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The inhibitory activity of *L. crispatus* against uropathogenes *in vitro*

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ABSTRACT: Bacterial interference refers to the use of bacteria of virulence to compete with and protect against colonization and infection by disease causing organisms. In this study, *L. crispatus* strain was used to detect its antibacterial activity towered five species (one Gram-positive and four Gram-negative) of the most common bacteria causing urinary tract infection. *L. crispatus* completely inhibited growth of *Staphylococcus aureus* , while it had no inhibitory effect on three of the other species.

Key Words: inhibitory activity, L. crispatus, uropathogens

I. INTRODUCTION

The microflora of the lower genital tract of healthy women is of interest because of its potentialas a reservoir for infections both of the normally sterile upper genital tract and of the neonate during delivery (Ison, 1990). The increase in estrogen at the onset of puberty cause a thickening of the vaginal epithelium with a concomitant deposition of glycogen, lactobacilli are thought to metabolize glycogen and produce large amount of lactic acid (Ison, 1990; Forbes *et al*., 2007). The resultant low pH would, therefore, select for acid tolerant microorganisms, predominately lactobacilli, and protect the vagina from colonization by pathogens (Boris and Barbés, 2000).

The prevalence of lactobacilli has been reported to be between 45 and 96% (Ison, 1990). Normal flora appears dominated by one or two species of *Lactobacillus* (Lamont *et al*., 2011). Studies showed that the most frequent *Lactobacillus* species are *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*(Hyman *et al*., 2005; Verhelst*et al*., 2005; Dong-hui*et al*., 2009).

L. crispatus is one of *Lactobacillus* species that have been used as probiotic agents (Darouiche and Hull, 2012), as it has several antimicrobial mechanisms, one of the most prevalent mechanism among its strains is the production of hydrogen peroxide (Song *et al*., 1999). Therefore, this species has been used in the treatment of certain conditions, such as urinary tract infections (Stapleton *et al*., 2011) and bacterial vaginosis (Ngugi*et al*., 2011).

The concept of bacterial interference embodies the use of bacteria of virulence to compete with and protect against colonization and infection by disease causing organisms. Passive bacterial interference is achieved when naturally present commensal bacteria help defend against host infection by pathogenic organisms, while active bacterial interference can be useful by administering into the human body bacteria of low virulence that could replace pathogens (Darouiche and Hull, 2012).

II. MATERIAL AND METHODS

The capacity of *L. crispatus* to inhibit the growth of five bacterial uropathogens species was studied in solid media according to the method described by Osset*et al*. (2001). Five uropathogens were isolated from patients with urinary tract infection, these were; *Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*and *Staphylococcus aureus*.The Bacterial isolates were inoculated in brain-heart infusion broth and incubated for 24 hrs. at 37°C. Gram-negative bacilli were studied in aerobic atmosphere, and Gram-positive cocci, i.e. *S. aureus*,was studied in 5% CO_2.

Eleven isolates of *Lactobacillus* recovered from women without bacterial vaginosis were selected to be diagnosed to species level using *L. crispatus* primer by RT-PCR method (a previous study).

L. crispatus was grown in MRS broth (Himedia) at 37°C with 5% CO_2 for 48 hrs. The assay was performed with the agar overlay method, where lactobacilli (2.5×10^6 cfu) were added to about 14 ml of Columbia agar (Oxford) with 5% yeast extract (Himedia) and were allowed to set in a Petri dish. Later about 7 ml of the same medium without lactobacilli was poured over the first layer and after it was solidified it was incubated at 37°C in 5% CO_2. Twenty four hours later, Gram-negative bacilli (1×10^4cfu) or Gram-positive cocci (4×10^4cfu) were inoculated onto the top layer.

After 24 hrs.of incubation, scores from 0 to 2 were assigned to the results, where 0 for no inhibition; 1 for partial inhibition; and 2 for complete inhibition of growth.

