Journal of Emerging Technologies and Industrial Applications
Vol. 2 No. 2 (2023) pp. 1-10
e-ISSN: 2948-507X

Journal homepage: https://jetia.mbot.org.my/index.php/jetia/index

Impact of Urinary Tract Infection (UTI) Related Microbial Strains' Bioactivity Kinetics in Difference Stages for Antimicrobial Susceptibility Testing

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Abstract: The emergence of UTI problem increasing in worldwide poses diagnostic and therapeutic challenges. Generally, antibacterial related research and product development begin from a laboratory scale, the bioactivity accuracy are crucial before scaling up testing till bioproduct development. In this study, eight strains were studied the impact of bioactivity in different stages with reaction time (0 – 240 minutes) via the Time-kill method. From study showed that all fresh stage strains' bioactivity are positive and maximum positive specific growth rate (SGR) (min^{-1}) in between 4 – 6 minutes while all aged stage strain's bioactivity showed negative and maximum negative SGR occurs in between 2 – 30 minutes respectively: *Enterococcus faecalis* (2.158744057 & -2.649158683), *Escherichia coli* (2.505317647 & -2.302585093), *Proteus vulgaris* (2.158744057 & -2.649158683), *Klebsiella pneumoniae* (2.158744057 & -0.191882091), *Candida albicans* (2.302585093 & -2.302585093), *L. acidophilus* (2.302585093 & -2.302585093), *L. sakei* (2.302585093 & -2.302585093) and *L. gassei* (1.956011503 & -2.302585093). UTI microbial strains' bioactivity kinetics were varied depending on stage under an active period with the same cultivation environment and within acceptable bioactivity range only suitable for UTI-related antimicrobial testing.

Received 09 October 2023; Accepted 30 November 2023; Available online 25 December 2023

Keywords: Bioactivity Kinetics, Urinary Tract Infection, Microbial Strains, Comparison, Stage.

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1. Introduction

Urinary tract infection (UTI) considers as the most common bacterial infections among children. According to research, around 8% of children from 1 to 11 years old will be infected at least 1 times urinary tract infection [1-2], and worse around 30% of children and infants faced recurrent infections during the first 6 to 12 months after infected urinary tract infection [3-4]. In the United States, pediatric ambulatory visits reached 1.5 million per year due to urinary tract infection related problems [5].

Also, in 2013 the United States already spent around $630 million in health care for urinary tract infection-related treatment and management [6]. The symptom of urinary tract infection such as dysuria, flank pain and fever in short term and possible cause long term renal injury such as permanent kidney scarring [7]

In addition, the children category faces the threat of urinary tract infections while women category as well. From the research of Salvatore et al. (2011) [8], 1/3 of women will experience at least one-time urinary tract infection problem during their lifetime and around 21-53% infected person reported asymptomatic reoccurrence within one year [9-10]. Biologic, pathogenic, and behavioral factors become individual susceptibility to recurrent urinary tract infection problems [11].

In this bioactivity kinetics research, eight microbial strain related urinary tract infections were selected in this study. First, surveillance research found that 46.9% of urinary tract infections caused by *E. coli* bacteremia and exposure to antibiotic therapy in the previous weeks have become the biggest risk factor [12-14].

Second, according to Faraina *et al*. (2009) [15] stated that *K. pneumoniae* considers the second potential caused urinary tract infection after *E. coli*, but the microbial strain's pathogenicity is higher than its counterpart. Around 12% of urinary tract infections happened due to *Klebsiella pneumoniae* and the case number still increasing which already reached an alarming rate all over the world. The reason is due to extended beta-lactamase strains and the spread of antibiotic-resistant [16]. Women's reproductive organs position and many of the infections remain asymptomatic for a prolonged period caused women 8 times more vulnerable to urinary tract infections [17].

Third, *Proteus vulgaris* considered bio-group two and has been proved that will cause urinary tract infections, burn infections, wound infection, respiratory tract infections, and bloodstream infections [18-19]. In addition, a study confirms that *Proteus vulgaris* causes brain abscesses [20].

