J. bio-sci. 20: 75-82, 2012 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index

COMPARATIVE STUDY ON GROWTH AND MORPHOLOGICAL CHARACTERISTICS OF A WILD TYPE STRAIN *RHIZOBIUM* SPP. (RCA-220) AND A GENETICALLY ENGINEERED *E. COLI* BL21

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Abstract

Context: Comparison between a wild type strain *Rhizobium spp.* (RCA-220) and a genetically modified strain *E. coli* BL21 in context of growth features.

Objective: To observe the comparative growth characteristics of a genetically modified *E. coli* BL21 and an isolated wild type strain *Rhizobium spp.* (RCA-220).

Materials and Methods: Different kinds of investigations were accomplished in both Luria-Bertani (LB) liquid and semi-solid media to observe the growth and maintenance of these strains. For the isolation of *Rhizobium spp.* selective Yeast Extract Manitol Agar (YEMA) was used. Colony morphology, pH, temperature, carbon source, salt concentration and light were taken under consideration and optimized for growth characteristics.

Results: For the strain *E. coli* BL21, the maximum growth rate was 1.9 at incubation time 72 h, pH 7.2, temperature 37°C (optimized) while for *Rhizobium spp.* the growth rate was significantly higher (OD 2) at pH 6.8, temperature 28°C (optimized). Among all used carbon sources, strains grown in the medium supplemented with peptone showed rapid and good performance. So, peptone was proved as the best carbon source for both strains. The maximum growths of these strains were observed at 0g/100ml NaCl salt concentration. RCA-220 strain was comparatively more tolerable to salt than *E. coli* BL21 strain. In this work, *E. coli* BL21 showed rapid and good performance in presence of light while *Rhizobium spp*. showed better performance in absence of light. Statistical analysis showed that the growth rate of *Rhizobium spp*. was significantly higher than *E. coli* BL21.

Conclusion: From the experimental results, it can be concluded that naturally obtained microbial strains were stable and could tolerate any stress condition where the modified strains lose their growth capability and the overall growth performances were reduced or slowed down than the wild type strain.

Key words: Genetically modified, RCA-220, Optimization, Morphological parameters.

Introduction

Bacteria are a large domain of prokaryotic microorganisms which can grow in soil, acidic hot springs, radioactive waste, root nodules of plants (Fredrickson *et al*. 2004). Most bacteria have not been characterized yet and only a few species can be grown in the laboratory (Rappe and Giovannoni 2003). *Rhizobium* belongs to the family Rhizobiaceae and found in soil and root nodules on leguminous plants and benefits the plant by nitrogen fixation (Lodwig *et al*. 2003). *Escherichia coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). *E. coli* belongs to the family Enterobacteriaceae and is the most widely studied prokaryotic model organism (Keseler *et al*. 2005). It was one of the first organisms to have its genome sequenced; the complete genome of *E. coli* K12 was published by *Science* in 1997 (Blattner *et al.*1997).

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E. coli BL21 is an engineered strain and is the most widely used for high-yield expression of recombinant protein (Sorensen and Mortensen 2005). In *E. coli* BL21 several modifications have performed like addition of T7 RNA polymerase gene and transduction of the "malB region" from the K-12 strain W3110 to make the strain $Mal^{+}\lambda^{S}$ (Studier *et al*. 2009). *E. coli* BL21 is a very versatile host for the production of heterologous proteins (Cornelis 2000), and various protein expression systems have been developed which allow the production of recombinant proteins. Researchers can introduce genes into the microbes using plasmids which permit high level expression of protein. As *E. coli* BL21 is a modified strain, so there would be significant difference between engineered and wild type strain. The differences may be in growth rate, carbon accumulation, growth temperature etc. In this research work, we used the genetically engineered *E. coli* BL21 strain and a normal *Rhizobium ciceri* (RCA-220) that nodulate chickpeas (*Cicer arietinum* L.) (Nour *et al*.1994). Considering all these viewpoints, an attempt was made to investigate the growth habit and adaptation capabilities of these bacterial strains under different conditions in the present study.

