined for its inhibitory effect against clinical urogenital bacterial strains and *Candida albicans* isolated from urinary tract infection infections. CFS obtained from these probiotic bacteriashowed a promising antibacterial and anticandidial results. *E. faeciumNM$_2$* possessed capability to be used as a probiotic against medical bacteria isolated from urogenital infections.

Keywords: Urogenital infections, Probiotics, Multi-drug resistance, Inhibitory effect.

Probiotics are beneficial that have provided health benefits when consumed. They are used to suppress carcinogenesis, reduce cholesterol level of blood serum, prevent bacteria-associated diarrhea, inhibit adhesion of pathogenic bacteria to epithelial tissues of human systems, and were also used to improve innate and acquired immunity, gastric function(Abdel-Shafi *et al.,* 2014; Agerholm-Larsen *et al*., 2000 and Turgis *et al.,* 2013).

An inflammatory syndrome caused by microorganism's invasion of the urinary tract. The microorganism's infection can affect a variety of patients ranging from healthy women to men and children according to their immunity. Most uncomplicated and complicated cases can be treated easily by antimicrobial agents and demand longer treatment periods in more complicated (Harry *et al*., 1996).

[#]Corresponding Author :
Affiliation: Benha University, Faculty of Science, Botany Department, Egypt
Address: 65, 13th Street, Elzohor District, Zagazig City, El-Sharkia Governorate, Egypt
Phone number: +2/01067140872
E-mail: dr.soheir_saad@yahoo.com

One or more structures in the urinary tract become infected after bacteria overcome its strong natural defenses, most cases of UT's bacterial infection that can multiply at the opening of the urethra and migrate to the bladder (known as the ascending route) (Harry *et al*., 1996 and Jackson, 2006). (Al-Sharif & Al-Run, 2008) reported that UTI is a wide definition that includes different clinical manifestations such as:

a) Asymptomatic bacteriuria: Bacteriuria with no symptoms.
b) Uncomplicated urinary tract infection: Infection with a normal, unobstructed genitourinary tract with least instrumentation.
c) Complicated urinary tract infection: Infection in men or pregnant women patient with presence of foreign body (urinary catheter, stone).
d) Relapse: Recurrence of bacteriuria with the same microorganism and implies failure to eradicate infection within seven days of therapy.
e) Reinfection: Recurrence of bacteriuria with a new microorganism, 80% of recurrent infections are due to reinfection. It is difficult to differentiate from relapse when infection occurs with the same species.
f) Cystitis: Infection of the bladder and inflammatory syndrome with dysuria, urgency, and supra-pubic tenderness.
g) Pyelonephritis: Acute or chronic bacterial infection of kidney characterized by flank pain, tenderness and fever.
h) Urethritis: Lower urinary tract inflammation with or without bacterial infection.
i) Prostatitis: Bacterial infection, inflammation and pain affecting on the prostate gland.
j) Probiotics (derived from Latin and Greek) means "for life" is defined according to FAO/WHO as: Live microorganisms had health benefit on the host.

Some *Lactobacillus* sp. and *Enterococcus faecium* have ability to prevent the attachment of some pathogenic bacteria to the intestinal mucosa, that's why, they are used as probiotics. *Enterococci* can resist the growth of pathogenic bacteria since they have the ability to lower the pH and produce organic acids especially, lactic acid and hydrogen peroxide, in addition to their capability to produce inhibitory substances called bacteriocins (Enan *et al.*, 2015). The intention of probiotics uses to control and inhibit the urogenital bacteria due to the resistance of many bacteria to antimicrobial agents and the development of the phenomenon of multidrug resistance bacteria (MDR bacteria).

This work aimed to preventing some clinical bacteria infecting the urinogenital system by the probiotic *Enterococcus faecium* and their cell free supernatant (CFS).

Materials and Methods

Bacterial strains and culture media

The probiotic bacterium *Enterococcus faecium* NM_2 was kindly provided by Prof. Dr. Gamal Enan, Faculty of Science, and Zagazig University. It was propagated and subculture on De Man, Rogosa and Sharpe (MRS, Oxoid) medium. It produced inhibitory activity as described by Enan *et al.,* (2015). The inhibitory substances were due to acids and bacteriocins which designated as Enterocin.

