Original Research Article

Comparative study of the effect of various parameters on growth and antimicrobial activity of probiotics

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ABSTRACT

The Lactobacilli show pronounced antibacterial activity between early logarithmic and early stationary phase. The maximum activity in composed medium was achieved at initial pH of 6, incubation temperature 37°C, and incubation period of 24h under static condition. Supplementation or replacement of nutrients demonstrated that larger quantities of growth could be produced. The compound produced by Lactobacilli was fully inactivated by some of the proteolytic enzymes, which confirms their proteinaceous nature. This study aimed to modify de Man Rogosa Sharpe culture medium (termed MRS) for selective cultivation of probiotics strain.

Introduction

Probiotics are an important part of the complex world of foods that are good for health. These bacteria have belong to the natural flora in order to be able to resist acid and bile, to survive during intestinal transit, to adhere to the intestinal mucosa and to produce antimicrobial substances in order to retain the characteristics that contribute to their beneficial health effects (Fuller, 1989). Probiotic organisms are thought to act through a variety of mechanisms which includes (a) Compete with potential pathogens for nutrients and growth factors or enterocyte adhesion sites or substract and involved in modifying the gut pH. (b) Degradation of toxins, production of antimicrobial substances, and antioxidants (Caglar et al., 2006). The lactic acid bacteria need to withstand varying environmental conditions including differences in temperature, pH and salinity, depending on the specific application. Glucose is frequently used as a carbon source in lactic acid fermentation. For instance, it had been used to promote lactic acid fermentation of shrimp wastes (Rao et al. 2000). For application of these strains in processes where high salt concentrations are required or are naturally present, their salt tolerance should be studied. Combined effect of low initial pH and salt addition may present additional advantages.
Materials and Methods

**Microorganisms:** Bacterial pure cultures *L. casei, L. fermentum, L. rhamnosus* and *S. thermophilus* were obtained from culture collection of National Dairy Research Institute (NDRI), Karnal, and the stock cultures were maintained by periodic subculture and Lactobacillus Man Ragosa& Sharp (MRS) Agar Medium (40°C).

**Culture media:** The cultures *L. casei, L. fermentum, L. rhamnosus* and *S. thermophilus* were inoculated on MRS agar and incubated at 370°C for 24 hr until extensive growth of colonies were seen.

**Preparation of inoculum:** Growth of organism appeared on Lactobacillus MRS Agar Medium after 24–48hrs of incubation, and the inoculum was transferred to 5% peptone water with inoculation loop. The inoculum strength was checked at 540nm and set at OD 0.3 to be used as inoculum when OD is 1.0 the dry weight is 0.28 (Rosemarei *et al.*, 1989).

**Growth analysis of probiotics using different parameters**

Comparative growth on different selective media

Different media selected were followed as:

1. MRS broth
2. Tomato juice broth (TJB)
3. Tryptone glucose yeast extract (TGYE)

With all standard and optimal conditions, various selective media were examined as optimal media for growth of probiotic bacteria. 1ml of inoculums, with OD 0.3 at 540 nm was added to various media (9ml) and incubated at 37°C. Variable media of same concentration are used, results were recorded for 4 days with an interval of 24 hrs compared with reference to control.

Comparative growth on variable carbon sources

Different organic carbon sources selected were as:

1. Glucose
2. Fructose
3. Maltose

With all standard and optimal conditions, an additional source of carbon was used to examine as nutrient sources for given cultures of bacteria. 20gm/L concentration of various sources was selected. 1ml of inoculum, with OD 0.3 at 540 nm was added to MRS media (9ml) and incubated at 37°C. Variable sources of same concentration were used, results were recorded for 4 days with an interval of 24 hrs compared with reference to control.

Comparative growth on various nitrogen sources:

An additional source of nitrogen (organic) was used to examine as nutrient for growth of given cultures of bacteria. Variable nitrogen sources (peptone 10 g/L, Yeast extract 5g/L, Casein 5g/L) of same concentration were used and 1ml of inoculum, with OD 0.3 at 540 nm was added to MRS media (9ml) and incubated at 37°C. Results were recorded for 4 days incubation with an interval of 24 hrs compared with reference to control.

