

# Evaluation of Antimicrobial Activity of *Saccharomyces boulardii* against *Clostridium difficile* In Vitro

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## Abstract

*Clostridium difficile* infection (CDI) has become a significant threat to public health. Although broad-spectrum antibiotic therapy is the primary treatment option for CDI, its use has evident limitations. Probiotics have been proved to be effective in the treatment of CDI and are a promising therapeutic option for CDI. In this study, strain of *Saccharomyces boulardii* was evaluated for their anti-*C.difficile* activity. The antibacterial activity of the standard isolated yeasts (biocodex, France) against pathogenic bacteria *C. difficile in vitro* was determined by Well's Diffusion method, the isolated yeast showed antibacterial activity against tested bacterium, the highest diameter of inhibition zone was (11 mm) . The study aimed to use the yeast *Saccharomyces boulardii* as probiotic for the prevention and treatment of diarrhea and colitis associated with antibiotics .These results may provide a basis for alternative therapies for the treatment of *C. difficile*-associated gut disorders.

**Keyword :** *C.difficile, Saccharomyces boulardii , in-vitro.*

## Introduction

*Clostridium difficile* (*C. difficile*) infections (CDI) are a global clinical concern and are one of the leading causes of nosocomial outbreaks (McFarland , 2015) .CDI is dramatically increasing in both the prevalence and clinical severity as a cause of antibiotic- and hospital-associated diarrhea in worldwide (Darkoh *et al.*,2015 ; Surawicz ,2015). Cases have been more severe with more complications, deaths, and higher healthcare-associated costs with the emergence of a hypervirulent strain of *C. difficile* and the increasing prevalence of community-acquired CDI among healthy patients without traditional risk factors, the epidemiology of *C. difficile* has been evolving (Vindigni and Surawicz ,2015).

Studies of CDI prevention and probiotics have been largely limited to CDI being evaluated as a secondary outcome of AAD studies, leading to

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under powerment for CDI outcomes (Johnson *et al.*, 2012) . Probiotics are effective in preventing AAD and may also be a beneficial strategy for the treatment of CDI .( McFarland , 2015).

By the World Health Organization, probiotic is defined as “live microorganisms, when administered in adequate amounts, confer a health benefit on the host(Hoffman *et al.*,2008; Oldfield and Johnson,2014). Probiotics containing one or several living beneficial microbes have been a addition to antibiotic therapy, and the benefits of probiotics are multiple mechanisms of action on pathogens, benefits to host immune system, survival to host colon, no drug interaction, and low risk to the patient and safe. ( Kelesidis and Pothoulakis,2012; Goldingberg,2013).

*Saccharomyces boulardii* has been found to support vancomycin therapy (McFarland ,2005). The mechanism by which *S. boulardii* inhibits toxin binding has been determined to be the production of a protease, which digests the toxin receptor in gut mucosal cells (McFarland ,2006). The best studied probiotic agents are *Saccharomyces boulardii* and *Lactobacillus*. Several studies showed that the mixtures of probiotics can be useful in the treatment and prevention .( McFarland ,2010).

*S. boulardii* shows promise for *C. difficile* infection control by several levels: produces a 54-kDa serine protease that directly degrades toxins and also directly destroys the colonic receptor site ( Pothoulakis ,2010) increases the immune response(Buts , 2009) in different animal models respond to this yeast . The *S. boulardii* possesses many properties that make it a potential probiotic survives transit through the GI tract, it's growth temperature optimum is 37°C both *in vitro* and *in vivo*, it inhibits the growth of a number of microbial pathogens ( AL-Samarraie,2013;AL-Zubaidy and Khidhr, 2014).

Since 1989,patients with recurrent *C. difficile* diarrhea treated with *S. boulardii* showed improvement.( Surawicz *et al.*,1989;Buts *et al.* , 1993; Hassett *et al.*,1995; Popoola *et al.*,2000; Surawicz and Macfarland ,2000) and found to be effective for primary prevention of CDI and recurrences(McFarland,2015; Mehdi and Al-Mossawei,2016 ),by effective combination of probiotic and antibiotics( McFarland,2010).

