Short Communication

GROWTH OF THIOBACILLUS FERROOXIDANS ON SOLID MEDIUM: EFFECTS OF SOME SURFACE-ACTIVE AGENTS ON COLONY FORMATION

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A number of solid media to cultivate Thiobacillus ferrooxidans have been developed with satisfactory results (2, 3, 5, 6, 8, 10, 11). In all of these reports, mineral salt solutions or acidified water have been used to prepare dilutions of T. ferrooxidans liquid cultures for plating and special emphasis was placed on medium composition and the type and brand of gelling agents used. However, regardless of medium composition or preparation procedure the T. ferrooxidans colonies are very small and frequently clustered. Therefore, their isolation or enumeration usually cause a difficult problem. Although the utilization of insoluble compounds as energy source (elemental sulfur or metallic sulfides) by Thiobacilli species is increased to some extent with addition of some surfactants to the culture medium (1, 4, 7, 9), the effects of these surface-active agents on T. ferrooxidans growth on solid medium have not been tested. In this paper we tested the utilization of some surfactants to prepare solutions for serial dilutions of T. ferrooxidans before plating. The results showed that at least one of these surfactants (Tween 80) can be used to improve the quality of T. ferrooxidans plates.

Bacterial strain. The T. ferrooxidans strain used throughout the study (Tf-LR) was isolated from acid leach liquor of Lagoa Real uranium ore from the State of Bahia, Brazil. For comparative purposes, we used four strains from our collection and the strains Tf-V3 and Tf-I35 kindly supplied by Dr. S. Groudev and Dr. O. H. Tuovinen, respectively.

Culture medium. The strain was routinely cultivated in a liquid medium described by Tuovinen and Kelly (10), with slight modifications: 0.1 g/l, K$_2$HPO$_4$, and initial pH 1.8. The solid medium was prepared by combining this medium

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(two-fold concentrated) with an equal volume of an aqueous solution of 0.9% (w/v) agarose (BRL, MD-USA). Basal salts solution of the medium was sterilized by autoclaving at 121°C for 20 min and the ferrous iron solution was filter-sterilized. Agarose solution was separately sterilized by autoclaving and allowed to cool to around 55°C, before mixing with the complete medium. The mixture was then poured immediately into Petri dishes. The gel obtained (0.45% w/v final agarose concentration) was sufficiently firm for spreading the inoculum with a bent glass rod.

**Surface-active agents.** The surfactants were only used to prepare the solutions for serial dilutions of the liquid bacterial cultures. The following agents were utilized: sodium dodecyl sulfate (SDS), octyl phenoxy polyethoxylated ethanol (Triton X-100), polyoxyethylene sorbitan monopalmitate (Tween 40) and polyoxyethylene sorbitan monooleate (Tween 80). Tubes containing 9 ml of an acid solution (pH 1.8) of each agent at concentrations of 0.1, 0.5 and 1% (w/v) were prepared; H₂O, pH 1.8, was used as control. The tubes were sterilized by autoclaving at 120°C for 20 min, and serial dilutions were made using 1 ml of a 48-h culture of Tf-LR. Aliquots of 0.1 ml from 10⁻⁵, 10⁻⁶ and 10⁻⁷ dilutions of each treatment were spread onto the plates, and these were incubated at 30°C for 10 days. The colonies were counted directly and their diameters were measured (10 colonies plate dilution, except in 10⁻⁷ dilution) with an ocular micrometer fitted to in a Zeiss Citoval-2 stereomicroscope (Carl Zeiss-Jena, RDA).

**Photography.** Photomacrographs of colonies were taken directly from the plates using a Plus-X pan film (Kodak) and an Asahi-Pentax model Spotmatic-F camera (Japan). Photomicrographs were obtained with the stereomicroscope cited above, equipped with an Automatic-2 model mf-AKS photographic apparatus (Carl Zeiss-Jena, RDA).

In a qualitative test we evaluated the influence of four surfactants on the growth of *T. ferrooxidans* on solid medium. SDS and Triton X-100 inhibited strongly colony formation even at the lowest concentration. The surfactants Tween 40 (0.5 and 1.0% w/v) and Tween 80 (1.0% w/v) also caused a reduction in the number of colonies. However, at the other concentrations (0.1% w/v Tween 40 and 0.1 and 0.5% w/v Tween 80) this effect was not detected. An interesting result observed in these conditions was that even when reduction in the number of colonies occurred, the colonies were larger and well spread onto the plates in comparison with those obtained in the control (H₂O, pH 1.8).

