



THE INHIBITION EFFECT OF ACIDS AND ALKALIS ON THE GROWTH OF MICROORGANISMS

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ABSTRACT

The over growth with TB culture slopes was a large problem that covers the mycobacterium growth in the culture and can be contaminated. This study is carried out to isolate and identify some of bacteria that overgrowth with mycobacterium on LJ slopes as well as to evaluate the efficacy of the oxalic acid and sodium hydroxide on the inhibition of this bacterial growth. A total of 107 samples of rectal swabs were collected during the period from March 2016 to March 2017. The contaminant bacteria grow on LJ media was recognized by usual method. The result shows that the bacterial contaminant was isolated from 107 samples of rectal swabs of pigeon. The result of the bacterial contaminant which was taken from LJ slopes showed that 9 samples were positive (*E. coli*, *Pasturellaspp*, *Klebsiellaspp*, *Bacillus spp*). Oxalic acid 5% and NaOH4% as decontaminant were used on these bacteria to demonstrate their inhibition effect on the growth. The result showed that these decontaminant reduced the growth of these bacteria except *Bacillus spp*.

KEYWORDS: TB, LJ, bacterial growth, efficacy, oxalic acid, sodium hydroxide.

INTRODUCTION

The contamination of TB cultures is a problem that could allow the overgrowth of another bacterium to a potentially mask the presence of Mycobacterium (Lumb *et al.*, 2006). The pH is the main factor which influences the bacterial growth. Most bacteria are developing well at pH values of 7 (Jay *et al.*, 2005). The decreasing of pH considered an inhibitor factor, which can inhibit the bacterial growth (Dubal *et al.*, 2004). Acids as oxalic acid or acetic acid are considered as growth inhibitors. Oxalic acid described as a solid and colorless crystalline and its organic compound is $C_2H_2O_4$. Typically, oxalic acid exist as a dihydrate ($C_2H_2O_4 \cdot 2H_2O$). Acetic acid has a bactericidal action resulted from the decreasing of pH in cell of bacteria which lead to inhibit of the *E. coli* population (Malicki *et al.*, 2004; Mufeed, 2013). Acetic acid has antibacterial efficiency because of decreasing substrate pH, intracellular inhibition of the pH is caused by ionization of the undissociated acid molecular permeability cell membrane alteration (Ikawa, 1995) (IFT, 1990) (Mufeed, 2013). Acetic acid and oxalic acid has more toxicity effect due to the pH change (Leon *et al.*, 1993). Alkalies such as Sodium hydroxide is widely used to clean, sanitize and store special media and systems because of its efficacy, low cost, easy of detection, removal, and disposal. The use of diverse concentrations of sodium hydroxide and hydrochloric acid, and measure the size of the particular inhibition zones in samples of *E. coli* bacteria was done to estimate the effect of these substances (Bjornsdottir, 2006). This study was aimed to identify such organisms that over growth on LJ media and cover mycobacterium growth and evaluate some acids and

alkalies capability to reduce these contaminant microorganisms growth.

E. coli is coliform bacterium of the genus *Escherichia*, a gram-negative, rod-shaped, non-capsulated, non-spore forming, facultative anaerobic, that can growth on ordinary media. It's a normal flora in intestine (Russell and Jarvis, 2001). *Pasteurella* is a genus of Gram-negative, facultative aerobic bacteria (Kuhnert and Christensen, 2008). *Pasteurella* species are nonmotile and pleomorphic, and often display bipolarity instaining (appearance as "safety pin"). Many *Pasteurella* species are zoonotic pathogens, its infect human and animals. In cattle, sheep, and birds, *Pasteurella* species ia the causative agent of pneumonia; in dogs and cats, however, *Pasteurella* cannot cause disease, *Pasteurella haemolytica* is a species that infects mostly horses and cattle. *P. multocida* is the mainly common causative agent in human *Pasteurella* infection (Barron, 1996). *Klebsiella pneumoniae* is a member of the *Klebsiella* genus of Enterobacteriaceae and belongs to the normal flora of the human mouth and intestine of the pathogenic *Klebsiella* species, *K. pneumoniae* is the most prevalent and clinically important. Infections with *K. pneumoniae* are usually hospital-acquired and occur primarily in patients with impaired host defenses (Fang, 2007). The main organic acid in Fructus mume extract is a citric acid which plays a major role in the inhibition of *K. pneumoniae* growth and CPS production. *Bacillus* is a genus of gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes. *Bacillus* species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic). Under stressful

environmental conditions, the bacteria can produce oval endo-spores that are not true 'spores', but to which the bacteria can reduce themselves and remain in a dormant state for very long periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of the Firmicutes (Madigan, 2005).