The sensitive microorganisms used were isolated from urogenital patients admitted to Fakous General Hospital, Al-Sharkia, Egypt. From both males and females at different ages,four isolates were obtained and designated *P. aeruginosa* (1U), *S.aureus* (2U), *K. pneumonia* (4U) and *E. coli* (6U). Another four bacterial isolates of urogenital origin were kindly provided by Prof. Dr. Ahmed Anwar Shahin from Faculty of Medicine, AL- Qasr Al- Aini. They were also designated, *Shigella* (31U), *C. albicans* (32U), *Salmonella paratyphiB* (33U) and *Proteus vulgaris* (34U).

Preparation of cell free supernatant (CFS)

Enterococcus faecium NM_2, the producer of inhibitory substances, as grown in De Man, Rogosa and Sharpe broth for 16h at 37°C. CFS was obtained by centrifuging the culture (10.000 x g for 15 min at 4 °C) then the supernatant was collected.

Inhibition test of microbial pathogens

Erlenmeyer flasks of 250 ml, each containing 100 ml BHI broth, were inoculated by 2.0x 10^4 CFU/ml of *E. faecium* NM_2 and about 2.0x 10^4 CFU/ml of each bacterial pathogen used. In another experiment MRS broth inoculated by *E. faecium* NM_2 (by 2.0x 10^4 CFU/ml) was inoculated also by about by 2.0x 10^4 CFU/ml of the C. albicans. Another series of 250 ml Erlenmeyer flasks, each containing 100 ml Brain Heart Infusion (BHI) broth, were inoculated either by 2.0x 10^4 CFU/ml of each bacterial pathogen or by 2.0x 10^4 CFU/ml of *C. albicans* used; and was treated by 1% v/v of CFS. The inoculated and treated Erlenmeyer flasks containing both test samples and controls were incubated at 37°C for 3 days (bacterial samples) or 6 days (Candidal test). After suitable time intervals 1 ml aliquots were removed, serially diluted and plated on to agar plates containing the selective and specific agar media for enumeration of each microbe used (Oxoid). These specific agar media were inoculated by sample dilutions and incubated at 37°C for 48h. Then CFU/ml was obtained as described previously for similar experimental conditions (Enan, 2000 and Enan *et al*., 2000).

Results and Discussion

It was essential to test the sensitivity of uropathogens to different types of antibiotics. Results are given in Table 1.

TABLE 1. Antibiotic susceptibility profile of selected clinical bacterial isolates.

Bacterial isolates	Tested antibiotics												
	IPM	**AK**	**CRO**	**RF**	**CTX**	**AZM**	**FEP**	**CFP**	**CN**	**AMC**	**E**	**OFX**	**LEV**
1U	R	R	R	R	R	R	R	R	R	R	R	R	R
2U	S	I	I	R	R	R	R	R	R	R	R	R	R
4U	S	I	R	R	R	R	R	R	R	R	R	R	R
6U	S	R	R	R	R	R	R	R	R	R	R	R	R
31U	S	I	R	R	R	R	R	R	S	R	R	R	R
32U	R	R	R	R	R	R	R	R	R	R	R	R	R
33U	S	R	S	I	R	R	R	R	R	R	R	R	R
34U	R	S	I	R	R	R	R	R	S	I	R	R	R

It was shown that all the bacterial isolates except *P. vulgaris* (isolate no 34U) were nearly resistant to all antibiotics excerpt for Imipenem (IPM) while *P. vulgaris* was sensitive for Gentamicin (CN) and Amikacin (AK). Table 1 illustrates that *C. albicans* (isolate no 32U) is completely resistant to all antibacterial agents. These were interesting results to evaluate bacterial isolates inhibition of urogenital system by the probiotic bacterium *E. faecium* NM_2.

The evaluation study of antibacterial activity CFS of *E. faecium* NM_2 against the 8 urogenital pathogens are showed in Fig. 1-8. Urogenital bacterial pathogens alone in control experiments grew vigorously and viable cells was 4-5 log cycles increase within 96 h in other samples which treated with CFS of *Enterococcus faecium* NM_2 thoroughly with urogenital pathogens, urogenital pathogens growth was decreased by 2 log cycles within 12hr and 48 or 72 h in case of *S. aureus* (isolate 2U), growth declined to $9.5x10^2$ within 12h by 2 log cycles while *E. coli* (isolate 6U), growth declined to $7.5x10^2$ by 2 log cycles within 96 h of incubation.

