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Low-Cost Purification Cultivation Process For Medical "Hirudinaria Manillensis"

Chen Chuang[1], Qin Ren[2], Farra Aidah Jumuddin[3]*

*[1, 3] *Lincoln University College, Petaling Jaya, Malaysia*
Email: [3] farraaidah@lincoln.edu.my
[2] Guangxi Zhuang Kang Ech Biotechnology Co. Ltd., China

***corresponding author:** *Farra Aidah Jumuddin*
**Lincoln University College, Petaling Jaya, Malaysia*
Email: farraaidah@lincoln.edu.my

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Abstract

Objective: To improve traditional leech farming and develop a low-cost purification cultivation process for medical leeches. Methods: Based on our preliminary research, a low-cost purification cultivation process for medical leeches (Hirudinaria manillensis) was developed, focusing on aspects such as the farming environment, site, water quality, feeding, sterilization, and detection requirements. Microbial tests were conducted to ensure the leeches were suitable for clinical or scientific use, and observations were made regarding the occurrence of complications such as allergies and microbial infections. Results: Medical Hirudinaria manillensis leeches cultivated using this purification process passed multiple batches of microbial tests and met medical standards. With the application of over 200 cases, no serious allergies or pathogenic microbial infections were reported, indicating a high level of safety.
Conclusion: Medical leeches produced through this purification cultivation process demonstrated good safety and met medical standards, providing a reference for the production of medical leeches.

Keywords: Hirudinaria manillensis, medical leeches, purification cultivation, safety, production process

1. INTRODUCTION

Medical leeches are invertebrate amphibious aquatic animals belonging to the phylum Annelida, class Hirudinea, order Arhynchobdellea, family Gnathobdellidae, genus Hirudo, species medicinal leech. They typically inhabit freshwater environments. Currently, about 600 species of leeches have been discovered globally, categorized into non-bloodsucking and bloodsucking leeches. To date, only a few species from the genera Whitmania Blanchard and Hirudo Linnaeus have been officially approved for medical purposes. Due to environmental and regional distribution differences, the types of leeches used for medical purposes vary among countries[1]. In Europe, the United States, Russia, Israel, Iran, and Turkey, three species of leeches—Hirudo medicinalis (European leech), Hirudo verbena Carena (Hungarian leech), and Hirudo orientalis Utevsky & Trontel (Eastern leech)—are utilized for medical applications. Currently, Hirudo verbena is the most commonly found species in legally authorized commercial leech farms.

Hirudinaria manillensis, also known as the Asian buffalo leech or Philippine medicinal leech, belongs to the order Hirudinida within the phylum Annelida, family Hirudinidae, genus Hirudo. This species is primarily distributed across Asia, especially in Southeast Asia, East Asia, and South Asia, including countries like India, the Philippines, Malaysia, Thailand, Indonesia, Vietnam, and regions in China such as Guangdong and Guangxi. They typically reside in the freshwater ecosystems of these countries. The back of Hirudinaria manillensis is usually dark brown or green, while the abdominal color may vary by location. Their size ranges from about 3 to 10 cm, with a spindle-shaped body that is tapered at both ends and wider in the middle. They have uniform annular segments and usually have a suction cup at both ends for attaching to the host's skin. In Asian countries such as Southeast Asia, South Asia, and China, Hirudinaria manillensis is widely used in traditional medicine. In 2018, the Health Commission of Guangxi Zhuang Autonomous Region in China approved Hirudinaria manillensis for leech therapy[2].

