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Effect of Antibiotics on Growth and L-glutamic Acid Fermentation by the Mutant *Micrococcus glutamicus* AB_{100}

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An experimental study was conducted to investigate the effects of varying concentrations (10-30 μg/ml) of different antibiotics namely penicillin G, erythromycin, chrompheniol, streptomycin, tetracycline and gentamicin added to the fermentation broth at different time intervals (0-48h) on growth and l-glutamic acid production by the biotin-auxotrophic mutant *Micrococcus glutamicus* AB_{100}. All these antibiotics showed determental effect on both growth & l-glutamic acid production.

Key words: Experimental, Antibiotics, Growth, L-glutamic acid.

The fermentative production of l-glutamic acid using l-glutamic acid was started by Kyowa hakko Kogyo Co. Ltd. Successfully in 1956[1]. The l-glutamate producing bacteria like *Micrococcus glutamicus*, *Brevibacterium roseum*, *Brevibacterium lactofermentum*, *Brevibacterium flavum* etc. are Gram+ve, non spore-forming and nonmotile and require biotin for growth[3]. The effects of different antibiotics on growth and L-amino acid production were reported by many authors[4-8].

Considering the reviews, the present study was intended to investigate the effects of different antibiotics namely penicillin G, erythromycin, chloramphenicopl, streptomycin, tetracycline and gentamicin on growth and l-glutamic acid accumulation by the mutant *Micrococcus glutamicus* AB_{100}.

MATERIAL AND METHODS

Microorganism

Micrococcus glutamicus AB_{100}, a biotin requiring auxotrophic mutant derived from a regulatory mutant *Micrococcus glutamicus* AB_1 by induced mutation in our laboratory was used throughtou the study[9].

Synthetic medium used for l-glutamic acid production

The composition of the synthetic medium used for l-glutamic acid production was as follows:

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glucose, 9.0%; $(NH_4)_2HPO_4$, 1.4%; K_2HPO_4, 0.15%; $MgSO_4.7H_2O$, 0.03%; $CaCO_3$, 0.04%; $FeSO_4.7H_2O$, 5.0 µg/ml; $ZnSO_4.7H_2O$, 10. µg/ml; $MnSO_4.4H_2O$, 1.0 µg/ml; and biotin, 0.2 µg/ml; pH 6.5 [10].

Fermentation was carried out using shake flask method on a rotary shaker (150 rpm) in 100 ml Erlenmayer conical flask containing 20 ml mineral salt medium for 72h at 29°C. The medium was inoculated with 4.0% (v/v) of 48h old seed culture (6.0×104 cells) of *Micrococcus glutamicus* AB_{100} [11].

Addition of antibiotics to the synthetic medium

The effects of varying concentrations (10.0 – 30.0 µg/ml) of different antibiotics namely penicillin, erythromycin, chloramphenicol, streptomycin, tetracycline and gentamicin added at different time intervals (0-48 h) on growth and l-glutamic acid production by this mutant were investigated.

Analysis of Amino acid

Descending paper chromatography was employed for detecting L-glutamic acid in culture medium and was run for 18h on a watman No. 1 chromatographic paper. Solvent system used include, n-butanol : acetic acid : water (2 : 1 : 1). The spots were visualized by spraying with a solution of 0.2% ninhydrin in acetone by spraying with a solution of 0.2% ninhydrin in acetone and quantitative estimation of L-glutamic acid in the suspension was done using colorimetric estimation method [12,13].

Estimation of Dry Cell Weight (DCW)

After centrifugation, a few ml of 1.0(M) HCl was poured into the precipitate of the bacterial cells and calcium carbonate to dissolve calcium carbonate. The remaining bacterial cells were washed with water and dried at 100°C until cell weight remain constant[14].

Statistical analysis

All data were expressed as mean ± SEM, were n = 6. The data were analyzed by one way ANOVA followed by Dunett's post hoc multiple comparison test using "prism 4.0" software (Graph pad Inc., USA). A "p" value less than 0.05 was considered significant and less than 0.01 as highly significant.