Materials and Methods

Isolation and Collection of Materials

Strain of *Rhizobium spp.* was obtained from pink colored root nodules of field grown chickpeas in Bangladesh Agricultural Research Institute (BARI). Collected nodules were sterilized in 0.1% (w/v) sodium hypochlorite (NaOCl) for 5min and followed by immersing in 95% (w/v) ethanol for 10sec, and then washed five to six times with distilled water. Then the nodules were crushed with the help of sterile forceps and then 100µL of milky fluid were spread on Yeast Extract Manitol Agar (YEMA) plate (Vincent 1970). After incubation for 3 days at 30°C, bacterial colonies were found to grow on the medium. Single colony was selected and restreaked on agar plate for purify. *E.* coli BL21 strain was obtained by the courtesy of Protein Biochemistry Laboratory, Gwangju, South Korea.

Culture Media

LB (Luria-Bertani) broth and agar medium was used for sub culturing, streaking and plating of these strains. Growth in LB broth incubated at 37°C on an orbital shaker at 160 rpm for 96h was determined by measuring the optical density (OD) at 660 nm every 2h. The generation time was calculated from the logarithmic phase of growth.

Colony Morphology

Colony morphology was evaluated by streaking a loop of the initial inoculum on LB agar and allowing the bacteria to grow at 37°C for 2 to 3 days (Vincent 1970). After 2-3 days of incubation, individual colonies were characterized based on their color, shape and size, and Gram stain reaction. Following plate counting method, cell density of these bacterial strains was calculated.

pH and Temperature Tolerance

The media were evaluated according to different pH variations ranging from 0-10 and tolerance to high temperatures were tested by typing on LB broth and incubating at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C and 50°C.

Growth with Different Carbon Sources

In this research work glucose, glycerol, peptone and sugar were used as carbon sources. Paliy and Gunasekera (2007) suggested that equal concentration of carbon sources is required for obtaining standard result. Following this suggestion, 10g/L carbon sources were added to the medium used in the present study.

Effect of Sodium Chloride (NaCl) and Light

The ability of these strains to grow in different concentrations of salt was tested by streaking strains on LB agar medium containing 0, 5, 10, 15 and 20g per 100ml NaCl concentration. The effect of light on the growth of these strains was also observed using LB broth.

Statistical Analysis

Different statistical approaches such as variance, standard deviation and co-efficient of variation were used as statistical analysis in the present study.

Formula:

Variance, $\sigma^2 = \sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{n}$

Standard deviation, σ = $\sqrt{\sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{n}}$

Co-efficient of variation, CV = $\frac{\sigma}{\bar{x}}$

Results

The bacterial strains were inoculated in LB media to observe their growth efficiency. The highest and the lowest growth rate were observed in 72h and 0hr, respectively. The decline phase was observed in 96h (Fig. 1). Colony of *E. coli*BL21 was transparent, round shaped and white in color (Fig. 2) while *Rhizobium spp*. was opaque, compact, sticky, granule shaped and off-white in color (Fig. 3). Both *E. coli*BL21 and *Rhizobium spp*. were Gram negative rod shaped bacteria and following plate counting method, the cell density of these strains were 5.76×10^{10} and 1.16×10^{15} cells/µl, respectively. Both the strains were grown in LB medium with pH values ranging from 0 - 10 and the maximum growth was observed at pH ranging from 6 to 8 (Fig. 4). Further, the research work was extended to optimize the pH variation and for *E. coli*BL21, the maximum growth was found at pH 7.2 (Fig. 5) while *Rhizobium spp*. showed maximum growth at pH 6.8 (Fig. 5).

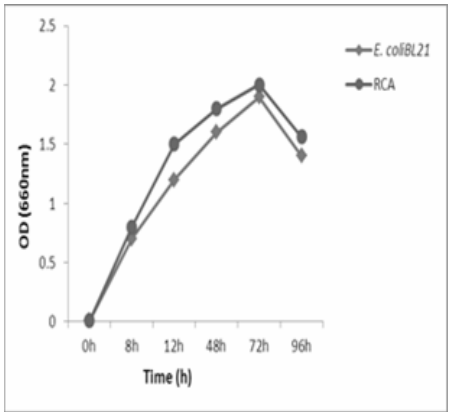

Fig.1. The growth habit of *E. coli*BL21 and *Rhizobium spp*. (RCA-220) at regular time intervals.