Comparative growth on various phosphorous sources:

Various source of phosphorous (KH₂PO₄, K₂HPO₄) inorganic, was used to examine as nutrient for growth of given cultures of bacteria. 1ml of inoculum, with OD 0.3 at 540 nm was added to MRS media (9ml) and incubated at 37°C. Variable sources of phosphorous of same concentration were used and results were recorded for 4 days.
incubation with an interval compared with reference to control.

**Comparative growth on various pH:**

An additional condition of variable temperature pH 5.5, pH 6.5 & pH 7.5 was used to examine as condition for growth of given cultures of bacteria. 1ml of inoculum, with OD 0.3 at 540 nm was added to MRS media (9ml) and incubated at 37°C. Variable temperature was used and results were recorded for 4 days incubation with an interval of 24 hrs compared with reference to control.

**Comparative growth on various temperatures:**

An additional condition of variable temperature 32°C, 37°C and 42°C was used to examine as condition for growth of given cultures of bacteria. 1ml of inoculum, with OD 0.3 at 540 nm was added to MRS media (9ml) and incubated at 37°C. Variable temperature was used and results were recorded for 4 days incubation with an interval of 24 hrs compared with reference to control.

**Determination of antimicrobial activity of Probiotics – test pathogens by well diffusion method and over lay method**

**Well Diffusion Method:** Two strains of pathogens were included in study (E. coli, S. typhi). Selective media were used to test the anti microbial activity against these pathogens. 0.1 ml dilution of each pathogen were tested by pour plate method, incubated anaerobically for 4 days for growth, then another layer of media having either pure culture and isolated culture was over laid on it, results were recorded after 4 days of anaerobic incubation.

**Results and Discussion**

It was inferred that L. casei grew well on peptone as additional organic nitrogen sources and rest three strains grew well on casein that is in contrast to Nebra and Blanch (1999) who observed no effect of casein on *Bifidobacterium* spp and there was little stimulation in case of yeast extract that is in accordance to Todorov and Dicks (2005) and they observed stimulation by tryptone and no stimulation by yeast extract.

**Studies on inorganic phosphorous sources**

(a) *L. casei*

Use of different inorganic phosphorous sources was examined and it was observed according to data given in Table 12(a). According to data growth in KH$_2$PO$_4$ is highest 64% in comparison to K$_2$HPO$_4$.

(b) *L. fermentum*

Use of different inorganic phosphorous sources was examined and it was observed according to data given in Table according to data growth in K$_2$HPO$_4$ is highest 66% in comparison to KH$_2$PO$_4$.

(c) *L. rhamnosus*

Use of different inorganic phosphorous sources was examined and it was observed according to data given below in Table according to data growth in KH$_2$PO$_4$ is
highest 64% in comparison to $K_2HPO_4$.

**S. thermophillus**

Use of different inorganic phosphorous sources was examined and it was observed according to data given below in Table 6.1(a). According to data growth in $K_2HPO_4$ is highest 35% in comparison to $KH_2PO_4$.

It was concluded that $L. casei$ and $L. rhamnosus$ gave high percentage increase in $KH_2PO_4$ as inorganic phosphorous sources and no stimulation by $K_2HPO_4$ that may be due to low concentration as observed by Todorov and Dicks (2005) and rest of strains gave high percentage increase in $K_2HPO_4$ as inorganic phosphorous sources. Mitchell (1954) showed that maximum inhibitory effect of probiotics by uptake of $H_3PO_4$ rather than $HPO_4$.

**Studies using inorganic carbon sources**

**L. casei**

Use of different inorganic carbon sources was examined and it was observed according to data given in table. According to data, growth in $CaCO_3$ is highest 135% in comparison to ammonium carbonate and $H_2CO_3$.

**L. fermentum**

Use of different inorganic carbon sources was examined and it was observed according to data given in table. According to data growth in ammonium carbonate is highest 135% in comparison to $CaCO_3$ and $H_2CO_3$.

**L. rhamnosus**

Use of different inorganic carbon sources was examined and it was observed according to data given in table. According to data growth in Ammonium carbonate is highest 135% in comparison to $CaCO_3$ and $H_2CO_3$.

**S. thermophillus**

Use of different inorganic carbon sources was examined and it was observed according to data given below in table. According to data growth in $CaCO_3$ is highest 82% in comparison to Ammonium carbonate and $H_2CO_3$.