The growth of *p. aeruginosa* (isolate 1U) treated with NM_2 declined to $6.6x10^2$ by 2 log cycles within 48h. *K. pneumonia* (isolate 4U) treated with NM_2 decreased by 2log cycles to $8.1x\ 10^2$ within 72h. In case of Shigella (isolate 31U) and *P. vulgaris* (isolate 34U), growth decreased by 2 log cycles within 72h, while in *S. paratyphi* B (isolate 33U), viable cells decreased by 2 log cycles within 48h to $3.8x10^2$*C. albicans* (isolate 32U) which proved to be resistant to all antibacterial agents decreased by 2 log cycles to $6.1x\ 10^2$ within 72h. Results are illustrated in the following Fig. (1-8).

Urinary tract infection is infectious diseases most of people during the span of their lifetime (Hoberman & Wald, 1997). The type of infection can cause serious consequences if remains untreated (Pezzlo, 1988). Although several different microorganisms are involved in causing urinary tract infection, including fungi and viruses, the role of bacteria in causing these infections can't be ignored (Bonadio *et al*., 2001).

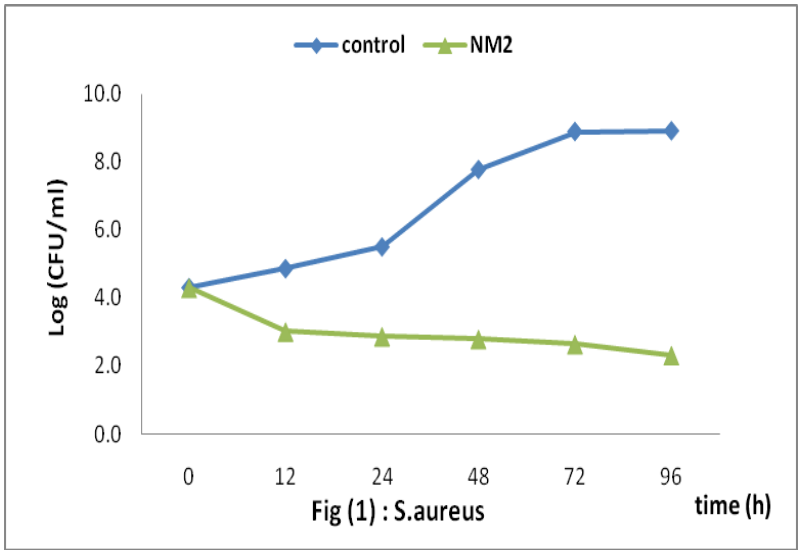

Fig.1. Inhibition of *S.aureus* (code 2U) by CFS *E.faecium* NM_2 .

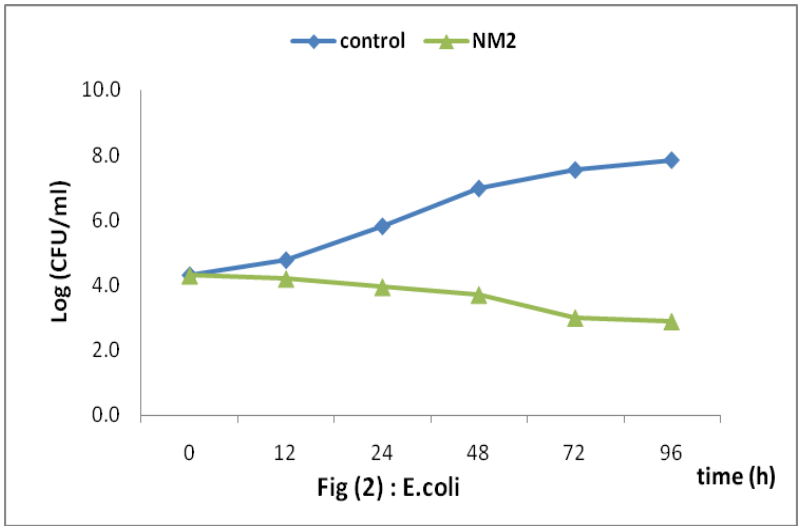

Fig.2. Inhibition of *E.coli* (code 6U) by CFS of *E.faecium* NM_2.