Medical Leech Therapy（MLT), also known as "leech treatment," is an ancient therapeutic method and one of the natural therapies in traditional medicine. It involves using leeches to bite a specific area of the patient's skin, treating diseases through the saliva secreted by the leeches during bloodsucking[3]. One or more medical leeches attach to the patient's skin area, aiming to harness the potential benefits of the saliva secreted during bloodsucking. The history of leech therapy can be traced back to ancient Egypt, Greece, Rome, India, China, and Anglo-Saxon cultures, all of which have records of leech therapy. In 17th-century Europe, leech therapy reached its peak application, used to treat almost all types of diseases, including infections, fevers, vascular lesions, joint disorders, neurological diseases, skin conditions, reproductive system diseases, and eye diseases. In ancient times, leech therapy was considered a form of "bloodletting therapy," based on the hypothesis by Hippocrates (circa 460~370 BCE) that diseases were caused by an imbalance of bodily fluids in the body. It wasn't until the late 19th century that Haycraft first proposed that leech saliva contains an anticoagulant, which he named "hirudin." Markwardt later isolated and identified hirudin[4], proving its anticoagulant effects, sparking significant interest in research on leech saliva. The mechanism of action of leech therapy is closely related to the effects of leech saliva. Leech therapy involves medical leeches drawing blood from the human body while releasing their saliva into the body, producing a series of reactions that lead to therapeutic effects. The anticoagulant effect of leech therapy, particularly in treating postoperative venous congestion, has attracted modern medical attention. In 2004, medical leeches were approved by the U.S. Food and Drug Administration (FDA) as a medical device for assisting in the treatment of venous congestion[5], mainly applied in reconstructive surgery, microsurgery, and trauma repair. In many countries, such as Europe, the United States, Russia, and Israel, there are companies specializing in the production of medical leeches that meet clinical requirements. Most of these countries use pure water cultivation for medical leeches, which is more reliable but also more expensive, with a single medical leech costing between $3 and $15. In developing countries like India, Iran, Turkey, China, and Southeast Asia, where traditional medical institutions operate, the high cost of medical leeches limits the development of leech therapy. Therefore, improving traditional leech farming techniques to reduce production costs is of significant importance. This article follows the traditional Chinese Hirudinaria manillensis farming techniques, with appropriate modifications, employing purification cultivation and sterilization treatment, achieving good results, as described below.

1. Purification Cultivation Process for Hirudinaria Manillensis

1.1 Selection of Farming Environment Abundant Water Source:

The ability to continuously access clean, unpolluted water is essential. Preference is given to sites with natural underground springs or mountain spring water, where irrigation and drainage are convenient, and water levels remain stable throughout rainy and dry seasons. The water used for farming must be purified and disinfected, meeting the "Standards for Drinking Water Quality" (GB5749-2006) with a post-chlorination pH value between 6.5 and 7.0, which is more beneficial for leech farming. Soil Quality Requirements: Must meet the "Environmental Quality Standards for Soils" (GB 15618) for second-level soil. Soil should be tested to ensure it is free from pesticide residues, and the content of heavy metals such as lead, chromium, cadmium, copper, and mercury does not exceed the standard limits. Farm Location: The farm should be situated far from industrial areas and landfills, free from water and air pollution. Generally, it is required to be away from cities, with convenient transportation, ample electricity, minimal human and livestock activity, and relatively isolated.

1.2 Construction of Farming Production Facilities

The entire production process, from seedling breeding, cocoon hatching, seedling cultivation, product development, purification farming, to sterilization packaging, is completed in a GMP workshop with cleanliness level above D grade. Indoor farming and breeding facilities are constructed according to the biological characteristics of Hirudinaria Manillensis, aiming to facilitate their growth and reproduction, winter conservation; ease manual operations; allow the inflow and outflow of farming water at any time; and effectively prevent escape. Mating Box: Compared to standard farming boxes, the mating box is square designed with a slight slope and well-equipped water inlet and outlet facilities for micro-flow water farming. Temperature Control Device: Mainly used to control water temperature, freely adjustable between 18~32℃. Another is the air temperature control system, generally using standard air conditioning to prevent indoor temperature from dropping below 16℃ during winter, keeping the daily temperature difference within 3℃. Cocoon Box: The box is over 30cm in height, capable of holding 15~25cm thick soil; typically, each box is designed to be 2~5 square meters, with a mesh top to prevent escape; the top mesh can be freely opened for daily management. Shading Facilities: Generally, black shading nets are used, as Hirudinaria Manillensis breeding peaks require a dark environment. Winter Conservation Facilities: Winter conservation is generally carried out in greenhouses or warm sheds within the farm to prevent rain and wind during winter. Indoor concrete pools or boxes are built according to specific needs, typically with each pool being 60~80cm wide, 100~200cm long, and 50~60cm high. The top of the pool is sealed with a 60~100 mesh nylon net to prevent escape, and the pool contains 30~40cm high loose, clean soil, with soil moisture maintained at 50~60%. Conditioned greenhouses or warm sheds may be equipped with heating lamps or use air conditioning.