**The comparative growth on various pH**

The data given below shows that optimum pH for $L. casei$, $L. rhamnosus$, $L. fermentum$, $S. thermophillus$ shows that optimum pH is 6.5.

It has been inferred that optimum pH for growth of these cultures is pH 6.5 but probiotics are taken orally and it has to pass through gastrointestinal tract and has to withstand various conditions i.e. acidic. So probiotics can not only persist in different conditions but can also grow Rishi et al (2008).

**The comparative growth on different temperatures**

The data given below shows those optimum temperatures for $L. casei$, $L. rhamnosus$, $L. fermentum$, $S. thermophillus$ is 37°C. It was concluded that optimum temperature for all four culture grow well at 37°C but $S. thermophillus$ showed good growth at 42°C.

**Determination of antimicrobial activity of Probiotics – Test pathogens by Well Diffusion Method and Overlay method.**

Data presented in table given involves the use of agar well method with reference to sensitivity of test pathogens in presence of pathogens. Antimicrobial sensitivity
showing zone of inhibition against *E. coli*. Result of the agar overlay method showed that all of the probiotic strains were showing inhibition against test microbial isolates. The spectrum of their antimicrobial activity varied.

**Table 1(a)** Studies using different growth media

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>TGYE</th>
<th>MRS</th>
<th>TJB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.3696</td>
<td>0.237</td>
<td>0.580</td>
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<tr>
<td>48</td>
<td>0.0047</td>
<td>0.0865</td>
<td>0.926</td>
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<tr>
<td>72</td>
<td>0.358</td>
<td>0.4284</td>
<td>0.7112</td>
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<tr>
<td>96</td>
<td>0.629</td>
<td>0.794</td>
<td>0.688</td>
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</table>

**Table 2(b)** Studies using different growth media

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>TGYE</th>
<th>MRS</th>
<th>TJB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
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</tr>
<tr>
<td>24</td>
<td>0.3696</td>
<td>0.414</td>
<td>0.823</td>
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<tr>
<td>48</td>
<td>0.0047</td>
<td>0.257</td>
<td>1.004</td>
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<tr>
<td>72</td>
<td>0.358</td>
<td>0.616</td>
<td>0.756</td>
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<td>96</td>
<td>0.629</td>
<td>0.628</td>
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**Table 3(c)** Studies using different growth media

<table>
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<th>Percentage increase in biomass</th>
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<th>MRS</th>
<th>TJB</th>
</tr>
</thead>
<tbody>
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<td>Time Period (hrs)</td>
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<td></td>
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<td>0.338</td>
<td>0.336</td>
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<td>48</td>
<td>0.003</td>
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<td>72</td>
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<td>96</td>
<td>0.629</td>
<td>0.435</td>
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**Table 4(d)** Studies using different growth media

<table>
<thead>
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<th>TGYE</th>
<th>MRS</th>
<th>TJB</th>
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</thead>
<tbody>
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<td>48</td>
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### Table 5(a) Studies using different organic carbon sources

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Maltose</th>
</tr>
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<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
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<tr>
<td>24</td>
<td>0.2452</td>
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<td>0.4816</td>
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<tr>
<td>72</td>
<td>0.387</td>
<td>0.5118</td>
<td>0.561</td>
</tr>
<tr>
<td>96</td>
<td>0.389</td>
<td>0.4566</td>
<td>0.588</td>
</tr>
</tbody>
</table>

### Table 6(b) Studies using different organic carbon sources

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
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<td>24</td>
<td>0.2198</td>
<td>0.279</td>
<td>0.2447</td>
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<td>48</td>
<td>0.426</td>
<td>0.455</td>
<td>0.430</td>
</tr>
<tr>
<td>72</td>
<td>0.4211</td>
<td>0.451</td>
<td>0.458</td>
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<td>96</td>
<td>0.408</td>
<td>0.4382</td>
<td>0.459</td>
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### Table 7(c) Studies using different organic carbon sources

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<tr>
<th>Percentage increase in biomass</th>
<th>glucose</th>
<th>lactose</th>
<th>Maltose</th>
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</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
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<tr>
<td>24</td>
<td>0.222</td>
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<td>0.3612</td>
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<td>48</td>
<td>0.447</td>
<td>0.449</td>
<td>0.503</td>
</tr>
<tr>
<td>72</td>
<td>0.4886</td>
<td>0.460</td>
<td>0.530</td>
</tr>
<tr>
<td>96</td>
<td>0.495</td>
<td>0.507</td>
<td>0.4919</td>
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</table>