RESULTS AND DISCUSSION

The effect of different antibiotics on growth and l-glutamic acid production by the biotin-auxotrophic mutant *Micrococcus glutamicus* AB_{100} were depicted in Fig. 1a-6c.

All these antibiotics studied showed determental effects on growth and l-glutamic acid production.

Penicillin G, being a member of β-lactam antibiotics inhibits the formation of peptidoglycan cross-links in the bacterial cell wall. The β-lactam moiety of the antibiotic binds to the enzyme-DO-transpeptidase which helps in cross linking between peptidoglycan strands of bacterial cell wall. Thus the growth of bacterial cell wall continue to function, leading to cytolysis. On the other hand, as peptidoglycan synthesis becomes inhibited, aminoglycosides penetrale the bacterial cell, allowing its disruption of protein synthesis within the cell[4,15].

Erythromycin shows bacteriocidal activity by binding to the 50S subunit of the bacterial ribosome. It interferes with aminoacyl translocation, preventing translocation of the t-RNA from A site to P site of the ribosome, leading to inhibition of polypeptide synthesis[16].

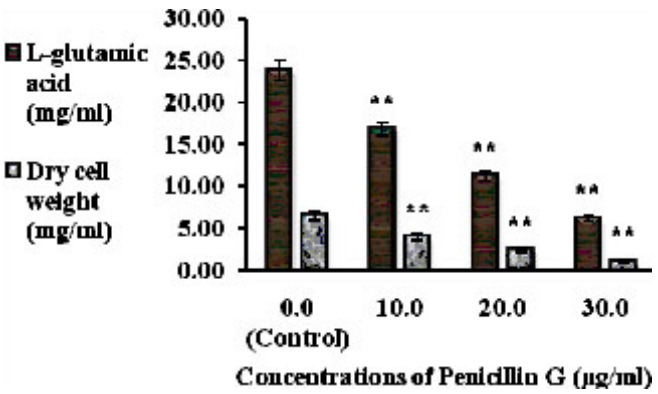
Chloramphenicol, bacteriostatic agent, binds to A2451 and A2452 residues in the 23S rRNA of 50S ribosomal sub unit, inhibits peptide bond formation [17].

Streptomycin binds to S12 protein of 30S ribosomal subunits, inhibiting the bond formation between formylmethionyl-tRNA and 30S subunit, preventing inhibition of protein synthesis which ultimately leads to death of microbial cells [18].

Tetracycline inhibits protein synthesis by blocking the attachment of aminoacyl-tRNA to the 30S subunit of 70S ribosome[19].

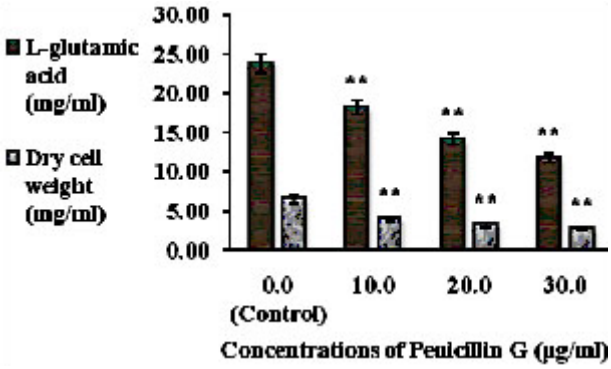
Gentamicin is a bacteriocidal antibiotic, binds to the 30S subunit of bacterial ribosome, thus, inhibits protein synthesis [20].

Thus, from this present study, it can be tentatively concluded that all the antibiotics studied adversely affects on growth and l-glutamic acid accumulation in the fermentation broth by the mutant *Micrococcus glutamicus* AB_{100}.