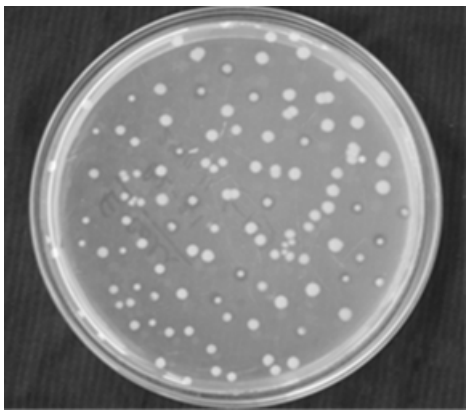

Fig.2. Plate showing the colony nature of *E. coli* BL21.

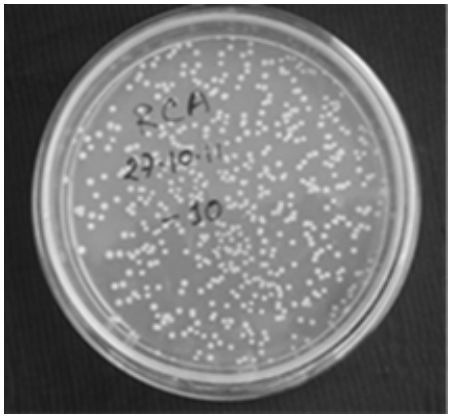

Fig. 3. Plate showing the colony nature of *Rhizobium spp*. (RCA-220).

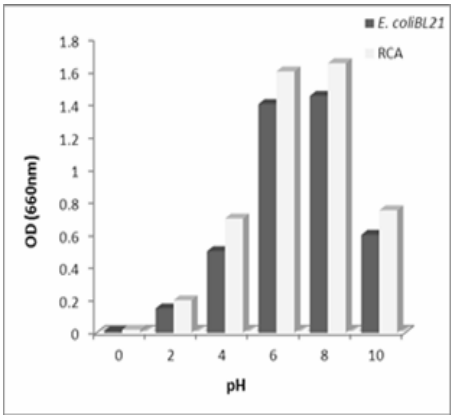

Fig. 4. Effect of pH variations on the growth of *E. coli* BL21 and *Rhizobium spp.* (RCA-220) in 24h of incubation period.

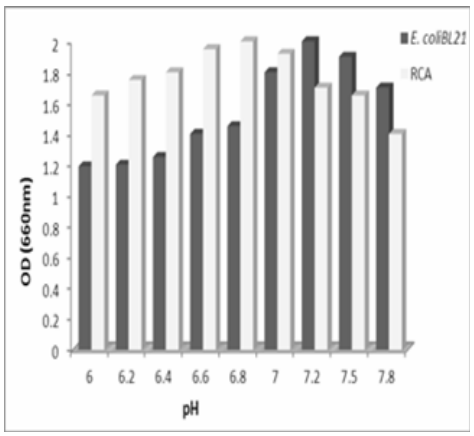

Fig. 5. Growth rates of *E. coli* BL21 and *Rhizobium spp.* (RCA-220) in 48h of incubation period in optimum pH.

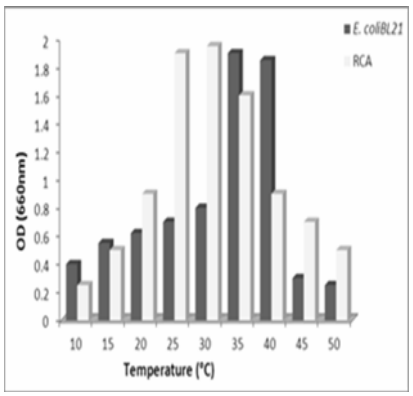

Fig. 6. Growth rates of *E. coli* BL21 and *Rhizobium spp.* in 48h of incubation in LB media with the variations of temperature.

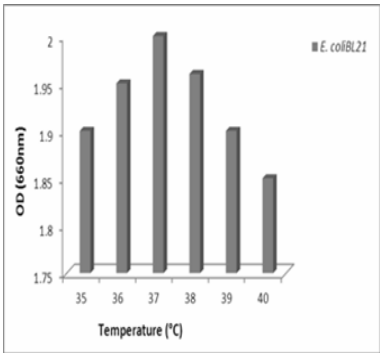

Fig. 7. Growth rates of *E. coli* BL21 in 48h of incubation in LB media in optimum temperature.