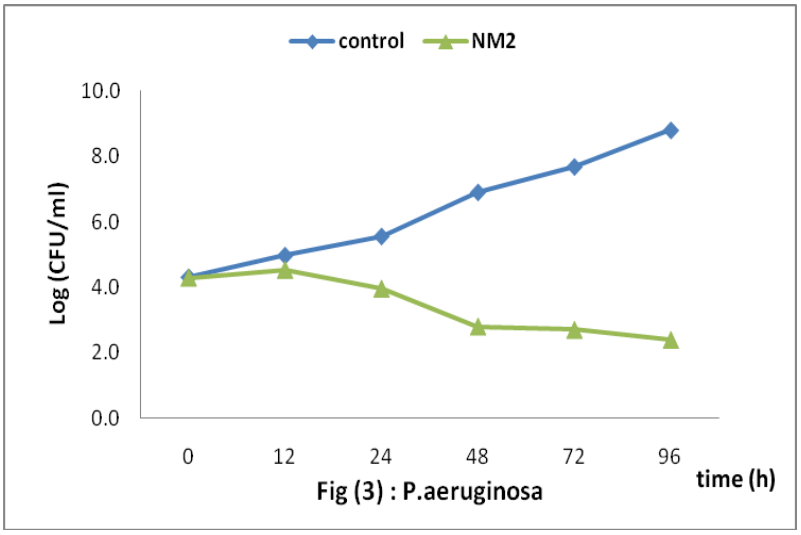

Fig. 3. Inhibition of *P. aeruginosa* (code 1U) by CFS of *E.faecium* NM_2.

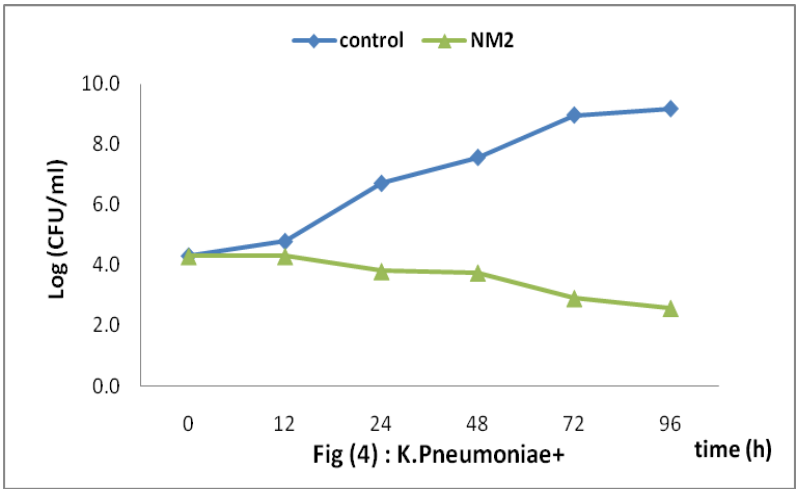

Fig. 4. Inhibition of *K. pneumonia* (code 4U) by CFS of *E.faecium* NM_2.

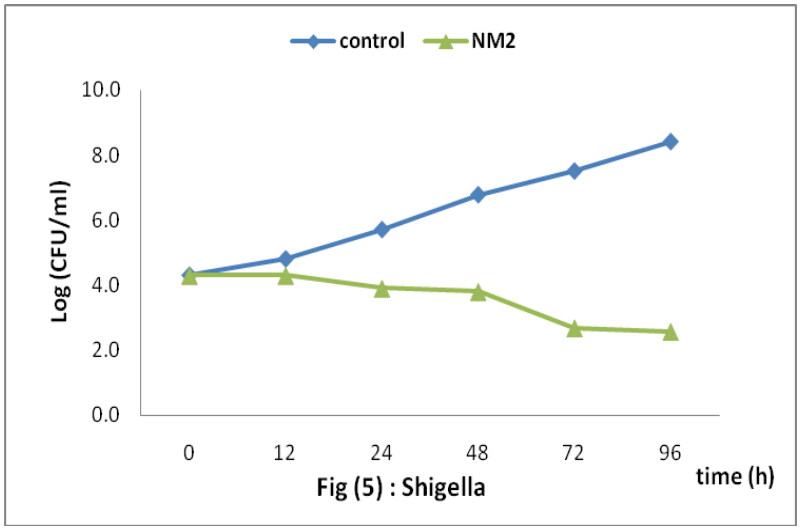

Fig. 5. Inhibition of *Shigella* (code 31U) by CFS *E.faecium* NM_2.