1.3 Farming Operation Techniques

1.3.1 Biological Characteristics of Hirudinaria Manillensis The optimal living temperature for Hirudinaria Manillensis is between 18~34°C, with the ideal range being 22~30°C. Below 18°C, they enter hibernation. In the wild, especially in southern regions, they are active, feed, mate, and reproduce from March to October, remaining dormant or hibernating during other times, essentially without feeding. Under natural growth conditions, juveniles can be cultivated to about 1~2 grams each within a year; with artificial feeding, they can reach ≥5 grams each within the same timeframe, essentially meeting the commercial size standards. Hirudinaria Manillensis are hermaphrodites that mate with other individuals, with each capable of producing cocoons and reproducing. The peak mating and reproduction period is from March to May, with a smaller amount of mating and reproduction occurring from July to October. Cocoon laying usually begins one month after mating, i.e., when the water temperature stabilizes above 20°C, they will burrow into moist soil 20-30 cm from the water surface to lay their cocoons. The cocoons hatch naturally in environmental conditions of 20~30°C, typically within about 20 days. They produce cocoons 1-2 times a year, with each cocoon containing 1~12 juvenile leeches, averaging 5~6, and each juvenile typically weighing 0.05~0.15 grams, though occasionally larger or smaller individuals are observed.

1.3.2 Preparation Before Seeding Clean and repair the site, and disinfect the breeding pools. Fifteen days in advance, use a bromine or chlorine solution with a concentration not exceeding 20ppm to disinfect the entire farm. Choose deep, clean soil as the breeding substrate, with a pH value of neutral to slightly acidic. Break up and disinfect the soil one month in advance, then sun-dry it to a dry powder for use. Prepare daily use tools such as feeding platforms, buckets for pig blood, gloves, dishes, and sausage casings. The feeding platform should be fixed in the water, maintaining 1-2 cm above the water surface throughout the feeding process, and promptly cleaned of any residual blood after feeding.

1.3.3 Parent Stock Release Preferably select Hirudinaria Manillensis individuals around 10 grams in flat state (i.e., first-year size), with uniform size, strong physique, and active movement, free from disease or external injuries. Feed them manually once before release. Density of leech seedlings: About 500~600 individuals per square meter, with individuals around 10 grams in flat state. Timing of release: Generally, seedlings are released when the air temperature stabilizes above 20°C, typically starting in early April in Guangxi, with April and May being the seeding season, April being optimal. Method of release: Place disinfected soil into the breeding pools, with a thickness of 15~25cm, graded from low to high; adjust the soil moisture to about 60% by spraying. Move the Hirudinaria Manillensis from the mating box that have reached the weight standard into the cocoon pool, allowing them to burrow into the soil to lay cocoons.

1.3.4 Cocoon Collection From April to June each year, collect the produced cocoons and transfer them to hatching boxes for artificial hatching. The specifications for the hatching boxes are as follows: about 15~20cm in height, 30~50cm in length, and 20~30cm in width, with a water level of about 2cm at the bottom. The cocooning period generally ends by the end of June, and after the leech juveniles hatch, they naturally crawl into the water to rest; a mesh is placed about 2~3cm above the water surface, with cocoons spread out on the mesh without overlapping. The box is covered with a mesh to prevent juveniles from escaping. Under conditions of 22~30°C, cocoons typically hatch into leech juveniles within 15-25 days. After hatching, juveniles can be transferred to farming boxes for artificial feeding. After 10~15 feedings, typically over about 4 months, they can reach the adult size of 6-8 grams per Hirudinaria Manillensis.

1.4 Purification Cultivation

After reaching adult size, Hirudinaria Manillensis are transferred to purification cultivation tanks to begin the purification process. Purification cultivation is a crucial phase in the entire farming environment, with specific requirements as follows:

(1) The purification cultivation space must be separate, with indoor temperatures controlled between 25~28°C.

(2) Cultivation tanks are designed to be 1-2 square meters each, with a stocking density of 1500-1600 individuals per square meter. The water level is maintained at 15-20cm, and the water must be changed daily.

(3) The cultivation tanks are manually cleaned daily, with a complete "tank change" every 15 days, including disinfection of the empty tanks with chlorine and potassium permanganate. Any deceased individuals must be promptly removed, accompanied by a "tank change."

(4) Purification cultivation should last ≥6 months, during which feeding is prohibited. After purification, individual Hirudinaria Manillensis should measure 1.5~3 grams, with internal blood content ≤10%.

(5) During the purification phase, each tank of Hirudinaria Manillensis is managed with a unique identification number, preventing mixing of leeches from different tanks.