Next, Van and Gilmore (2014) [21] stated that enterococci are gastrointestinal flora members and found in the 1970s as common causes of multidrug-resistant hospital infections. Infected of enterococcal may cause in asymptomatic bacteriuria and overt urinary tract infection [22]

Furthermore, during the past decade, a fungal infection caused urinary tract infections appeared a challenge in therapy and diagnosis to researchers. *Candida albicans* was proved that the one of most common microbial strain caused urinary tract infection to humans [23]. Fortunately, according to Reid et al., 1990; Redondo-Lopezet et al., 1990 and Hudault et al., 1997 [24-26] studies found that *Lactobacilli* play an important role in the vagina against genital organs infection in mammals.

A lot of research using *Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Candida albicans, L. acidophilus, L. sakei* and *L. gassei*. as target microbial strain in disease, infection, and biochemical related researches [14,15, 17, 20-23, 27-31] and diagnostic with risk factor related researched [32-34].

In recent studies the bioactivity kinetics of microbial strain which related urinary tract infection in different stages has not been investigated yet. Therefore, in this research eight urinary tract infection-related microbial strains (*Enterococcus faecalis*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Candida albicans*, *L. acidophilus*, *L. sakei*, and *L. gassei*) with variety of cultivation duration (0 – 240 minutes via the Time-kill method) and its bioactivity kinetics were studied.

2. Materials and Methods

2.1 Inoculum preparation

Fresh bacteria *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* (ATCC 8739), *Proteus vulgaris* (ATCC 33420), and *Klebsiellla pneunmoniae* (ATCC 13883) had been cultured on suitable agar until it was ripe and fruiting at 35ºC for 18 – 24 hours. Yeast *Candida albicans* (ATCC10231) had been cultured on suitable agar till it was ripe and fruiting (44 – 52 hours) at 25ºC. Bacteria *Lactobacillus acidophilus* (ATCC 4356), *Lactobacillus sakei* (ATCC 15521), and *Lactobacillus gassei* (ATCC 19992) had been cultured on suitable agar until it was ripe and fruiting at 35ºC anaerobically for 3 – 5 days. By scraping the fruity culture, the spore collected had been transferred to 10 mL sterilized tryptone sodium chloride solution in a universal bottle to obtain a microbial count of about $1.0x10^8$ CFU/mL. Each test microorganism will be prepared in a different universal bottle. The universal bottle was vortex for 10 seconds to bring the spores into

suspension. This suspension was then used as the inoculum for the experiment.

Table 1 Urinary tract infection related microorganisms.

Name of microbial strain	Type of cell	Type of microorganism
Enterococcus faecalis (ATCC 19433)	Prokaryote	Gram-positive bacteria
Escherichia coli (ATCC 8739)	Prokaryote	Gram-negative bacteria
Proteus vulgaris (ATCC 33420)	Prokaryote	Gram-negative bacteria
Klebsiellla pneunmoniae (ATCC 13883)	Prokaryote	Gram-negative bacteria
Lactobacillus acidophilus (ATCC 4356)	Prokaryote	Gram-positive beneficial bacteria
Lactobacillus sakei (ATCC 15521)	Prokaryote	Gram-positive beneficial bacteria
Lactobacillus gassei (ATCC 19992)	Prokaryote	Gram-positive beneficial bacteria
Candida albicans (ATCC10231)	Eukaryote	Pathogenic yeast

2.2 Microbial growth analysis

The concentration of viable microorganisms in the test preparation was determined by the plate count method. The contact times were 0 minutes (as control), 2 minutes, 4 minutes, 6 minutes, 30 minutes, 60 minutes, and 240 minutes. After inoculation and 0 minute assayed, the inoculated sample was incubated in 22.5ºC until the end of contact time. Each experiment was performed in duplicates to ensure reproducibility of results. Then the experiment repeated with aged stage microbial strains which more than 6 months compared with fresh stage microbial strains. The microbial strain growth rate was calculated from the Eq. 1 -4:

$$Mean\ nuumber\ of\ cell\ (m), \frac{CFU}{g} = \frac{Sample\ 1 - Sample\ 2}{2} \quad (1)$$

