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EXTRACTION OF BIOSURFACTANT FROM *ENTEROCOCCUS* SPP. AND EFFECT ON *CANDIDA* SPP.

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Abstract

The aim of this study was determination the antimicrobial activity of biosurfactant that produced from *Entercoccus* spp. against some medical fungus such as *Candida spp*.80 isolates of *Enterococcus faecium* and *E. faecal* were collected from 120 stool and raw milk samples from healthy patients and cow and buffalo and 10 Isolates from skin, vagina and ear swaps samples to *Candida* spp. during the period 2/1/2019 to 30/4/2019. Inhibition of isolates *Candida glabrata, C. albicans* and *C. parapsilosis*. by Biosurfactant that produced from *Enterococcus* spp. Results revealed that the three *Candida* spp. were sensitive and will be inhibition by BS in several MIC concentration and the concentration 200 is the most effective agents against the isolates when we use several MIC concentration (200, 20, 2, 0.2, 0.02). On the other hand, the results showed that all *Candida* spp. isolates were have the same affected in all Biosurfactant concentration when Next day culture the dilution on muellar agar to observe the effect of these concentration on growth fungus.

Key word: Biosurfactant, *Enterococcus* spp., *Candida* spp.

Introduction

Enterococci are normal commensals in the gastrointestinal tract, oral cavity, vagina etc. They are organisms of low virulence, but are known to cause various clinical infections. *Enterococcus* is considered as an important nososcomial pathogen because of its intrinsic as well as acquired antibiotic resistance (Franz *et al.,* 1999) and (Domig *et al.,* 2003). The increasing importance of *Enterococcus* is largely due to their resistance to many antimicrobials particularly intrinsically resistant *Enterococci*, which is the cause of changing pattern of Enterococcal infection resulting in treatment failures. Some bacterial species belonging to genera such as *Serratia, Arthrobacter, Nocardia, Lactobacillus, Rhodococcus, Thiobacillus* (Jett *et al.,* 1994). Biosurfactants are surface-active biomolecules produced by microbes (bacteria, fungi and yeast) and have several advantages over the chemical surfactants, such as lower toxicity, higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity under extreme conditions such as temperature, pH and salinity. Almost all the surfactants now available in the market are chemically synthesized. Recently, attention toward the biosurfactants was doubled, which is mainly due to their wide range of functional properties and the diverse synthetic capabilities of the microbes (Fracchia *et al.,* 2010). Microbial biosurfactants are found to have a wide range of applications in environmental protection, which include enhancing oil recovery, controlling oil spills, biodegradation, the increased interest in the biosurfactant producing lactobacilli is related to the well-known probiotic effects of these microorganisms which are natural components of human microbiota (Sharma *et al.,* 2014). Biosurfactants produced by microorganisms have potential applications in pharmaceutical/ medicine, food, cosmetic, pesticide, oil and biodegradation industries as well as to the ability of such bacteria to inhibit pathogenic bacteria and fungi (Cornea *et al.,* 2014).

Prevention and control of spread of multi drug resistant Candidias infections in the hospital require a coordinated effort between the various departments and this can only be achieved by educating the hospital staff, vigilant use of antimicrobials, early detection and reporting by laboratories and immediate implementation of

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appropriate infection control measures. In this study we focus on *Enterococcus* spp. Like *E. faecium* and *E. faecalis* that producing biosurfactant and their ability to inhibition of fungus growth such as *Candida glabrata, C. albicans* and *C. parapsilosis*.

Materials and Methods

Specimens were processed as follows:

80 specimens were collected include 40 stool from healthy child and 30 milk from cow and buffalo and 10 from skin, vagina and ear swap from healthy patient (Koneman *et al*., 2006) and (Koneman *et al.,* 1997). Stool will cultured immediately in tetrathionate broth media to disposal normal flora in stool and then after over night will be cultured on Blood agar and Milk will be cultured in 9 ML of brain heart infusion broth with 1 ml of milk then incubated in incubator for 24 h in 37^{0}C storage until it use and *Candida* will cultured and after 24 h in 27^{0}C it will tested by Microscope then it will stored to will be used to tested later (Forbes *et al.,* 2007).

-after 24 h the culture of all specimens will be tested by API test and Vitek 2 system. Specimens were collected according to the standard recommended methods (Collee and Marr, 1996) and (Ross, 2006).

