



THE STATE OF BEING ALIVE AND SEPARATION OF BACTERIAL STRAIN WITH HIGH CELLULOSE ACTIVITY FROM LEFT OVER MULBERRY PULP EXCRETED BY SILKWORMS, *BOMBYX MORI* L.

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ABSTRACT

The theme of this work revolves around the utilization of beneficial bacterial strains in biotechnological application chiefly related to agri-waste degradation process as investigated. These bacterial strains are obtained from animal wastes like excreta serves as the material for such studies. The excreta of the popular mulberry silkworm, *Bombyx mori* L. may serve as the instant source of these bacterial strains proving immense research scopes learning to remarkable discovery of the betterment of human life. Silkworm excrement is the collective name of the left over mulberry pulp and excreta produced by the silkworm during larval periods. It is a type of semisolid substance containing chiefly leaf juices, cellulose and protein. The cellulose content of silkworm excrement has been recorded high-up as 20%. It is well established earlier that cellulolytic functional bacteria are responsible to accelerate the biodegradable process of cellulose and for which it was assumed to be helpful during conversion process of agricultural wastes. Therefore, stepwise studies are imperative, for achieving an end on the confirmation about the procedures of isolation, identification of high efficiency of cellulose degrading bacteria, their selective culture suitability, the process of cellulose degradation, screening, sorting and application trials. Till date, experimental work progress and initial encouraging results related to separation of bacterial forms and distinctness of their colonies from other colonies of microorganisms, appearance, texture, identification, basic biochemical tests, standardization of culture media etc. has been tested and two useful strains have been verified as *Bacillus* and *Pseudomonas* sp. among collected samples. Huge microbial growth has been observed in each sample against excreta of bivoltine and multivoltine silkworm breeds. The growth load have reached near about 1×10^8 cfu/ml of sample in each case using non-selective culture media at this initial study. So, relevant data collection about these assessable microbial cells present in the silkworm excrement is anticipated with this for the safe use in the degradation process of agro-wastes comprising of mostly cellulose.

KEYWORDS: Agricultural Waste, Silkworm Excrement, Cellulose Converting Bacteria, Cultural Practice and Separation.

INTRODUCTION

Biological treatment and management of agricultural waste are essential for sustainable resource conservation and environmental suitability. This process involves the activity of useful microbes to break down organic substances. In India, large amounts of sericultural waste are produced due to silk-producing activities. Silkworm excrement is a semisolid multi-component substance produced by *Bombyx mori* L. larvae during all

developmental stages. This cellulose, rich silkworm excrement is pointed as the source of microbial existence of useful bacteria (H Li et al, 2019, 2023). According to them cellulose degrading functional bacteria isolated from cellulose rich waste environment can be utilized for the biodegradation of cellulose and for the improvement of conversion process of agricultural waste composting. Therefore, first task for the separation and isolation of new high yielding cellulose producing 'the state of being

alive' microbial cells, particularly bacteria from cellulose rich waste environment and the study of their ability to produce enzymes and degrade cellulose efficiency which is important for the research and development of microbial inoculants for the safe treatment of cellulose rich agricultural waste such as silkworm excrement. R Kumar and DK Vatsa (2021) conducted studies on the details of cellulolytic bacteria collected from Yak dung where dung manure and soil samples were collected from Spiti region of Lahol, Himachal Pradesh, India and they isolated and cultured on CMC (Carboxy Methyl Cellulose) AGAR medium. Another interesting work carried out by Q R Li (2021) on the silkworm excrement and this study focused on isolation and identification of possible phosphates solubilising bacteria from the sewage fed East Kolkata wetland, a prospective water body resource for fishes. Based on above valuable literatures, the key intent of this work is to address the gap through a comprehensive study on the existence of bacteria with high cellulose activity from different environment as investigated started here in West Bengal.

Cellulose is important limiting cause that restricts the resource utilization effectiveness of silkworm excrement via composting. Proper understanding of separation, isolation, screening, sorting, enzymatic activities, conversion process etc. in Indian context will be required to utilize high quality strains of bacteria for the environment safe treatment as well as for the development of soil conditioner through these processes. Based on present investigation, our primary report has been interpreted about the availability of convenient sources of cellulolytic bacteria strains.

MATERIALS AND METHODS

- a. **Silkworm Breeds Rearing:** Both bivoltine (SK6 x SK7) and multivoltine (Nistari) silkworm seeds were collected and reared at the Department of Sericulture, Murshidabad University, as recommended by Krishnaswami S (1978), for better understanding of microbial sources
- b. **Excreta Collections:** Fresh samples of 5th-stage larval silkworm excrement were collected from

rearing beds using sterilized containers with proper hygienic care and stored at 4°C.

- c. **Gram staining for study:** Differential staining procedure was followed to ensure visibility the in first step.
- d. **Culture media for bacterial growth:** Samples were homogenized in 0.9% saline solution. For general isolation of all gram positive rods organisms Nutrient Agar Media was used and Soyabean Casein Digest Media (SCDA) was used for growth load comparison.
- e. **Preparation of sample:** In each case of sample dissolved particular amount of excreta collected as 0.3 g and 0.5 g in a sterile container and added 9 times of its weight dilution solution. Then further diluted up to 10^{-6} to found countable colonies.
- f. **Inoculation on Media:** 1ml. from each dilution of each samples plated on Nutrient ager media and Soyabean casein digest media.