$$Mean\ log = log_{10}\, m \quad (2)$$

$$Growth\ percentage, \% = \frac{Initial\ value - Sample\ value}{Initial\ value}\ X\ 100\% \quad (3)$$

$$log\ growth\ = \log_{10} Initial\ value - \log_{10} Sample\ value \quad (4)$$

2.2.1 Positive specific growth rate, positive division rate, negative specific growth rate, and negative division rate

The cultures were sampled 0 minutes, 2 minutes, 4 minutes, 6 minutes, 30 minutes, 60 minutes, and 240 minutes then the microbial counting technique was used to monitor the microorganism growth by counting the microbial number. The microbial concentration was determined by a colony counter (Funke Gerber, Colony Star 8502-3952). Eq. 5 & 6 was used for the determination of the specific growth rate:

$$\mu = \frac{\ln(N_2 - N_1)}{t_2 - t_1} \quad (5)$$

$$\mu = -\frac{\ln(N_1 - N_2)}{t_2 - t_1} \quad (6)$$

where N_2 and N_1 represent cell number concentrations at time t_2 and t_1, respectively whereas k represents the time taken to duplicate the microbial division rate, evaluated according to the Eq. 7 [35].

$$k = \frac{\mu}{\ln 2} \quad (7)$$

3. Results and Discussions

3.1 Analysis of microbial strains growth percentage

3.1.1 Gram-positive bacteria

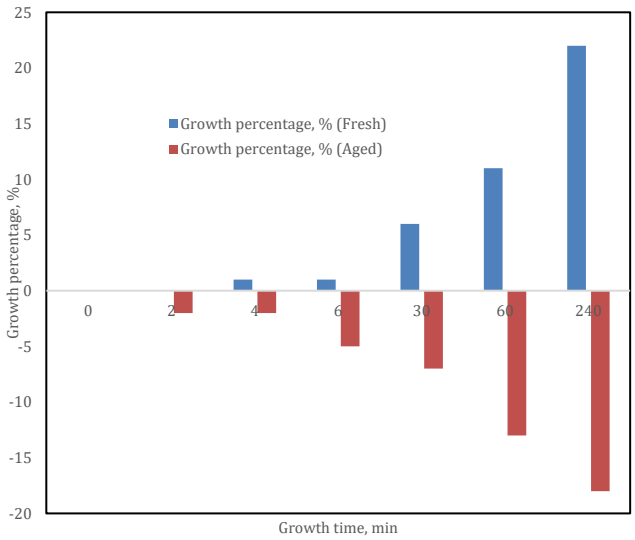

Fig. 1 Growth percentage over growth time of *Enterococcus faecalis* 19433 (Fresh strain and aged strain).

From the Fig. 1 showed that gram-positive bacteria (*Enterococcus faecalis* 19433) the growth percentage indicating positive growth trend from 0 minutes to 240 minutes (0% to 22%) to fresh stage microbial strain while to the aged microbial strain showed negative growth trend from 0 minutes to 240 minutes (0% to -18%) although this microbial strain culture in the same condition with the different the difference stages only. This showed that *Enterococcus faecalis* 19433 after 6 months or more than 6 months if need to use for anti- microorganism testing in product development, have to test its bioactivity prior and suggested *Enterococcus faecalis* 19433's growth

percentage has to reach around 22% or above and not less than 1%.