In order to assure that colony size increased and cell viability did not decrease by addition of surfactant, we carried out a quantitative experiment utilizing only 0.5% (w/v) Tween 80 for serial dilutions of the culture in comparison with the control; in this test we utilized flasks containing 45 ml of the surfactant or H₂O, pH 1.8, instead of 9 ml tubes. Table 1 shows that control colonies had a mean diameter of 0.32 and 0.56 mm (10⁻⁵ and 10⁻⁶ dilutions, respectively) whereas 0.5% (w/v) Tween colonies had 0.82 and 1.36 mm, corresponding to six-fold increase in colony area. The sizes of *T. ferrooxidans* colonies reported in other publications (2, 5, 11)
never exceeded 0.5 to 0.7 mm in diameter after the same period of incubation. As also shown in Table 1, there were no differences in the number of colonies obtained in both conditions, indicating that Tween 80 did not affect cell viability. The number of colonies obtained in this study (ca. \(2 \times 10^5\) ml) is in agreement with data reported by others (3,10,11). Furthermore, reproducibility was good both within the same dilution and among dilutions (\(10^{-5}\), \(10^{-6}\) and \(10^{-7}\)) in both situations. This reproducibility was not found in the experiments where we utilized tubes containing 9 ml of solution to dilute the culture for plating.

Figure 1 shows plates for both treatments at \(10^{-5}\), \(10^{-6}\) and \(10^{-7}\) dilutions. We can see that with Tween 80, even at the lowest dilution (\(10^{-5}\); ca. 200 colonies/plate), colonies were spread out and easily countable. Similar results were

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### Table 1. Effects of surfactant addition on the growth of *T. ferrooxidans* (TF-LR) on the agarose plates.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Dilution</th>
<th>Number of colonies&lt;sup&gt;a&lt;/sup&gt; (Relative)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Diameter of colonies&lt;sup&gt;c&lt;/sup&gt; (Relative)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control with</td>
<td>(10^{-3})</td>
<td>204.7±7.8</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O, pH 1.8</td>
<td>(10^{-4})</td>
<td>20.1±0.6</td>
<td>0.56±0.03</td>
</tr>
<tr>
<td></td>
<td>(10^{-7})</td>
<td>1.9±0.2</td>
<td>—</td>
</tr>
<tr>
<td>0.5% (w/v) Tween 80</td>
<td>(10^{-5})</td>
<td>194.3±8.5</td>
<td>0.82±0.01</td>
</tr>
<tr>
<td></td>
<td>(10^{-6})</td>
<td>(0.95)</td>
<td>(2.56)</td>
</tr>
<tr>
<td></td>
<td>(10^{-7})</td>
<td>(1.03)</td>
<td>(2.42)</td>
</tr>
<tr>
<td></td>
<td>(10^{-7})</td>
<td>2.3±0.3</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean values±standard deviation for 10 plates/dilution (inoculum size: 0.1 ml/plate).

<sup>b</sup> Relative values to the data for control experiment.

<sup>c</sup> Mean values in mm±standard deviation, for 10 colonies/plate.

<sup>d</sup> Relative values to the data for control experiments.

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![Fig. 1. Growth of *T. ferrooxidans* (TF-LR) on solid medium after dilution for plating in H<sub>2</sub>O, pH 1.8 (A) and 0.5% (w/v) Tween 80 (B). Dilutions from left to right: \(10^{-5}\), \(10^{-6}\) and \(10^{-7}\). Inoculum size: 0.1 ml.](image)
obtained with other agarose brands such as Bio-Rad (CA-USA), Sigma (MO-USA) and Inlab (SP-Brazil), utilized to solidify the medium. Moreover, the other strains of *T. ferrooxidans* tested showed the same behavior when diluted with 0.5% (w/v) Tween 80 before plating. The mechanism by which Tween 80 stimulated the growth of *T. ferrooxidans* on solid medium is yet under investigation in our laboratory.

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REFERENCES


