Soy protein isolate: A substitute of fetal bovine serum for the in vitro cultivation of Leishmania donovani

Arushdeep Sidana1,2, Afroz Alam3 and Umar Farooq1,3*

Molecular and Immuno-parasitology Laboratory, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan -173 229, Himachal Pradesh, India.
Received: 14-05-2016 Accepted: 17-09-2016 DOI: 10.18805/LR-3730

ABSTRACT
Fetal Bovine Serum (FBS) is an expensive source of macronutrients which are required for proper nutrition of Leishmania parasite in the culture medium. An alternative, cost effective source of macronutrients which can replace the use of FBS in tissue culture medium is required. The potential of Soy Protein Isolate (SPI) to replace FBS in RPMI-1640 medium for the in vitro cultivation of Leishmania donovani was evaluated. Commercially available SPI powder was used in RPMI-1640 medium as a substitute of FBS to cultivate L. donovani promastigotes. The growth, multiplication and morphology of cultivated parasites was observed in conventional RPMI-1640 with 10% FBS (v/v) and RPMI-1640 containing 10% SPI (v/v) by using light microscopy, measurement of absorbance and cell counting. The growth of Leishmania promastigotes in the medium containing 10% SPI was slower in initial phase; however, the parasites were morphologically larger as compared to those in RPMI-1640 medium containing 10% FBS. Cell count in the SPI-containing RPMI-1640 medium was 2.3 × 10^8 cells/ml whereas it was 1.9 × 10^7 cells/ml in RPMI-1640 with 10% FBS. This study concludes that RPMI-1640 may be supplemented with SPI instead of FBS for the in vitro cultivation of Leishmania donovani promastigotes to decrease the culture maintenance cost in developing countries.

Key words: FBS, Leishmania cultivation, Leishmania donovani, Medium designing, Soy protein isolate.

INTRODUCTION
Leishmaniasis is a major public health problem and causes high morbidity and mortality in tropical and subtropical countries of the world including India. It is caused by an obligate intracellular protozoan parasite of the genus Leishmania. Research is going on worldwide to solve the problems related to the diagnosis, treatment and prevention of leishmaniasis. The basic requirement for the on-going research is in vitro cultivated Leishmania parasite. The cultivation of Leishmania is commonly carried out in the media requiring fetal bovine/calf serum as a source of protein and other macronutrients without which the parasite cannot grow and multiply. Fetal bovine serum (FBS) is the most expensive component required for the cultivation of Leishmania parasite and is not readily available in most of the developing countries. FBS also require deep freezers for storage and get contaminated very rapidly if proper storage conditions are not provided.

There is a need to replace FBS with some other cost effective and readily available source of macronutrients. Soy bean (Glycine max), belonging to the plant family Fabaceae, is native to East Asia and is cultivated for its edible bean. It is widely used for oil extraction from the beans, as animal feed, in infant foods and meat and dairy alternatives (Dixit et al. 2011). The present study was designed to assess the potential of commercially available Soy Protein Isolate (SPI) to replace fetal bovine serum in RPMI-1640 medium for the in vitro cultivation and maintenance of Leishmania donovani promastigotes.

MATERIALS AND METHODS
Parasite stock culture: The Axenic culture of L. donovani (LdMIPL-1) was maintained at 25°C in RPMI-1640 medium supplemented with 10% heat inactivated FBS, streptomycin (150 µg/ml), penicillin G (100 µg/ml) and gentamycin (150 µg/ml) at pH 7.2.

Preparation of 2% SPI stock solution: For preparing SPI stock solution, 2 grams of SPI powder (Modi-Mundipharma, New Delhi) was dissolved in 100 ml of autoclaved double distilled water and then filtered twice through Whatman filter paper No.1. Each 2 grams of SPI powder contained 1.69 grams of soy protein, significant amounts of essential and non-essential amino acids and minerals such as sodium, potassium and calcium.