3.1.2 Gram-negative bacteria

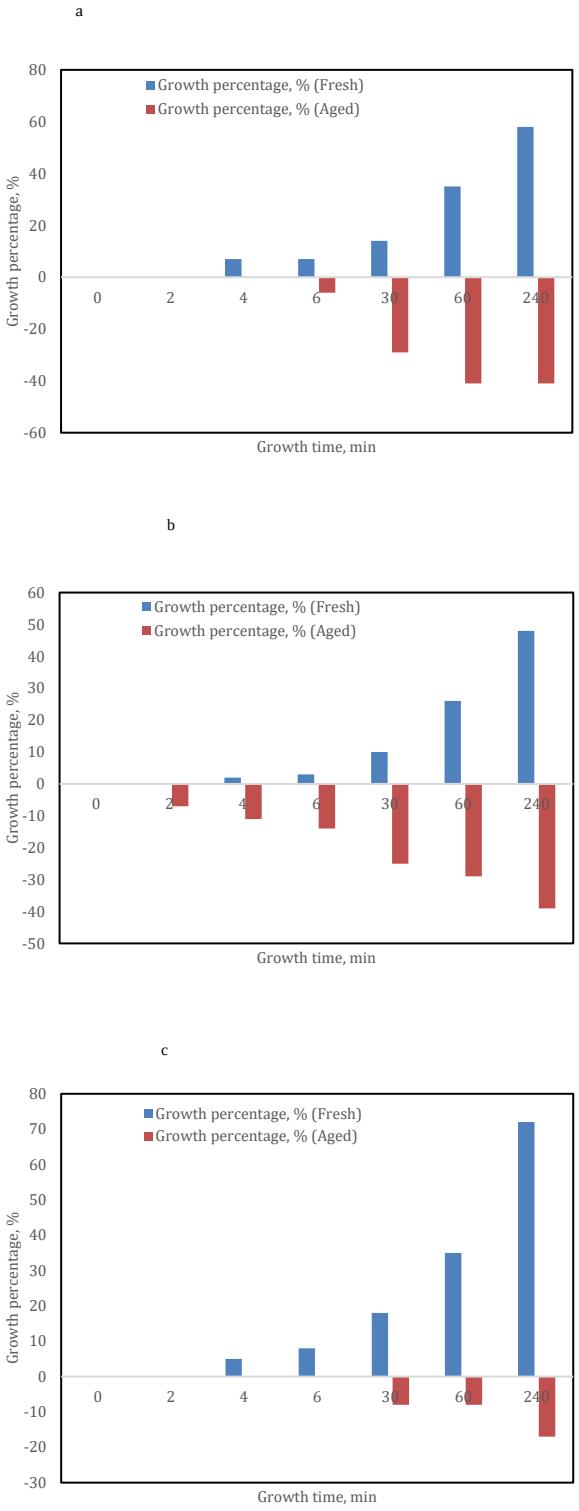

Fig. 2 Growth percentage over growth time of (a) *Escherichia coli* 8739 (b) *Proteus vulgaris* 33420 (c) *Klebsiellla pneunmoniae* 13883 (Fresh strain and aged strain).

From the Fig. 2 showed that gram-negative bacteria (2a: *Escherichia coli* 8739, 2b: *Proteus vulgaris* 33420 and 2c: *Klebsiellla pneunmoniae* 13883) the growth percentage indicating positive growth trend from 0 minutes to 240 minutes (*Escherichia coli* 8739: 0% to 58%, *Proteus vulgaris* 33420: 0% to 48%; *Klebsiellla pneunmoniae* 13883: 0% to 72%) to fresh stage microbial strain while to the aged microbial strain showed negative growth trend from 0 minutes to 240 minutes (*Escherichia coli* 8739: 0% to -41%; *Proteus vulgaris* 33420: 0% to -39%; *Klebsiellla pneunmoniae* 13883: 0% to -17%) although this microbial strain culture in similar condition with the different the difference stages only. This showed that *Escherichia coli* 8739, Proteus vulgaris 33420 and *Klebsiellla pneunmoniae* 13883 after 6 months or more than 6 months if need to use for antimicrobial susceptibility testing in bioproduct development, have to test its bioactivity prior and suggested Escherichia coli 8739, *Proteus vulgaris* 33420 and *Klebsiellla pneunmoniae* 13883's growth percentage has to reach around 58%, 48%, and 72% or above and not less than 7%, 2% and 5% respectively.