### Table 8(d) Studies using different organic carbon sources

<table>
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<tr>
<th>Percentage increase in biomass</th>
<th>glucose</th>
<th>lactose</th>
<th>Maltose</th>
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</thead>
<tbody>
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<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
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<tr>
<td>24</td>
<td>0.229</td>
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<td>0.313</td>
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<td>48</td>
<td>0.435</td>
<td>0.497</td>
<td>0.456</td>
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<tr>
<td>72</td>
<td>0.447</td>
<td>0.479</td>
<td>0.4312</td>
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<tr>
<td>96</td>
<td>0.447</td>
<td>0.482</td>
<td>0.4244</td>
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### Table 9(a) Studies using different organic nitrogen sources

<table>
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<th>lactose</th>
<th>Maltose</th>
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<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
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<tr>
<td>24</td>
<td>0.856</td>
<td>0.276</td>
<td>0.245</td>
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<td>48</td>
<td>0.7896</td>
<td>0.337</td>
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<td>72</td>
<td>0.5596</td>
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<td>96</td>
<td>0.2904</td>
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**Table 10(b)** Studies using different organic nitrogen sources

<table>
<thead>
<tr>
<th>Time Period (hrs)</th>
<th>Concentration of biomass (mg dry wt/ml)</th>
<th>Percentage increase in biomass for glucose</th>
<th>Percentage increase in biomass for lactose</th>
<th>Percentage increase in biomass for Maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.275</td>
<td>0.437</td>
<td>0.267</td>
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<td>48</td>
<td>0.491</td>
<td>0.573</td>
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<tr>
<td>72</td>
<td>0.515</td>
<td>0.634</td>
<td>0.600</td>
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<tr>
<td>96</td>
<td>0.454</td>
<td>0.572</td>
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**Table 11(c)** Studies using different organic nitrogen sources

<table>
<thead>
<tr>
<th>Time Period (hrs)</th>
<th>Concentration of biomass (mg dry wt/ml)</th>
<th>Percentage increase in biomass for glucose</th>
<th>Percentage increase in biomass for lactose</th>
<th>Percentage increase in biomass for Maltose</th>
</tr>
</thead>
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<td>0.535</td>
<td>0.525</td>
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<tr>
<td>48</td>
<td>0.501</td>
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<td>0.684</td>
<td>0.576</td>
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<td>0.490</td>
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**Table 12(d)** Studies using different organic nitrogen sources

<table>
<thead>
<tr>
<th>Time Period (hrs)</th>
<th>Concentration of biomass (mg dry wt/ml)</th>
<th>Percentage increase in biomass for glucose</th>
<th>Percentage increase in biomass for lactose</th>
<th>Percentage increase in biomass for Maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.313</td>
<td>0.595</td>
<td>0.336</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>0.456</td>
<td>0.628</td>
<td>0.414</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>0.506</td>
<td>0.659</td>
<td>0.620</td>
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<tr>
<td>96</td>
<td>0.313</td>
<td>0.595</td>
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**Table 13(a)** Studies using different inorganic phosphorous sources

<table>
<thead>
<tr>
<th>Time Period (hrs)</th>
<th>Concentration of biomass (mg dry wt/ml)</th>
<th>Percentage increase in biomass for KH2PO4</th>
<th>Percentage increase in biomass for K2HPO4</th>
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<tbody>
<tr>
<td>24</td>
<td>0.279</td>
<td>0.560</td>
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<tr>
<td>48</td>
<td>0.382</td>
<td>0.625</td>
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<tr>
<td>72</td>
<td>0.5118</td>
<td>0.561</td>
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</tr>
<tr>
<td>96</td>
<td>0.458</td>
<td>0.588</td>
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**Table 14(b)** Studies using different inorganic phosphorous sources

<table>
<thead>
<tr>
<th>Time Period (hrs)</th>
<th>Concentration of biomass (mg dry wt/ml)</th>
<th>Percentage increase in biomass for KH2PO4</th>
<th>Percentage increase in biomass for K2HPO4</th>
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<tbody>
<tr>
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<td>72</td>
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<tr>
<td>96</td>
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**Table 15(c)** Studies using different inorganic phosphorous sources