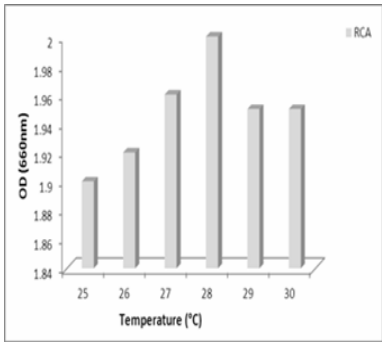

Fig. 8. Growth rates of *Rhizobium spp. (RCA-220)* in 48h of incubation in LB media in optimum temperature.

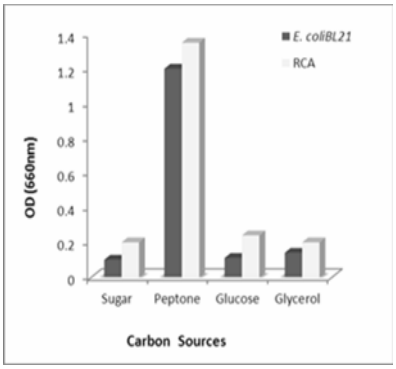

Fig. 9. Growth rates of *E. coli* BL21 and *Rhizobium spp*. (RCA-220) in 72h of incubation period in different carbon sources.

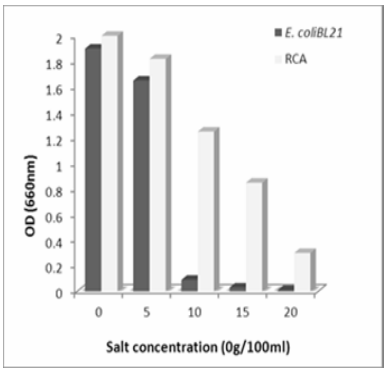

Fig. 10. Growth rates of *E. coli* BL21 and *Rhizobium spp.* (RCA-220) in 24h of incubation period in different salt concentrations.

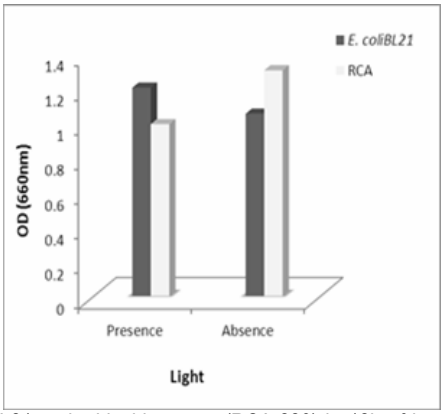

Fig. 11. Growth rates of *E. coli* BL21 and *Rhizobium spp*. (RCA-220) in 12h of incubation period in the presence and absence of light.

E. coli BL21 showed better performance at 35° - 40°C (Fig. 6) and *Rhizobium spp*. showed better growth at 25° - 30°C (Fig. 6). Then, the research work was extended to optimize the temperature variation and for *E. coli*BL21 the highest growth was found at 37°C (Fig. 7) while the maximum growth of *Rhizobium spp*. was found at 28°C (Fig. 8). The growth of these strains at different carbon sources was assessed and it was observed that peptone containing medium showed maximum growth (Fig. 9). On the other hand, strains grown in the medium containing sugar, glucose and glycerol showed poor and slow growth performance. The effect of salt on the growth of these strains was observed and maximum growth was found at 0g/100ml NaCl salt concentration (Fig. 10). In this work, *E. coli* BL21 showed rapid and good performances in presence of light while *Rhizobium spp*. showed better performance in absence of light (Fig. 11). It is also found that the overall growth performance of *Rhizobium spp.* is greater than the engineered *E. coli*BL21. The growth superiority of *Rhizobium spp.* is also confirmed by statistical analysis like calculation of variance (σ^2), standard deviation (σ) and coefficient of variation (CV). At optimum culture conditions (optimized pH, temperature, carbon sources, salt concentration and light) all the CVs for *Rhizobium spp.* is smaller than the CVs for *E. coli* BL21 which proves that the growth of *Rhizobium spp.* followed normal distribution and its growth rate is better than *E. coli* BL21 (Table 1).