3.1.3 Pathogenic yeast

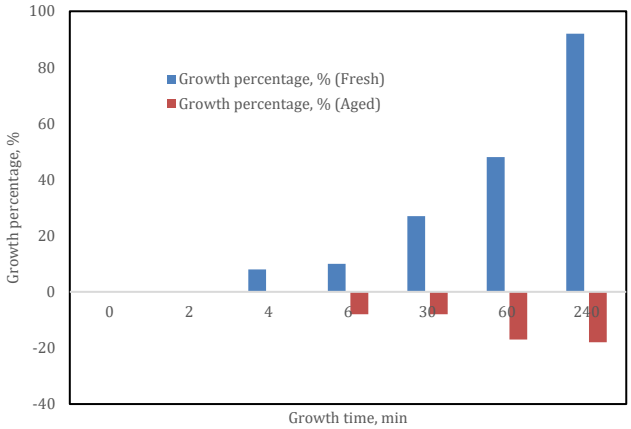

Fig. 3 Growth percentage over growth time of *Candida albicans* 10231 (Fresh strain and aged strain).

From the Fig. 3 showed that pathogenic yeast (*Candida albicans* 10231) the growth percentage indicating positive growth trend from 0 minutes to 240 minutes (0% to 92%) to fresh stage microbial strain while to the aged microbial strain showed negative growth trend from 0 minutes to 240 minutes (0% to -18%) although this microbial strain culture in the same environment with the different the difference stages

only. This showed that *Candida albicans* 10231 after 6 months or more than 6 months if need to use for anti-microorganism testing in product development, have to test its bioactivity prior and suggested *Candida albicans* 10231's growth percentage have to reach around 92% or above and not less 8%.

3.1.4 Gram-positive beneficial bacteria

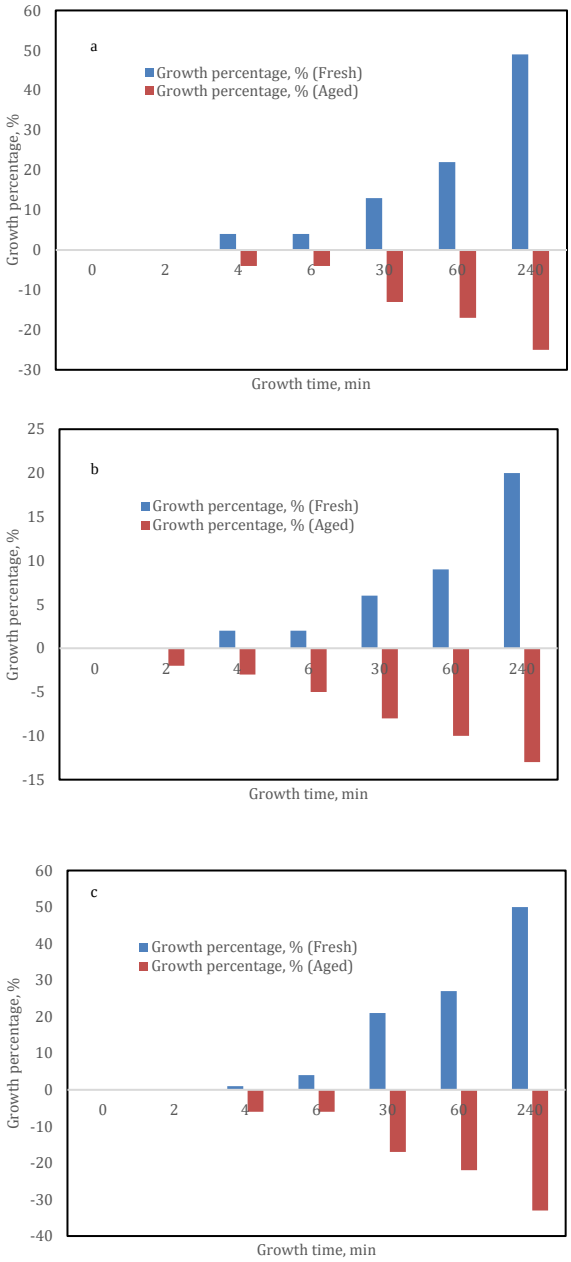

Fig. 4 Growth percentage over growth time of (a) *Lactobacillus acidophilus* 4356 (b) *Lactobacillus sakei* 15521 (c) *Lactobacillus gassei* 19992 (Fresh strain and aged strain).