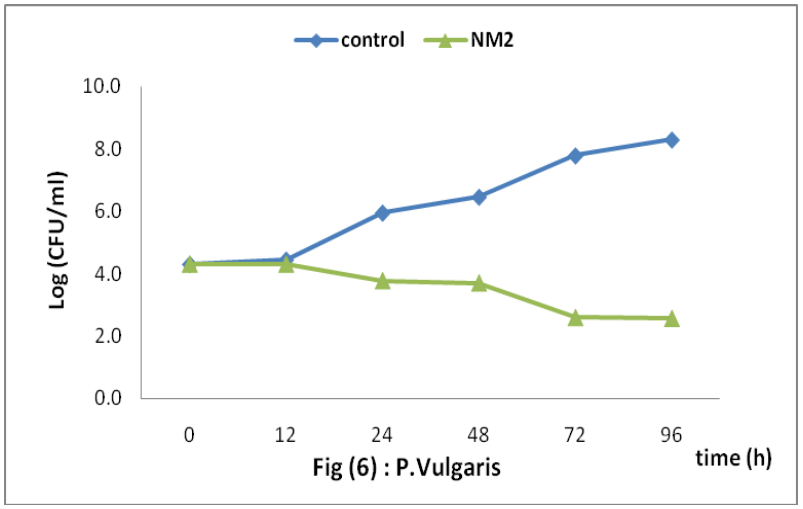

Fig. 6. Inhibition of *P. vulgaris* (code 34U) by CFS of *E.faecium* NM_2.

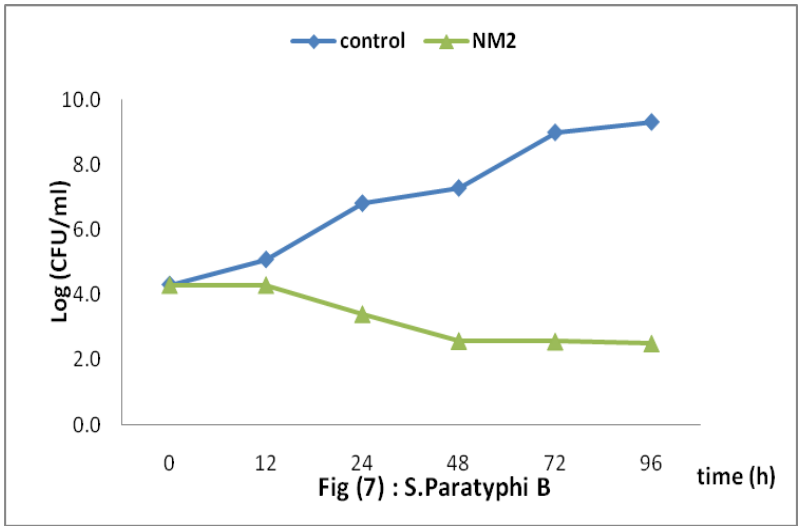

Fig. 7. Inhibition of *S. paratyphi B* (code 33U) by CFS of *E.faecium* NM_2.

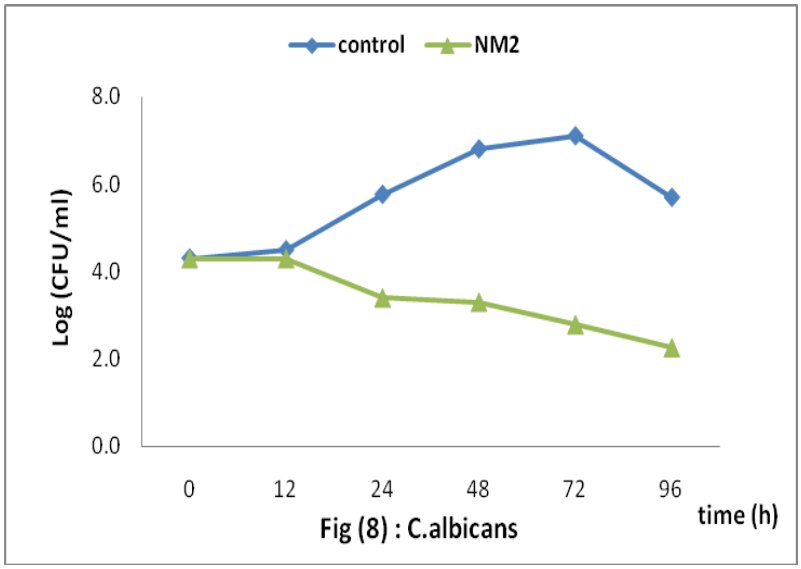

Fig . 8. Inhibition of *C. albicans* (code 32U) by CFS of *E.faecium* NM_2 .