Preparation of RPMI-1640 medium supplemented with SPI: Ten millilitres of 2% SPI stock solution was dissolved...
in 90 ml of autoclaved double distilled water followed by addition of 1.03 g of RPMI-1640 powder along with 30 mg L-Glutamine and 200 mg of sodium bicarbonate. After completely dissolving the contents, the pH of the solution was adjusted to 7.2. The solution was then filtered through Whatman filter paper No. 1 in sterile conditions. The medium was supplemented with gentamycin (150 μg/ml), streptomycin (150 μg/ml) and penicillin (100 μg/ml). It was then filtered with 0.2 μm syringe filters (Chromaphil) and poured into sterile culture bottles.

*Leishmania donovani* cultivation in SPI-substituted RPMI-1640 medium: Five millilitres of the prepared medium was poured into 10 ml bijou bottles to fill half of its volume aseptically. *L. donovani* inocula were then added into the medium and incubated at 25°C. Each bottle was shaken gently once every day to get uniform growth of the parasites throughout the culture medium. The RPMI-1640 medium without FBS or SPI was also inoculated with *L. donovani* culture and used as a negative control.

Observation of growth and morphology of the parasites: The growth and multiplication of *L. donovani* promastigotes was observed microscopically and by measurement of absorbance at 595 nm using ELISA plate reader (BioTek, USA) after three and seven days of incubation. Cell counting was done after seven days using Neubauer chamber in which five squares were counted. The viability of *Leishmania* promastigotes cultivated in SPI-substituted medium was assessed by sub-culturing the promastigotes in conventional RPMI-1640 medium supplemented with 10% FBS after seven and thirty days of incubation.

**RESULTS AND DISCUSSION**

The growth of *L. donovani* promastigotes was examined microscopically after three and seven days of inoculation in RPMI-1640 medium supplemented with 10% SPI as well as in conventional RPMI-1640 supplemented with 10% FBS. Slow growth of *L. donovani* was observed in SPI-substituted medium as compared to conventional RPMI-1640 on day 3 of incubation. However, on day 7, the growth and cell count was similar in both the media (Fig. 1 and 2). This may be due to the adaptation of the *L. donovani* promastigotes in an unusual environment. After a week of incubation, the promastigotes in SPI-substituted medium were larger in size than those in conventional RPMI-1640 medium supplemented with 10% FBS. No growth of *L. donovani* was observed in negative control i.e., RPMI without FBS or SPI (Fig. 3).

![Fig. 1. *L. donovani* promastigotes under light microscope, after seven days of incubation in RPMI-1640 + 10% SPI; a) 10X, b) 40X and c) 100X magnification](image1)

![Fig. 2. *L. donovani* promastigotes under light microscope, after seven days of incubation in RPMI-1640 + 10% FBS; a) 10X, b) 40X and c) 100X magnification](image2)
Measurement of absorbance at 595 nm also supported the observation of microscopic analysis as in case of SPI-substituted medium the absorbance was lesser as compared to conventional RPMI-1640 medium after three days of incubation, but it was similar after a week (Table 1).

Table 1: Absorbance in conventional and SPI-substituted RPMI-1640 medium after 3 and 7 days of incubation at 25°C

<table>
<thead>
<tr>
<th>Absorbance at 595 nm</th>
<th>After 3 days</th>
<th>After 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI + FBS</td>
<td>0.155</td>
<td>0.169</td>
</tr>
<tr>
<td>RPMI + SPI</td>
<td>0.123</td>
<td>0.158</td>
</tr>
<tr>
<td>RPMI + FBS</td>
<td>0.156</td>
<td>0.169</td>
</tr>
<tr>
<td>RPMI + SPI</td>
<td>0.121</td>
<td>0.161</td>
</tr>
<tr>
<td>RPMI + FBS</td>
<td>0.164</td>
<td>0.174</td>
</tr>
<tr>
<td>RPMI + SPI</td>
<td>0.124</td>
<td>0.166</td>
</tr>
<tr>
<td>RPMI + FBS</td>
<td>0.159</td>
<td>0.166</td>
</tr>
<tr>
<td>RPMI + SPI</td>
<td>0.122</td>
<td>0.160</td>
</tr>
<tr>
<td>RPMI + FBS</td>
<td>0.159</td>
<td>0.167</td>
</tr>
<tr>
<td>RPMI + SPI</td>
<td>0.123</td>
<td>0.166</td>
</tr>
<tr>
<td>RPMI + FBS</td>
<td>0.156</td>
<td>0.168</td>
</tr>
<tr>
<td>RPMI + SPI</td>
<td>0.121</td>
<td>0.165</td>
</tr>
<tr>
<td>RPMI + FBS</td>
<td>0.154</td>
<td>0.167</td>
</tr>
<tr>
<td>RPMI + SPI</td>
<td>0.131</td>
<td>0.170</td>
</tr>
</tbody>
</table>