From the Fig. 4 showed that gram-positive beneficial bacteria (4a: *Lactobacillus acidophilus* 4356, 4b: *Lactobacillus sakei* 15521 and 4c: *Lactobacillus gassei* 19992) the growth percentage indicating positive growth trend from 0 minutes to 240 minutes (*Lactobacillus acidophilus* 4356: 0% to 49%, *Lactobacillus sakei* 15521: 0% to 20%; *Lactobacillus gassei* 19992: 0% to 50%) to fresh stage microbial strain while to the aged microbial strain showed negative growth trend from 0 minutes to 240 minutes (*Lactobacillus acidophilus* 4356: 0% to -25%, *Lactobacillus sakei* 15521: 0% to -13%; *Lactobacillus gassei* 19992: 0% to -33%) although this microbial strain culture in the same parameter with the different the difference stages only. This showed that *Lactobacillus acidophilus* 4356, *Lactobacillus sakei* 15521 and *Lactobacillus gassei* 19992 after 6 months or more than 6 months if need to use for antimicrobial susceptibility testing in bioproduct development, have to test its bioactivity prior and suggested *Lactobacillus acidophilus* 4356, *Lactobacillus sakei* 15521 and *Lactobacillus gassei* 19992's growth percentage has to reach around 49%, 20% and 50% or above and not less than 4%, 2% and 1% respectively.

3.2 Analysis of microbial strains log growth, specific growth rate, and division rate
3.2.1 Analysis gram-positive bacteria

Table 2 Log growth, specific growth rate and division rate of gram-positive bacteria (Fresh strain and aged strain).

Time, min	Log growth (Fresh)	Log growth (Aged)	SGR (Fresh), □ □(min^{-1})	SGR (Aged), □ □(min^{-1})	Division rate (Fresh), (min^{-1})	Division rate (Aged), (min^{-1})
***Enterococcus faecalis* 19433**						
0	-	-	-	-	-	-
2	0	-0.007968930	-	-2.302585093	-	-3.321928095
4	0.005829544	-0.007968930	2.158744057	-	3.114409345	-
6	0.005829544	-0.024359346	-	-2.649158683	-	-3.821928095
30	0.024714888	-0.032792513	0.230060872	-0.191882091	0.331907679	-0.276827341
60	0.044582133	-0.059121452	0.187225703	-0.190126082	0.270109594	-0.274293956
240	0.085010790	-0.087150176	0.035538498	-0.031687680	0.051271215	-0.045715659

Table 2 illustrated the log growth, specific growth rate, and division rate of gram-positive bacteria (Fresh strain and aged strain). The maximum log growth (0.085019790 at 240 minutes), highest specific growth rate (2.158744057 at 4 minutes) and highest division rate (3.114409345 at 4 minutes) to fresh stage microbial of *Enterococcus faecalis* 19433 however aged stage microbial strain showed all negative trend, the maximum negative log growth (-0.087150176 at 240 minutes), highest negative specific growth rate (-2.649158683 at 6 minutes) and highest negative division rate (-3.821928095 at 6 minutes).

3.2.2 Analysis gram-negative bacteria

Table 3 Log growth, specific growth rate and division rate of gram-negative bacteria (Fresh strain and aged strain).

Time, min	Log growth (Fresh)	Log growth (Aged)	SGR (Fresh), □ □(min^{-1})	SGR (Aged), □ □(min^{-1})	Division rate (Fresh), (min^{-1})	Division rate (Aged), (min^{-1})
***Escherichia coli* 8739**						
0	-	-	-	-	-	-
2	0	0	-	-	-	-
4	0.029963223	0	2.505317647	-	3.614409345	-
6	0.029963223	-0.026328939	-	-2.302585093	-	-3.321928095
30	0.057991947	-0.151267675	0.208776471	-0.249644356	0.301200779	-0.360160675
60	0.128799157	-0.230448921	0.201736306	-0.176610579	0.291043968	-0.254795206
240	0.199572355	-0.230448921	0.034525601	-	0.049809913	-
***Proteus vulgaris* 33420**						
0	-	-	-	-	-	-
2	0	-0.032184683	-	-2.649158683	-	-3.821928095
4	0.010219165	-0.049218023	2.158744057	-2.302585093	3.114409345	-3.321928095
6	0.013572807	-0.066946790	1.609437912	-2.302585093	2.321928095	-3.321928095
30	0.039508541	-0.124938737	0.220763224	-0.237657603	0.318494008	-0.342867445
60	0.101026579	-0.146128036	0.208779942	-0.153505673	0.301205787	-0.221461873
240	0.171471061	-0.216709110	0.036394891	-0.031687680	0.052506728	-0.045715659
***Klebsiellla pneunmoniae* 13883**						
0	-	-	-	-	-	-