Culture

Specimens were inoculated onto blood agar and Bile esculine agar (As their procedures) and then the plates were incubated over night at 37^{0}C. After that the inoculated plates were examined for *Enterococci* growth (Koneman *et al.,* 1997) as follows:

Screening of isolates for biosurfactant production

All the pure isolates were subjected to screening for their ability to produce biosurfactants That will be determined by disc diffusion method.

Biosurfactant activity assay

Three methods were used to assay the biosurfactants produced by the isolates, which are the emulsification index, oil spreading, and drop collapse methods as described by (Kosaric, 2001). Isolates were grown in MRS media. The culture was incubated for seven days at 37°C with regular shaking. After the incubation period, the broth of each isolate was centrifuged at 6000 rpm for 10 min and the supernatants were filtered to obtain cell-free supernatants. The supernatants were used for the assay.

Oil spreading method

Oil spreading technique was carried out according to the method described by (Maneerat and Phetrong, 2007). 40 mL of distilled water was added to Petri dishes followed by the addition of 100 µL of oil on the surface of the water. Then, one drop of the supernatant was put on the oil surface. The diameter of the clear zone on oil surface was measured using a meter rule. The time taken to achieve the spread was also noted.

Determination of emulsification index

4 ml sample of the cell free supernatant was taken in a small test tube along with 4 ml of oil. To ensure homogenous mixing of both the lipids, the mixture was vortexed at high speed for 2 minutes and incubated at room for 24h. The emulsification index (E24) was calculated by dividing the measured height of the emulsification layer by the mixtures total height and multiplying by 100. (Sharma *et al.,* 2014).

$$E24 = \frac{height\ of\ the\ emulsion\ layer}{total\ height\ of\ the\ mixture} \times 100$$

Drop collapse test

25µl of biosurfactant was pipetted as a droplet onto a parafilm, the spreading of the droplet on the parafilm was observed. Similarly 25µl of water was pipetted on a parafilm and the changes were observed. The biosurfactant spreads and get flattened whereas water stays intact without any change. (Tabmourespour *et al.,* 2011).

Biosurfactant production

Culture of 20 ml MRS broth was prepared with *Enterococcus* and incubated for 24 h at 28^{0}C is static condition . after 24 h of incubation the 20 ml broth starter culture was inoculated into 1000 ml broth and incubated at 28^{0}C for 24 h. After incubation, the cells were removed by centrifugation at 8000 rpm for 30 min the supernant was collected the supernant after centerifuge was collected and kept at 4^{0}C overnight.

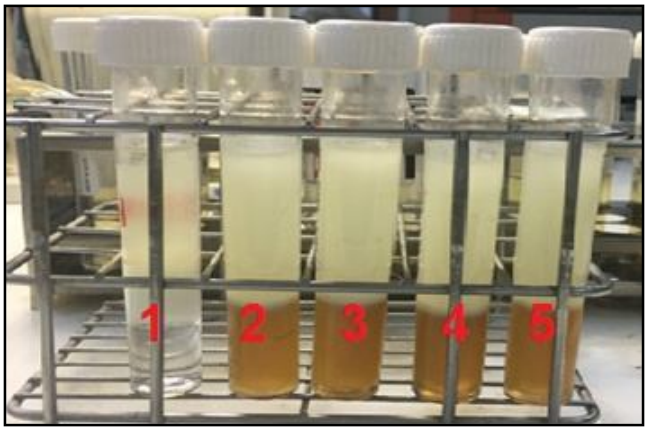

Fig. 1: Detection of biosurfactant from *Enterococcus* spp. by emulsification index assay (1)-Oil with DW as control and (2, 3, 4, 5)- Oil with biosurfactant.

Results and Discussion

Total of 80 train of *Enterococcus* and *Candida* were isolated from a total of 120 clinical specimens processed. (40 are *Enterococcus faecium*, 30 *Enterococcus faecalis*, 10 were *Candida* spp. Such as *C. parapsilosis, C. glabrata* and *C. albicanus*, this isolated were obtained from stool, Throat swap, raw milk, vagina, skin and ear swap.

This present study was conducted to check the antimicrobial activity of biosurfactant produced by *Enterococcus* against pathogenic such as *Candida* spp. (*C. parapsilosis*, *C. glabrata and C. albicans*) the antimicrobial activity of BS were measure it ability to kill or reduce the growth of fungus *Candida* spp. Before that it was compared it activity against other harmful bacteria in ancient studies by forming different concentration 0.1, 0.5, 1, 1.5% the zone of inhibition was observed for BS confirming their antimicrobial activity against harmful bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli*. The microscopic examination after 6 hours of BS treatment should that the number harmful bacteria decreased as the concentration of BS increased. (Sharma *et al.,* 2014).