Table 1. Estimation of variance, standard deviation and co-efficient of variation by the OD of *E. coli*BL21 and *Rhizobium spp.* that grew at optimum culture condition, σ^2_E indicate the variance of *E. coli*BL21 and σ^2_R indicate the variance of *Rhizobium spp.* (RCA-220).

Growth conditions	Variance (σ^2)		Standard deviation (σ)		Co-efficient of variation (CV)	
	σ^2_E	σ^2_R	σ_E	σ_R	CV_E	CV_R
pH	0.5772	0.6603	0.7597	0.8126	0.8340	0.8134
Temperature	0.4972	0.5688	0.7051	0.7542	0.8315	0.8145
Carbon sources	0.1912	0.3120	0.4373	0.5585	0.8942	0.8178
Salt concentration	0.4922	0.5634	0.7073	0.7506	0.8282	0.8079
Light	0.4294	0.4897	0.6553	0.6998	0.7489	0.7297

Discussion

In this research work, both strains showed the highest growth rate in 72h, while the lowest growth rate was in 0h and the decline phase in 96h. The similar findings have also been reported by Nguyen (2006). *Rhizobium spp*. (RCA- 220) showed better growth performance than *E. coli* BL21 at regular time intervals (Fig. 1). Differences and similarities between two strains were verified using some morphological parameters like shape, size and color. Kucuk *et al*. (2006) reported that the colony of *Rhizobium spp*. is opaque, granule shaped and off-white in color which matches the results of the present study. Considering the cell density of both strains, it is generally comparable that the multiplication ability of *Rhizobium spp.* was much higher than the modified *E. coli* BL21. It was reported that the *E. coli* BL21 grew better at pH 6, 7 and 8 and did not grow at pH 4 and 5 (Schilling 2008), but moderate growth was observed at pH 4, 5 and 10. Similarly, in case of *Rhizobium spp*., reported poor growth was detected at pH 3 and 9 (Kucuk and Kivanc 2008), but moderate growth at pH 10 was found in this research. Both the strains were tolerant at high temperature (Fig. 6) (Soussi *et al.* 2001) and either lower or higher temperature could stall cell growth (Zhu 2007). Previous research of Thakur *et al.* (2010) reported that *E. coli*BL21 showed maximum growth in glycerol supplemented medium but in this research work, among all the

used carbon sources, peptone containing medium showed the rapid and good performance on growth at regular time intervals and the cell density was 1.2 after 72h of incubation (Fig. 9). The present study observed that *Rhizobium spp.* also showed the best performance on growth in peptone supplemented medium (Fig. 9) and the optical density was 1.4. Considering both strains, it was found that the growth rate of *Rhizobium spp.* was significantly higher than *E. coli* BL21. Both strains showed a significant increase in growth at 0g/100ml NaCl salt concentrations (Abdulkarim *et al.* 2009). With further increase in the NaCl concentration, growth was found to steadily decrease but interestingly, *Rhizobium spp*. was able to grow in NaCl as high as 10g/100ml concentration indicating as more tolerant than *E. coli* BL21. Rationale from this finding agreed that *E. coli*BL21 was a modified strain and unable to grow in natural condition, whereas *Rhizobium spp*. occurred in soil and were better adapted in natural conditions like salinity. In case of light, we found that *E. coli* BL21 grew better in presence of light but *Rhizobium spp*. responded well in absence of light. Key finding was that naturally occurring *Rhizobium spp.* showed better growth performances in absence of light as the strain lives in or under the soil where light is not available.

Conclusion

From the above discussions it can be concluded that, if a bacterial strain is modified (*E. coli* BL21) its overall growth performance become reduced than a normal bacterial strain (*Rhizobium spp.*). As certain genetic elements have been changed or removed by genetic engineering techniques to modify a bacterium might lead to its slow growth rate. The obtained results may help the researchers to know further differences and similarities between engineered bacteria and normal bacterial strain and the information might be valuable for researchers to do future experiments in the field of microbiology.

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