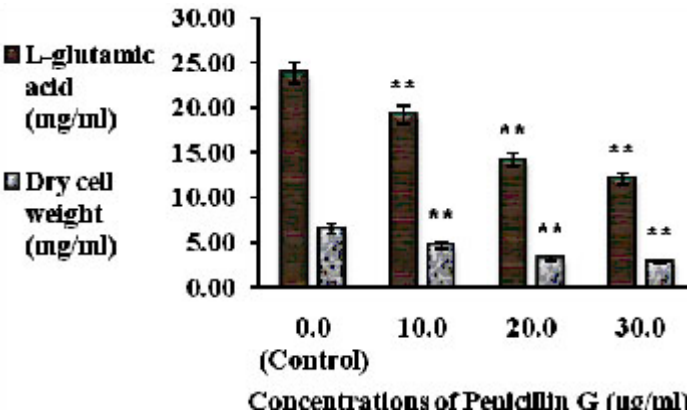
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 1(a). Effect of Penicillin G on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 0hr



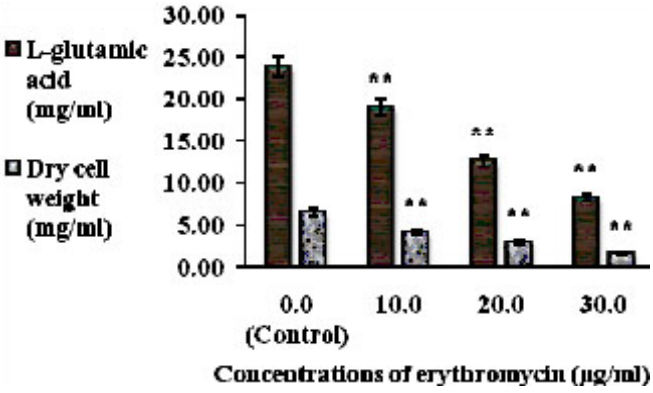
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 1(b). Effect of Penicillin G on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 24hr



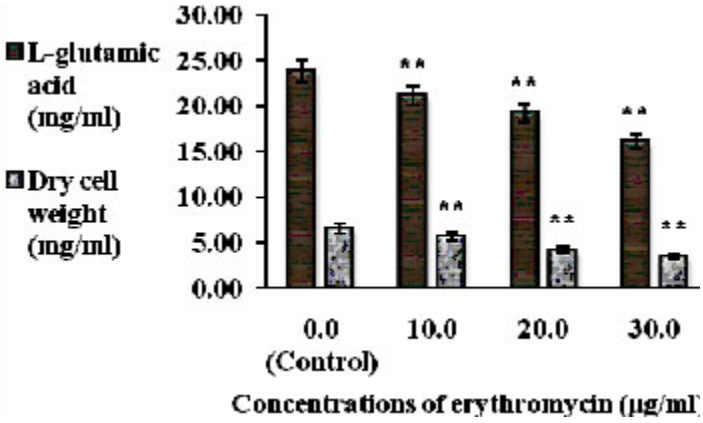
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 1(c). Effect of Penicillin G on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 48hr



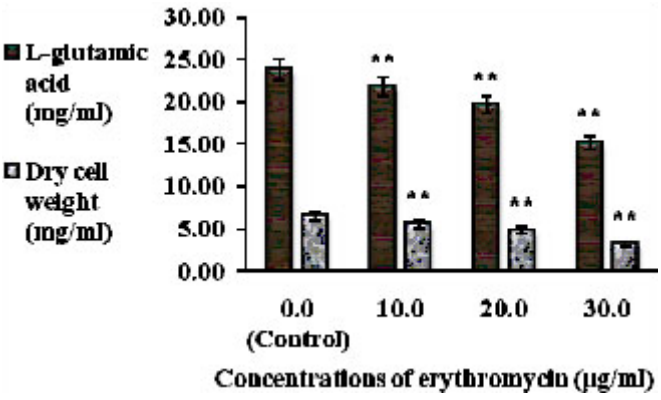
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 2(a). Effect of erythromycin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 0hr