### Materials and Methods

#### *C.difficile* isolate:

*C. difficile* was isolation and identification from stool samples was collected from hospital in Baghdad previously by :1-selective media(CCFA) Cycloseren-Cefoxitin-Fructose Agar (Sigma Aldrich,UK) . 2-Gram stain ,Malachite green for spore,Api20A Kit(BioMerieux,USA). 3-

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detection of two toxins A&B in stool samples by ELISA Kit (primer toxin A&B from Meridian Bioscience ,USA),according to (Mehdi and Al-Mossawei,2015)<sup>a</sup>.Yeast Strain *Saccharomyces boulardii* from company Biocodex(France).

### Antimicrobial activity of *S.boulardii* against *C.difficile* .(in vitro)

The standard isolated yeasts( biocodex, France) were inoculated in Yeast Extract Glucose peptone (YEGP) (Forbes *et al.*, 2007;AL-Samarraie,2013), and incubated at 37°C for( 18-24) hr, 50ml of the same medium was inoculated by 5ml of yeast suspension and incubated at 37°C for (18-24) hr, three different concentration of yeast ( $1 \times 10^7$ ,  $1 \times 10^8$ ,  $1 \times 10^9$ ) CFU/ml were used to determine the best concentration . *C.difficile* isolate showed that it was resistant to many antibiotics (Mehdi and Al-Mossawei,2015)<sup>b</sup> and cultured in nutrient broth at 37°C under anaerobic incubation for (18-24) hrs, Plates were inoculated by 0.1 ml of *C.difficile*, left to dry for 15 minutes at 37°C, (3-4) wells in each plate (with diameter 5mm) were done using Cork Borer and filled with 0.1ml of yeast suspension , the plates were incubated for (18-24) hrs under anaerobic at 37°C then the inhibition zones were measured (Izgu *et al.*, 1997).

### Results and Discussion

The antibacterial activity of the yeasts against pathogenic bacteria *in vitro* was determined by Well's Diffusion method. yeasts showed antibacterial activity against selected local *C.difficile* isolate that given resistance to many antibiotics used in sensitivity tested , the higher diameter of inhibition zone was (11 mm) at concentration of yeast  $1 \times 10^8$  CFU/ml and  $1 \times 10^9$  CFU/ml ,while lower inhibition zone at concentration of yeast  $1 \times 10^7$  CFU/ml was (9mm),(Qamar *et al.*,2001; Mehdi and Al-Mossawei,2016)<sup>c</sup>.The previous study showed that *S. boulardii* has ability to inhibit bacteria that causes diarrhea by release of certain antimicrobial metabolites,( Neelayadatchi *et al.*,2012; AL-Zubaidy *et al.*,2014 ).

Previous studies (Castagliuolo *et al.*,1999) illustrated it secretes a 54-kDa protease, that had been degraded toxins A and B secreted from *C. difficile* and inhibit their binding to receptors along the brush border, leading to a reduction in the enterotoxic and cytotoxic effects of *C. difficile*. (Johnson *et al.*, 2012).

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### تقييم استخدام خميرة *Saccharomyces boulardii* كمضاد

### لبكتريا *Clostridium difficile* خارج الجسم الحي

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الخلاصة :

أصبحت عدوى المطثية العسيرة *Clostridium difficile* خطرا كبيرا على الصحة العامة, على الرغم من أن العلاج بالمضادات الحيوية واسعة الطيف هو خيار العلاج الرئيسي ، واستخدامه له حدود واضحة, فقد أثبتت المعززات الحيوية فعاليتها في العلاج. في هذه الدراسة تم تقييم النشاط المضاد للبكتيريا لسلالة قياسية من خميرة (*boulardii*(biocodex,France) *S.* في مكافحة *C.difficile* خارج الجسم الحي وبطريقة Well's Diffusion method. وأظهرت الخميرة قابليتها لتنشيط نمو البكتريا المرضية ,حيث بلغ قطر منطقة التنشيط (11 ملم) . وهدفت الدراسة إلى تقييم استخدام خميرة *S. boulardii* كمعزز حيوي لمنع وعلاج الإسهال والتهاب القولون المرتبط بالمضادات الحيوية, و قد توفر هذه النتائج أساسا لعلاجات بديلة لعلاج اضطرابات الأمعاء .

الكلمات المفتاحية : المطثية العسيرة , الخميرة , خارج الجسم الحي .