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>KH$_2$PO$_4$</th>
<th>K$_2$HPO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
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**Table 16(d)** Studies using different inorganic phosphorous sources

<table>
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<tr>
<th>Percentage increase in biomass</th>
<th>KH$_2$PO$_4$</th>
<th>K$_2$HPO$_4$</th>
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</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
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<td>0.432</td>
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</tr>
</tbody>
</table>

**Table 17(a)** Studies using different inorganic carbon sources

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>CaCO$_3$</th>
<th>Ammonium carbonate</th>
<th>H$_2$CO$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.267</td>
<td>0.238</td>
<td>0.272</td>
</tr>
<tr>
<td>48</td>
<td>0.460</td>
<td>0.322</td>
<td>0.336</td>
</tr>
<tr>
<td>72</td>
<td>0.848</td>
<td>0.440</td>
<td>0.738</td>
</tr>
<tr>
<td>96</td>
<td>0.563</td>
<td>0.375</td>
<td>0.096</td>
</tr>
</tbody>
</table>

**Table 18(b)** Studies using different inorganic carbon sources

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>CaCO$_3$</th>
<th>Ammonium carbonate</th>
<th>H$_2$CO$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.245</td>
<td>0.275</td>
<td>0.247</td>
</tr>
<tr>
<td>48</td>
<td>0.2979</td>
<td>0.181</td>
<td>0.327</td>
</tr>
<tr>
<td>72</td>
<td>0.769</td>
<td>0.343</td>
<td>0.401</td>
</tr>
<tr>
<td>96</td>
<td>0.362</td>
<td>0.397</td>
<td>0.264</td>
</tr>
</tbody>
</table>

**Table 19(c)** Studies using different inorganic carbon sources

<table>
<thead>
<tr>
<th>Percentage increase in biomass</th>
<th>CaCO$_3$</th>
<th>Ammonium carbonate</th>
<th>H$_2$CO$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.250</td>
<td>0.257</td>
<td>0.247</td>
</tr>
<tr>
<td>48</td>
<td>0.2979</td>
<td>0.181</td>
<td>0.327</td>
</tr>
<tr>
<td>72</td>
<td>0.769</td>
<td>0.343</td>
<td>0.401</td>
</tr>
<tr>
<td>96</td>
<td>0.362</td>
<td>0.397</td>
<td>0.246</td>
</tr>
</tbody>
</table>
### Table 20(d) Studies using different inorganic carbon sources

<table>
<thead>
<tr>
<th>Time Period (hrs)</th>
<th>CaCO₃</th>
<th>Ammonium carbonate</th>
<th>H₂CO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.213</td>
<td>0.274</td>
<td>0.217</td>
</tr>
<tr>
<td>48</td>
<td>0.387</td>
<td>0.306</td>
<td>0.2614</td>
</tr>
<tr>
<td>72</td>
<td>0.589</td>
<td>0.496</td>
<td>0.362</td>
</tr>
<tr>
<td>96</td>
<td>0.387</td>
<td>0.371</td>
<td>0.258</td>
</tr>
</tbody>
</table>

### Table 21(a) Studies using different pH

<table>
<thead>
<tr>
<th>pH</th>
<th>pH5.5</th>
<th>pH6.5</th>
<th>pH7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME PERIOD (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.470</td>
<td>0.5516</td>
<td>0.434</td>
</tr>
<tr>
<td>48</td>
<td>0.966</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>72</td>
<td>1.038</td>
<td>1.07</td>
<td>1.753</td>
</tr>
<tr>
<td>96</td>
<td>0.190</td>
<td>0.229</td>
<td>0.221</td>
</tr>
</tbody>
</table>

### Table 22(b) Studies using different pH

<table>
<thead>
<tr>
<th>pH5.5</th>
<th>pH6.5</th>
<th>pH7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage increase in biomass</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
</tr>
<tr>
<td>Time Period (hrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.644</td>
<td>0.63</td>
</tr>
<tr>
<td>48</td>
<td>0.988</td>
<td>1.008</td>
</tr>
<tr>
<td>72</td>
<td>1.055</td>
<td>1.07</td>
</tr>
<tr>
<td>96</td>
<td>0.794</td>
<td>0.263</td>
</tr>
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</table>