MATERIALS & METHODS

Bacterial strains: all strains of bacteria were isolated from previous research that contaminated Low neistian-Jenson media for isolation of mycobacterium from fecal samples of pigeon during the period from March 2016 to March 2017.

Chemicals

1-Preparing of oxalic acid 5% by dissolving 5g of oxalic acid in 100ml of distal water .

2-Preparing NaOH 4% by dissolving 4g of NaOH in 100ml distal water.

Culture media

Preparing MacConky agar, Eosin Methylen blue agar, Blood agar and Nutrient agar.

All isolated bacteria were activated by subculturing on these media, then harvesting by Pbs, the harvesting bacteria divided to two tubes and centrifugation at 3000 rpm. The supernatant was discarded while the pellet in each tube treated with oxalic acid 5% and NaOH 4% the first pellet tube treated with 5% oxalic acid for 15 min after that centrifugation was made and supernatant was discarded then washing with pbs three times. After washing the pellet, it cultured on specific media to each bacteria. The same procedure was done with these bacteria that treated with NaOH 4%.

RESULTS & DISCUSSION

The result of our study showed that bacterial contaminant which isolated from LJ slopes were (*E. coli*, *Pasturella*, *Klepsiallaspp*, *Bacillus* spp). Therefore the effect of oxalic acid 5% on these bacteria were positive to inhibit of these growth except bacillus and with use of NaOH 4% also have obvious effect to slow down growth of these bacteria except bacillus spp have negative result. Most bacterial growth was at pH 7.00 (Jay *et al.*, 2005). Decrease of pH is very important to restrain the bacterial growth (Dubal *et al.*, 2004). It mean that the direct bactericidal activity of acidic acid or oxalic acid resulted from decrease of pH within bacterial cell due to decrease of *E. coli* population (Maliki *et al.*, 2004). These results were agreement with Anang *et al.* (2006) who found the oxalic acid effect for reducing growth count organisms isolated from one raw chicken. When treated with oxalic acid 5%. Also May and Shafeek (2011) revealed that using of citric acid for 30 minutes lowering bacterial growth to 40-25 cfu/ml. Andrew T. Schilling (2008) concluded that the development of *E. coli* was best at pH of 6-8but they do not grow at pH of 4 or 5. The recommendation to add acids to food appear to be an active way to inhibition of *E. coli* growth, he also stated that It is likely that acetic acid is toxic to *E. coli*. Sharma and Beuchat (2004) found alkaline cleaners were inhibited a

higher numbers of *E. coli* O157:H7 strain FRIK 816-3) and strain EDL 1933). Inhibit of *E. coli* O157:H7 when exposed to alkaline cleaners were expected. Gram-negative bacteria when exposed to high pH lead to destroy of cell membranes and causes escape of the internal contents of cells (Mendonca, 1994). Membranes of Cell may dislodge by solubilization of proteins or saponification of lipids. Gram-negative cells are also affected by alkaline pH, in part due to lose of the peptidoglycan layer structural integrity then ruptures. Use Alkaline with high (CO_3^{-2}) carbonate concentration and ammonia (NH_3) are efficient in killing $6 \log_{10}$ CFU of *E. coli* O157:H7 (Park, and Diez Gonzalez ... 2003).

Tien *et al.* (2013) use prunusemume extract are organic acids, and revealed that the Fructusmume has the ability of antibacterial efficacy against pathogenic *K. pneumonia* strains, fructose mume extracts including major organic acid such as citric acid, tartaric acid, oxalic acid, he was stated that have an effect to inhibit on growth and CPS biosynthesis in *K. pneumoniae* and also inhibits bacterial CPS biosynthesis and facilitate of pathogen exclusion by the immune system of the host.

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