III. RESULTS

Results showed the ability of *L. crispatus* to completely inhibit growth of *S. aureus* and to partially inhibit growth of *P. aeruginosa*, while there was no inhibition in the growth of other tested organisms i.e., *E.coli*, *K.pneumoniae*, and *P. mirabilis*

Table (1). Inhibition of the growth of uropathogens by *Lactobacillus crispatus*

Uropathogen	Score of inhibition
Escherichia coli	0
Klebsiellapneumoniae	0
Pseudomonas aeruginosa	1
Proteus mirabilis	0
Staphylococcus aureus	2

Inhibition scores: 0, no inhibition;
1, partial inhibition;
2, complete inhibition

IV. DISCUSSION

Previous studies have showed the ability of *L. crispatus* to inhibit *S. aureus* and *P. aeruginosa* (Ocaña*et al.*, 1999; Osset*et al.*, 2001; Aslim and Kilic, 2006), in addition to other urogenital tract pathogens such as *E. coli*, *Enterococcus spp*., *G. vaginalis*, *K. pneumoniae*, *N. gonorrhoeae*, and *Staphylococcus saprophyticus* (Aslim and Kilic, 2006; Muench*et al.*, 2009; Ngugi*et al.*, 2011).

Urogenital cells are covered by dense bacterial biofilms whose composition constantly changes in which lactobacilli predominate, uropathogenic organisms emerge from the intestine and come into contact with these biofilms on vaginal and urethral cells (Marelli*et al.*, 2004). A different mode of action, control of the pathogens, applies to *Lactobacillus* species which replace virulent vaginal flora that could migrate from the vagina to the bladder and cause symptomatic UTI in women (Darouche and Hull, 2012).

There is a variation among studies in the recorded capacity of *L. crispatus* strains to interfere with urogenital pathogens. In our study, the failure of *L. crispatus* to inhibit the growth of most tested pathogens might be attributed to one or more of the following reasons: first, in studies conducted *in vitro* there were differences among lactobacilli strains in the antimicrobial mechanisms that they have, e.g.; lactic acid production, bacteriocins, and/or hydrogen peroxide production (Corr*et al.*, 2007; Martin and Suarez, 2010, O'Hanlon *et al.*, 2011). Even though there are differences within the same defense mechanism possessed by different lactobacilli strains.

Second, although the hydrogen peroxide production has been considered as an important antimicrobial component contributing to the colonization resistance provided by lactobacilli (Martin and Suarez, 2010), and although there seems to be a link between H_2O_2 producing lactobacilli and normal vaginal flora (Antonio *et al.*, 1999), some studies do not support this role for H_2O_2 ,*in vitro*, as for other mechanisms such as lactic acid and low pH (O'Hanlon *et al.*, 2010; O'Hanlon *et al.*, 2011), since the vagina tends toward low level oxygen tension thus lactobacilli would be expected to favor lactate rather than H_2O_2 as the major metabolic end product *in vivo*.

Third, *in vivo* the matter is definitely different where mechanisms such as H_2O_2 and lactic acid production may not be important as others like blockage of pathogens adherence to vaginal epithelial cells, which represent the first step in pathogens establishment. It was found that *L. crispatus* strains had higher exclusion activity than *L. jensenii*; 61.9 vs. 49.5%; (Osset*et al.*, 2001).

Fourth, differences in the procedures used for detection of interference ability, e.g.; type of inhibition assay, kind of cultivation media, type of lactobacilli inocula (whole cells, cell wall fragments, or culture filtrate), and size of lactobacilli and pathogens inocula. For example, Osset*et al.* have found that *Lactobacillus* showed greater inhibitory power in liquid medium rather than solid medium (Osset*et al.*, 2001).

Choice of *Lactobacillus* strains for probiotic regime require further investigations regarding the effective mechanism that they utilized *in vivo* against urogenital pathogens. In addition, *in vivo* studies has showed that a cocktail of normal flora was better than a single strain at colonizing the vagina and interfering with uropathogens (Reid and Bruce, 2001).

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