1.5 Feed Selection

Collect fresh, clean blood from pigs, cows, sheep, etc., from slaughterhouses, ensuring it is uncontaminated and without any additives. Feeding should be completed within 4 hours from the time of slaughter. Do not feed blood that has been contaminated, smells bad, or has spoiled.

1.6 Feeding Tools and Methods

(1) Artificial Sausage Casings: Use artificial collagen casings 2-4cm wide in a flat state. Feeding Platform(Typically made of wood or plastic)designed with a shallow concave groove to prevent blood from leaking into the water, fixed so that the surface is 1~2cm above the water. Place the casings on the platform for feeding.

(2) Blood Processing: Convert the collected pig or cow blood into a slurry, filter out clots and impurities using a 60-mesh screen, then fill the blood into the artificial casings, tying them off into 20~40cm segments.

(3) Feeding Quantity and Method: Leeches have a high feeding capacity, requiring 3-5 pounds of fresh blood for every pound of hungry leeches. Feed early before 10:00 AM in good weather, avoiding feeding on rainy days. Place the blood-filled casings on the feeding platform, then stir the water around the platform to attract the leeches by the scent of blood. Feeding lasts 30~60 minutes until the casings are nearly empty or flattened, then remove and clean the casings.

1.7 Routine Management

Maintain stable water levels, checking the inflow and outflow ports and ditches 2-3 times a month to prevent blockages. Ensure the water level fluctuates within a 5cm range. Keep a micro-flow state or change the water frequently. Control Indoor Temperature: Keep indoor temperatures between 22~30°C. Inspect the escape prevention facilities 2~3 times a month, repairing any damages promptly. Regularly disinfect various areas of the farm, disinfecting each cultivation tank more than twice per cultivation cycle. Any tanks with deceased leeches must be cleaned and disinfected before reuse.

1.8 Pre-shipment Processing

Hirudinaria Manillensis that have undergone purification cultivation and meet the specifications must be sterilized before shipment. The sterilization process involves a 48-hour bath in a 50ppm concentration of gentamicin. Each batch is sampled for microbial testing, which must show no pathogenic bacteria before the leeches can be shipped.

2. MICROBIAL TESTING AND CLINICAL USAGE

Using this production process, approximately 10,000 medical leeches were produced in three batches, with random samples sent for third-party testing. The primary bacterial types tested for included Salmonella, Staphylococcus aureus, Shigella, Escherichia coli, hemolytic Streptococcus, Vibrio parahaemolyticus, Listeria monocytogenes, enterohemorrhagic E. coli, and Pseudomonas aeruginosa, covering nine professional-level tests, with none of these pathogenic bacteria detected. These leeches were utilized by laboratories of medical institutions and traditional Chinese medicine clinics in China, totaling 200 cases and approximately 5,000 leeches, with no reports of severe allergies or microbial infections. This indicates that Hirudinaria Manillensis cultivated through this process meet a higher safety standard.

3. DISCUSSION

Leech therapy is a traditional treatment with a long history, applied in traditional medicine for conditions such as arthritis, vascular diseases, chronic wounds, gout, and edema. In modern medicine, it is primarily used for treating venous congestion following plastic and trauma surgery. As leeches are living organisms, there have been reports of severe infections following treatments with uncertified leeches[6], hence the safety of leech therapy remains somewhat controversial. Historically, leech therapy has been safe, and recent applications have not reported serious medical incidents involving regulated leeches. Research indicates that complications from leech therapy can be categorized into five types: infections, allergies, continuous bleeding, migration, and others (meningitis, acute renal failure), with infection being the most common complication related to leech therapy[7]. Sterile operation and the use of qualified medical leeches can control bacterial infections, significantly reducing the incidence of infections caused by leeches. Purified cultured leeches are crucial for reducing bacterial infections, making microbial testing an essential indicator for medical leech standards. However, purification cultivation techniques in Western developed countries are costly. This study adopts purification cultivation and sterilization processes, validated by third-party testing and clinical use, achieving a higher safety level. We have calculated our production costs: the average cost for producing 10,000 leeches is $0.8 per leech. Through analysis of the structure of our invested funds, the use of conventional water cultivation and low labor costs are the main factors in reducing costs. Thus, this study provides a reference for low-cost medical leech purification cultivation. There is some controversy over using antibiotic solutions for sterilization, as some scholars suggest it may increase bacterial resistance[8]. Yet, this method is reliable and low in cost, currently considered a favorable option. We are also continuously researching new sterilization methods to address potential issues of bacterial resistance.

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