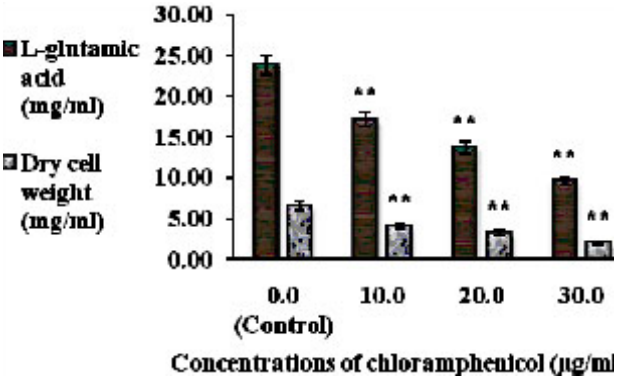
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 2(b). Effect of erythromycin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 24hr



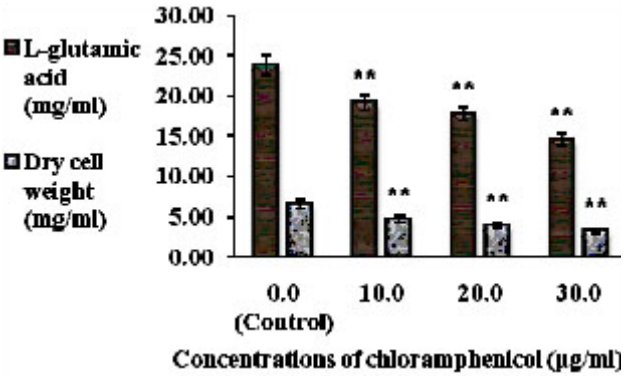
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 2(c). Effect of erythromycin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 48hr



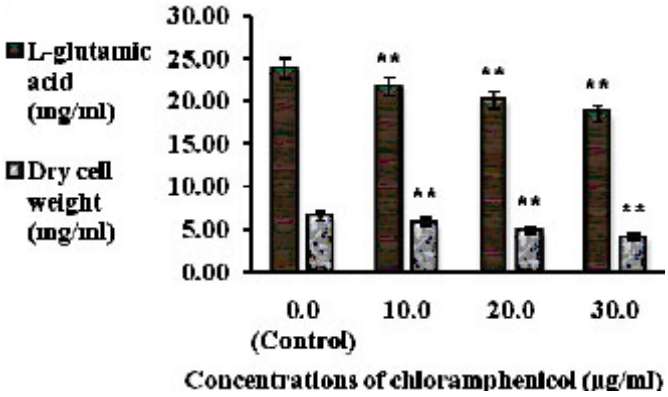
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 3(a). Effect of chloramphenicol on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 0hr



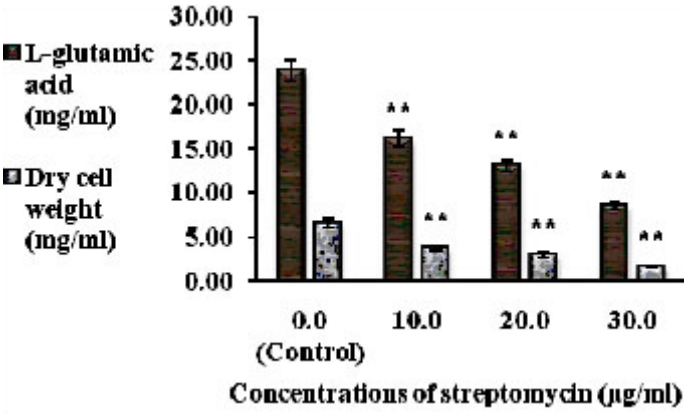
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 3(b). Effect of chloramphenicol on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 24hr