2	0	0	-	-	-	-
4	0.021189299	0	2.158744057	-	3.114409345	-
6	0.034762106	0	1.956011503	-	2.821928095	-
30	0.073107098	-0.037788561	0.208776471	-0.191882091	0.301200779	-0.276827341
60	0.130333768	-0.037788561	0.184048697	-	0.265526143	-
240	0.234685974	-0.079181246	0.035055102	-0.025584279	0.050573821	-0.036910312

Table 3 listed the log growth, specific growth rate, and division rate of gram-negative bacteria (Fresh strain and aged strain) of *Escherichia coli* 8739, *Proteus vulgaris* 33420 and *Klebsiellla pneunmoniae* 13883 respectively: The maximum log growth (0.199572355 at 240 minutes, 0.171471061 at 240 minutes, 0.234685974 at 240 minutes), highest specific growth rate (2.505317647 at 4 minutes, 2.158744057 at 4 minutes, 2.158744057 at 4 minutes) and highest division rate (3.614409345 at 4 minutes, 3.114409345 at 4 minutes, 3.114409345 at 4 minutes) to fresh stage microbial strain however aged stage microbial strain showed all negative trend, the maximum negative log growth (-0.230448921 at 60 minutes, -0.216709110 at 240 minutes, -0.079181246 at 240 minutes), highest negative specific growth rate (-2.302585093 at 6 minutes, -2.649158683 at 2 minutes, -0.191882091 at 30 minutes) and highest negative division rate (-3.321928095 at 6 minutes, -3.821928095 at 2 minutes, -0.27682734 at 30 minutes).

3.2.3 Analysis pathogenic yeast

Table 4 Log growth, specific growth rate and division rate of pathogenic yeast (Fresh strain and aged strain).

Time, min	Log growth (Fresh)	Log growth (Aged)	SGR (Fresh), □ □(min^{-1})	SGR (Aged), □ □(min^{-1})	Division rate (Fresh), (min^{-1})	Division rate (Aged), (min^{-1})
***Candida albicans* 10231**						
0	-	-	-	-	-	-
2	0	0	-	-	-	-
4	0.034762106	0	2.302585093	-	3.321928095	-
6	0.043034632	-0.037788561	1.609437912	-2.302585093	2.321928095	-3.321928095
30	0.104088598	-0.037788561	0.220763224	-	0.318494008	-
60	0.170017111	-0.079181246	0.184048697	-0.153505673	0.265526143	-0.221461873
240	0.282546590	-0.087955170	0.034796657	-0.016642957	0.050200965	-0.024010712

Table 4 illustrated the log growth, specific growth rate, and division rate of pathogenic yeast (Fresh strain and aged strain). The maximum log growth (0.282546590 at 240 minutes), highest specific growth rate (2.302585093 at 4 minutes) and highest division rate (3.321928095 at 4 minutes) to fresh stage microbial of *Candida albicans* 10231 however aged stage microbial strain showed all negative trend, the maximum negative log growth (-0.087955170 at 240 minutes), highest negative specific growth rate (-2.302585093 at 6 minutes) and highest negative division rate (-3.321928095 at 6 minutes).

3.2.4 Analysis gram-positive beneficial bacteria

Table 5 Log growth, specific growth rate and division rate of gram-positive beneficial bacteria (Fresh strain and aged strain).