The *Candida* resistant to most of antibiotics *Candida* is a predominant species found in human body and case infection in some location of body. *Enterococcus* spp. bacteria have ability to produce antimicrobial agents. The use of BS is a biologically derived active and product over the chemical surfactant. The results of the BS inhibition assays suggest that the BS produce has on inhibitory effect on *Candida* forming (Sanglard and Odds, 2002) and (Pfaller *et al*., 2001).

Candida **inhibition assay**

-Over night culture of three *Candida* spp. was inoculate in to five microfuge tube containing 5ml of Saparoid broth.

-work different concentration of Bs (200, 20, 2, 0.2, 0.02) that prepared from the original concentration 1000 of BS then reserved and use next day.

-Work several dilution of *Candida* spp. (6 tubes for each *Candida* spp.) 0.5 McFarland leave one for control. Then inoculate on saparoid broth.

-Inoculation 10 µl of this dilution that have *Candida* in to 1ml concentration of Biosurfactant

-Incubation all of these tubes that have the fungus and BS in 37^{0}C in 24 h.

-Next day culture the dilution on muellar agar to observe the effect of these concentration on growth fungus.

-Result must only be read when there is sufficient growth of the test organism (no growth in un-inoculated or negative growth and when a purity plate shows that the test organism was pure. The amount of growth in each tube is compared with that in the positive growth control and the MIC recorded as the lowest concentration of the agent that completely inhibits growth. (Plaza *et al*., 2006) and (Zakaria 2013).

Inhibitory activity of biosurfactant produced from *Enterococcus* spp. against *Candida parapsilosis* That

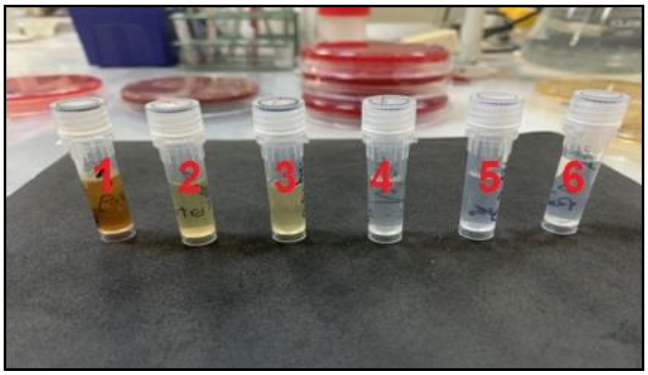

Fig. 2: Dilution of biosurfactant produced from *Enterococcus* spp. by emulsification index assay (1)- dilution of *Candida parapsilosis* without BS as control and (2, 3, 4, 5, 6)- dilution of *Candida parapsilosis* with different concentration of biosurfactant.

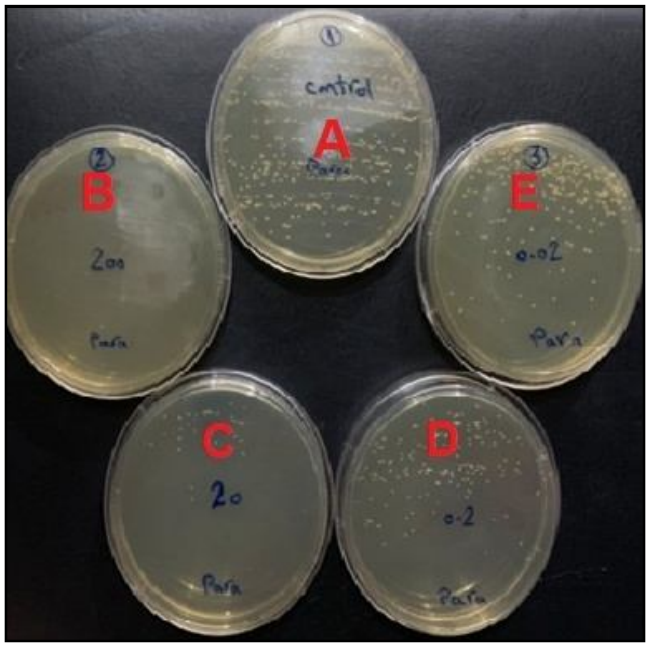

Fig. 3: We will see the number of colony of *Candida spp.* that formed in each plate and compare the result to see which one the concentration of BS that consider MIC to inhibition the growth of *Candida* spp. (A- Coltrol, B- Concentration 200, C- Concentration 20, D- Concentration 0.2, E- Concentration 0.02).

cultured on Mueller agar.

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