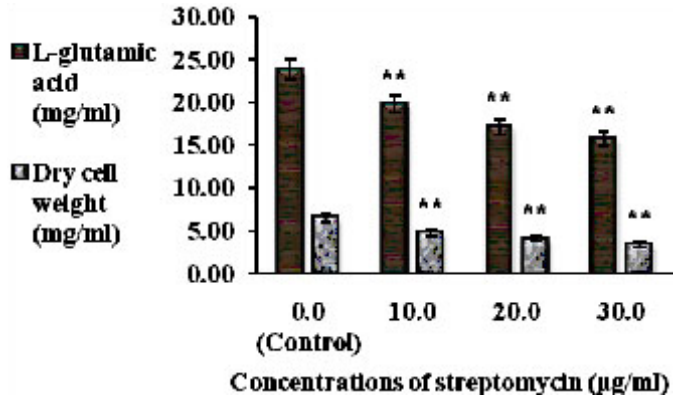
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 3(c). Effect of chloramphenicol on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 48hr



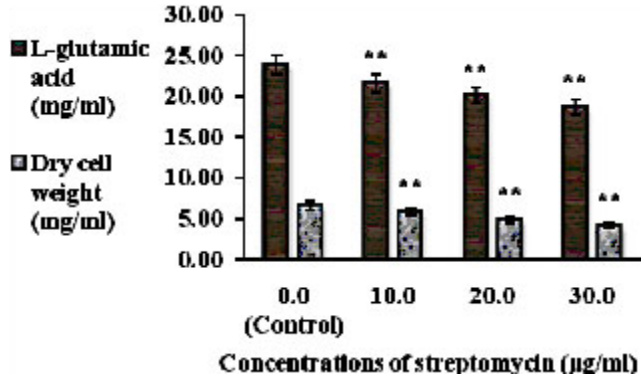
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 4(a). Effect of streptomycin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 0 hr



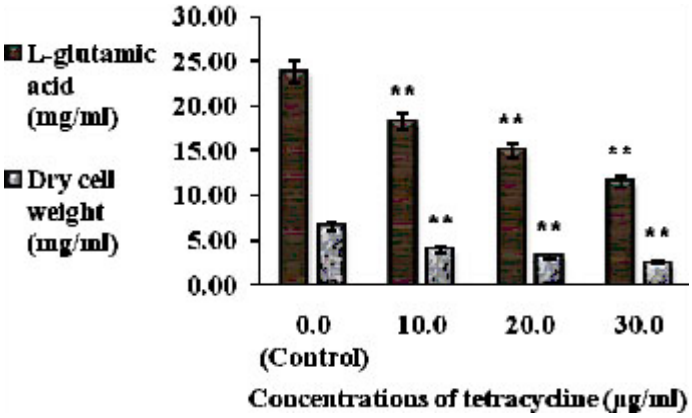
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 4(b). Effect of streptomycin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 24 hr



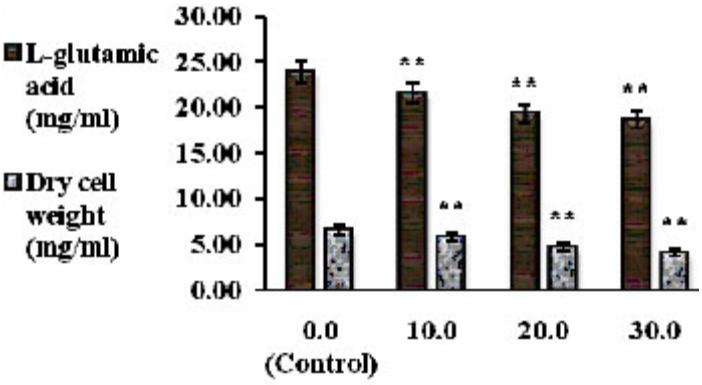
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 4(c). Effect of streptomycin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 48 hr