Treatment of urinary tract infection patients is often started empirically. Therapy is based on doing of urine culture to recognize the pathogen responsible for such injury and its susceptibility to different antibiotic agents. However, because of the evolving antibiotic resistance phenomenon, an urgent necessity to face such problem (Lim *et al*., 2009).

Enterococcus faecium is a lactic acid bacterium that had antibacterial effect *in vitro*, It's a gram positive bacterium that has good microbiological characteristics such as short generation time and bacteriocin production (Lewenstein *et al.,* 1979) and also has effect in gastrointestinal disorders (Salminen & Deighton, 1992).

Enterococcus faecium NM_2 isolated from urine of healthy people decreased the growth of urogenital pathogen used in this study qualitatively. This was proven in other studies due to the action of bacteriocins. Hence, *E. faecium* NM_2 may be used as an appropriate probiotic (Enan *et al*., 2015) and can reduce pH to pH 3.8 after 20h. (Zakaria, 2013).

Certain characters should be available for any bacterial organism to be used as probiotic adjuvant such as production of inhibitory acids and proteins, production of H_2O_2, adherence to genital surfaces and good growth and survival in presence of antimycotic and antibacterial agents. *E. faecium* NM_2 possess most of these criteria; so, it used as probiotic to prevent the urogenital pathogens (Ronnqvist *et al*., 2005).

Gram positive and negative bacteria are inhibited by the production of antimicrobial substances, such as organic acids, hydrogen peroxide , reuterin and bacteriocins (Silva *et al*., 1987; Vandenbergh, 1993; Meurman *et al*., 1995 and O'Hara *et al.,* 2007). CFS has antimicrobial substances may induce an antagonistic action to pathogens (Balcazar *et al*., 2007). The accumulation of such metabolites such as short chain fatty acids can reduce the pH which can inhibit the growth of clinical pathogens.

Cell free supernatant has an antibacterial activity against urinary tract infection pathogens as it contains organic acids that decrease the pH where uropathogens can't survive (Sawa *et al*., 2009 and Enan *et al*., 2014). These acids may acidify cytoplasm of susceptibility bacteria leading to collapsing proton gradient which causes bacterial death (Tharmaraj & Shah, 2009). Because of *E. faecium* NM_2 probiotic characters showed previously and so, in our work, this organism could be used as probiotic bacteria (Enan *et al.,* 2015).

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المكافحه الحيويه للبكتيريا السريريه التى تصيب الجهاز البولى التناسلى بواسطة بكتيريا البروبيوتك

طلعت إبراهيم السيد، سهير سعد عبدالسلام ،جمال عبد العزيزعنان*، أحمد انور شاهينوهيثم محمد أحمد**

قسم النبات - كلية العلوم- جامعة بنها – بنها ، * قسم النبات- كلية العلوم- جامعة الزقازيق و**قسم الميكروبيولوجي- كلية الطب- جامعة الزقازيق- الزقازيق– مصر.

يعتبر التهاب المسالك البولية (UTI) التي تسببها البكتيريا واحدة من الإصابات الأكثر شيوعا التي تصيب كلا من الذكور والإناث. تطوير ظاهرة المقاومة المتعدده للعقاقير في البكتيريا يؤدي إلى ضرورة إيجاد بديل. اقترح البروبيوتيك حلا مناسبا وملائما. في هذه الدراسة،حيث يتم اختبار المستخلص الخلوي الحر(CFS) من Enterococcus faeciumNM2 للتأثير المثبط ضد سلالات البكتيرية السريرية للجهاز البولي التناسلي وCandida albicans المعزولة عن التهابات المسالك البولية(UTI) .المستخلص الخلوى CFS من بكتيريا البروبيوتيك أظهر نتائج واعده مضادة للبكتيريا والكانديدات . faeciumNM2 . E يمتلك القدرة ضد البكتيريا السريرية المعزولة من الالتهابات التناسلية.