Cell counting of L. donovani promastigotes cultivated in both conventional RPMI-1640 and SPI-substituted RPMI-1640 media was done using Neubauer chamber by counting the cells in 5 squares after one week of incubation. The total number of cells was $2.3 \times 10^8$ cells/ml in SPI-substituted RPMI-1640 medium whereas the number of cells in conventional RPMI-1640 medium with 10% FBS was $1.9 \times 10^7$ cells/ml.

Leishmania parasite has a dimorphic life cycle and comprises of promastigote stage which is an extracellular flagellated stage present in the mid gut of the sandfly vector and transmitted to the human host at the time of a blood meal. The promastigote within the human host is engulfed by the phagocytic cells and differentiate into the amastigote stage which is the infectious stage of this parasite (Estevez et al. 2007). To conduct continuous research, large quantities of in vitro cultivatrd Leishmania promastigotes are required. The cultivation of Leishmania requires at least 10% FBS as a supplement in the culture media, it becomes a difficult task because of its high cost and low availability in developing countries where leishmaniasis is endemic. There should be some substitute of FBS which may be used as a supplement in culture media for macronutrients and support the long term growth and survival of Leishmania parasite.

Several attempts have been made to replace FBS by bovine serum albumin or mixture of purine bases, vitamins, and bovine albumin fraction IV for the cultivation of Leishmania (Steiger and Steiger, 1976; Steiger and Steiger, 1977; Berens and Marr, 1978). The concentrated RPMI-1640 medium and medium 199 containing salts, glucose, and tryptose were observed satisfactory for the cultivation of L. chagasi and L. amazonensis promastigotes (Sadigursky and Brodskyn, 1986). Beef extract and yeast extract along with inorganic salts have also been used for the cultivation of Leishmania promastigotes to replace FBS (Ali et al. 1998). Several other reports suggest the use of Human urine, milk of cattle and some selected vitamins and minerals to replace FBS for the in vitro cultivation of Leishmania spp. (Singh et al. 2000; Muniaraj et al. 2007; Tasew et al. 2009). The present work was aimed to replace FBS with a cost effective nutritional supplement to cultivate L. donovani. We have used Soy Protein Isolate as a substitute of FBS as it is a cost effective product and is stable at room temperature. It supported the growth and survival of L. donovani up to one month of incubation. The parasite multiplication rate was slow in the beginning, while it was faster as compared to RPMI-1640 supplemented with 10% FBS on the seventh day of incubation. No bacterial or yeast contamination was observed in the SPI-substituted RPMI medium. The cost of SPI for preparing 100 ml SPI-substituted RPMI medium was INR 1.55 whereas that of FBS (Himedia) was INR 325. All these factors make SPI an efficient substitute of FBS for the in vitro cultivation of Leishmania parasite.
CONCLUSION
The present study has evaluated the potential of Soy Protein Isolate as an acceptable alternative of Fetal Bovine/Calf Serum in the liquid culture medium for the cultivation of *L. donovani*. The exploration of SPI as a substitute of FBS in media other than RPMI such as Medium 199 and Schneider’s insect medium would be beneficial and cost effective for the cultivation of *L. donovani* and other clinically significant species of *Leishmania*.

CONFLICT OF INTEREST
Authors declare no conflict of interest.

REFERENCES