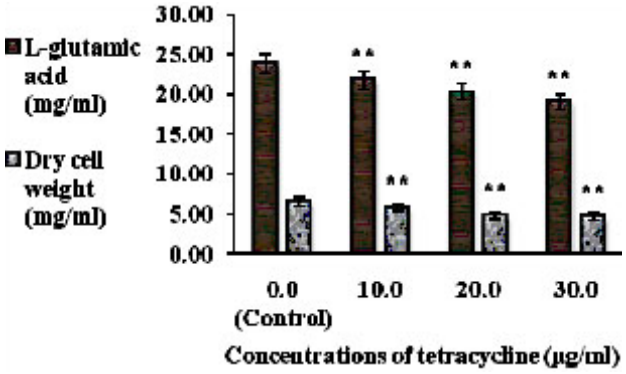
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 5(a). Effect of tetracycline on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 0hr



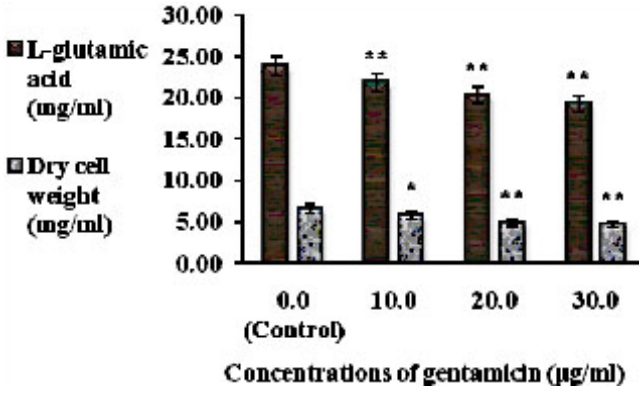
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 5(b). Effect of tetracycline on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 24 hr



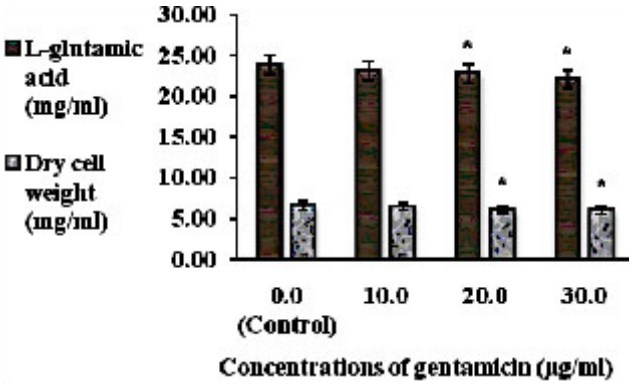
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 5(c). Effect of tetracycline on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB100 added to the fermentation broth at 48 hr



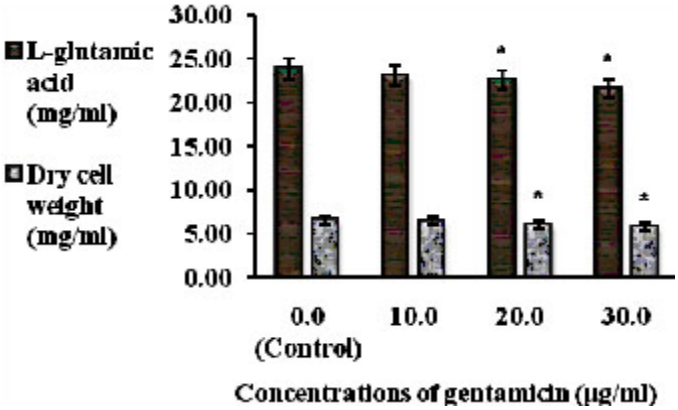
(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 6(a). Effect of gentamicin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 0hr



(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 5(b). Effect of gentamicin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 24 hr



(Values were expressed as mean ± SEM; where n-6; **p < 0.01 when compared to control).

Fig. 6(c). Effect of gentamicin on growth & L-glutamic acid production by *Micrococcus glutaqmicus* AB_{100} added to the fermentation broth at 48 hr

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