### Table 23(c) Studies using different pH

<table>
<thead>
<tr>
<th>pH5.5</th>
<th>pH6.5</th>
<th>pH7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME PERIOD (hrs)</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.63</td>
<td>0.627</td>
</tr>
<tr>
<td>48</td>
<td>0.99</td>
<td>1.01</td>
</tr>
<tr>
<td>72</td>
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<td>1.1</td>
</tr>
<tr>
<td>96</td>
<td>0.19</td>
<td>0.243</td>
</tr>
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</table>

### Table 24(d) Studies using different pH

<table>
<thead>
<tr>
<th>pH5.5</th>
<th>pH6.5</th>
<th>pH7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage increase in biomass</td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
</tr>
<tr>
<td>TIME PERIOD (hrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.512</td>
<td>0.512</td>
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<tr>
<td>48</td>
<td>0.9716</td>
<td>0.9716</td>
</tr>
<tr>
<td>72</td>
<td>1.09</td>
<td>1.09</td>
</tr>
<tr>
<td>96</td>
<td>0.222</td>
<td>0.222</td>
</tr>
</tbody>
</table>
### Table 25(a) Studies using different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>32°C</th>
<th>37°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TIME PERIOD (hrs)</strong></td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.442</td>
<td>0.613</td>
<td>0.476</td>
</tr>
<tr>
<td>48</td>
<td>0.926</td>
<td>0.996</td>
<td>0.994</td>
</tr>
<tr>
<td>72</td>
<td>1.10</td>
<td>1.052</td>
<td>1.10</td>
</tr>
<tr>
<td>96</td>
<td>0.227</td>
<td>0.213</td>
<td>0.225</td>
</tr>
</tbody>
</table>

### Table 26(b) Studies using different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>32°C</th>
<th>37°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TIME PERIOD (hrs)</strong></td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.554</td>
<td>0.618</td>
<td>0.554</td>
</tr>
<tr>
<td>48</td>
<td>1.005</td>
<td>1.03</td>
<td>1.02</td>
</tr>
<tr>
<td>72</td>
<td>1.12</td>
<td>1.153</td>
<td>1.3</td>
</tr>
<tr>
<td>96</td>
<td>0.1988</td>
<td>0.170</td>
<td>0.21</td>
</tr>
</tbody>
</table>

### Table 27(c) Studies using different temperature

<table>
<thead>
<tr>
<th>Temperature</th>
<th>32°C</th>
<th>37°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TIME PERIOD (hrs)</strong></td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.621</td>
<td>0.537</td>
<td>0.585</td>
</tr>
<tr>
<td>48</td>
<td>1.02</td>
<td>1.036</td>
<td>1.008</td>
</tr>
<tr>
<td>72</td>
<td>1.14</td>
<td>1.122</td>
<td>1.052</td>
</tr>
<tr>
<td>96</td>
<td>0.24</td>
<td>0.2184</td>
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</tr>
</tbody>
</table>

### Table 28(d) Studies using different temperature

<table>
<thead>
<tr>
<th>Temperature</th>
<th>32°C</th>
<th>37°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time Period (hrs)</strong></td>
<td>Concentration of biomass (mg dry wt/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.504</td>
<td>0.453</td>
<td>0.1792</td>
</tr>
<tr>
<td>48</td>
<td>0.952</td>
<td>1.03</td>
<td>0.9408</td>
</tr>
<tr>
<td>72</td>
<td>1.08</td>
<td>1.11</td>
<td>1.117</td>
</tr>
<tr>
<td>96</td>
<td>0.1876</td>
<td>0.204</td>
<td>0.229</td>
</tr>
</tbody>
</table>
Figures 16 a, b, c & d Results of Agar Well method (Figures a&b) and overlay method (Figures c&d)

Table 29 Zone of inhibition against test pathogens

<table>
<thead>
<tr>
<th>Test organism</th>
<th>ZONE (Diameter) OF INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L.casei</td>
</tr>
<tr>
<td>E.coli</td>
<td>17mm</td>
</tr>
<tr>
<td>S.typhi</td>
<td>15mm</td>
</tr>
</tbody>
</table>

References