Time, min	Log growth (Fresh)	Log growth (Aged)	SGR (Fresh), □ □(min^{-1})	SGR (Aged), □ □(min^{-1})	Division rate (Fresh), (min^{-1})	Division rate (Aged), (min^{-1})
***Lactobacillus acidophilus* 4356**						
0	-	-	-	-	-	-
2	0	0	-	-	-	-
4	0.016867925	-0.018483406	2.302585093	-2.302585093	3.321928095	-3.321928095
6	0.016867925	-0.018483406	-	-	-	-
30	0.052583478	-0.057991947	0.225670850	-0.220763224	0.325574216	-0.318494008
60	0.085583738	-0.079181246	0.180536680	-0.153505673	0.260459373	-0.221461873
240	0.171769885	-0.124938737	0.036192848	-0.029435096	0.052215243	-0.042465868

***Lactobacillus sakei* 15521**						
0	-	-	-	-	-	-
2	0	-0.006948860	-	-2.302585093	-	-3.321928095
4	0.006580345	-0.014010714	2.302585093	-2.302585093	3.321928095	-3.321928095
6	0.008209970	-0.021189299	1.609437912	-2.302585093	2.321928095	-3.321928095
30	0.025743505	-0.035912556	0.234032129	-0.220763224	0.337636992	-0.318494008
60	0.038064742	-0.043465694	0.176610579	-0.153505673	0.254795206	-0.221461873
240	0.078628357	-0.058977860	0.036394891	-0.029435096	0.052506728	-0.042465868
***Lactobacillus gassei* 19992**						
0	-	-	-	-	-	-
2	0	0	-	-	-	-
4	0.005264240	-0.024823584	1.609437912	-2.302585093	2.321928095	-3.321928095
6	0.015605073	-0.024823584	1.956011503	-	2.821928095	-
30	0.081821342	-0.079181246	0.244080548	-0.220763224	0.352133796	-0.318494008
60	0.104053867	-0.109144469	0.162251148	-0.153505673	0.234078927	-0.221461873
240	0.176091259	-0.176091259	0.034181848	-0.029435096	0.049313983	-0.042465868

Table 5 listed the log growth, specific growth rate, and division rate of gram-positive beneficial bacteria (Fresh strain and aged strain) of *Lactobacillus acidophilus* 4356, *Lactobacillus sakei* 15521 and *Lactobacillus gassei* 19992 respectively: The maximum log growth (0.171769885 at 240 minutes, 0.078628357 at 240 minutes, 0.176091259 at 240 minutes), highest specific growth rate (2.302585093 at 4 minutes, 2.302585093 at 4 minutes, 1.956011503 at 6 minutes) and highest division rate (3.321928095 at 4 minutes, 3.321928095 at 4 minutes, 2.821928095 at 6 minutes) to fresh stage microbial strain however aged stage microbial strain showed all negative trend, the maximum negative log growth (-0.124938737 at 240 minutes, -0.058977860 at 240 minutes, -0.176091259 at 240 minutes), highest negative specific growth rate (-2.302585093 at 6 minutes, -2.302585093 at 2 minutes, -2.302585093 at 4 minutes) and highest negative division rate (-3.321928095 at 4 minutes, -3.321928095 at 2 minutes, -3.321928095 at 4 minutes).

Conclusion

In this study proved that UTI-related microbial strains' bioactivity kinetics were varied depending on stage under an active period with the same cultivation environment and the acceptable microbial strain's bioactivity range will not only determine antimicrobial testing's susceptibility level but also reduce false positive/ false negative's possibility while reduce cost and time-consuming. Bioactivity range in growth percentage: *E.faecalis* (22% or above and not less than 1%); *E.coli*, *P.vulgaris* and *K.pneunmoniae* (58%, 48% and 72% or above and not less than 7%, 2% and 5%); *C.albicans* (92% or above and not less 8%); *L.acidophilus*, *L.sakei* and *L.gassei* (49%, 20% and 50% or above and not less than 4%, 2% and 1%). Thus, these microbial strains' bioactivity range as references for prior antimicrobial testing.

Acknowledgement

This research is supported by Agri Season Sdn. Bhd. The author acknowledged Agri Season Sdn. Bhd. approved fund which makes this important research viable and effective.

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