RESULTS AND DISCUSSION

Initial studies yielded positive results regarding the separation of bacteria from the semi-solid components sourced from silkworm excrement (*Bombyx mori* L) and to utilize them in the biodegradation processes. Bacterial smear involves gram staining procedure which is the first step, in microbiology thus two types of microbial cells have been verified and still identification details are in progress. The bacillus cells (Dark purple) are rod shaped gram +ve occur in singly or in a chain along with many oval and spherical cells are present in other prepared slides. Among these one of them appeared as *Pseudomonas* (pink/red) which is a slender gram -ve bacterium. The shape and rigidify of both the cells at a glance have been judged repeatedly to determine their existence in both dried and fresh left over mulberry pulp consider as silkworm excrement.

The presence of both gram +ve and gram -ve bacteria have been observed in majority of the stained slides preparing from the both bivoltine and multivoltine breeds of silkworm *Bombyx mori* L. (Fig 8-Fig 10).

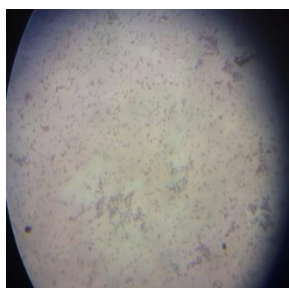


Fig-8: Gram +ve rod shaped bacteria. Fig-9: Gram-ve short rod bacteria. Fig-10: Chain of Bacillus cells.

Two non- selective culture media viz. Nutrient Agar and Soyabean Casein Digest media have been tried to examine their presence through CFU-Colony Forming

Units at 3 hours interval for consecutive 3 days as shown in Fig 1 – Fig 6, after incubation at 37°C for 24 hours.

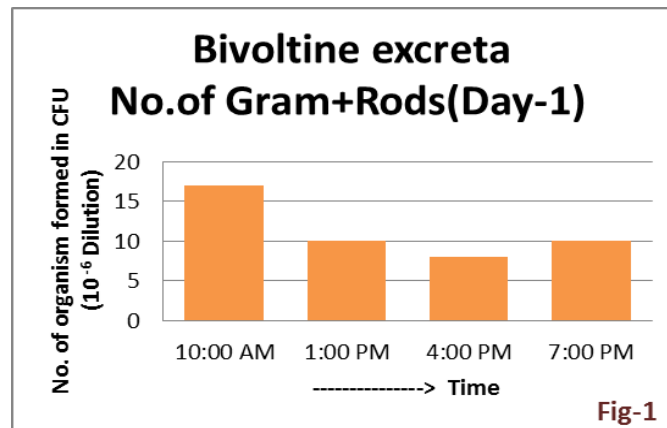


Fig-1: Excreta from Bivoltine Silkworm and showing number of Gram+ve rods (Day-1).

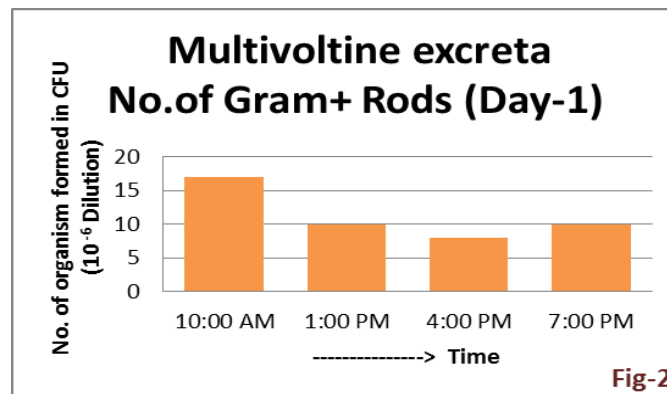


Fig-2: Excreta from Multivoltine Silkworm showing gram+ve rods (Day-1).

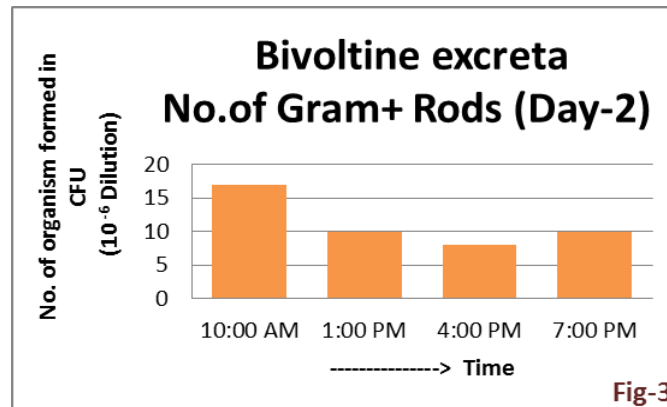


Fig-3: Excreta from Bivoltine Silkworm showing number of Gram+ve rods (Day-2).

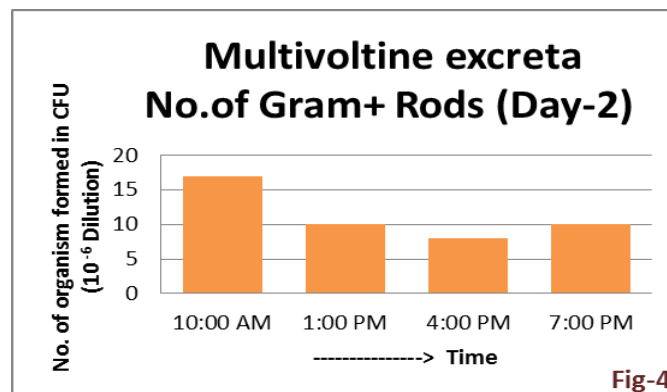


Fig-4: Excreta from Multivoltine Silkworm showing gram+ve rods (Day-2).

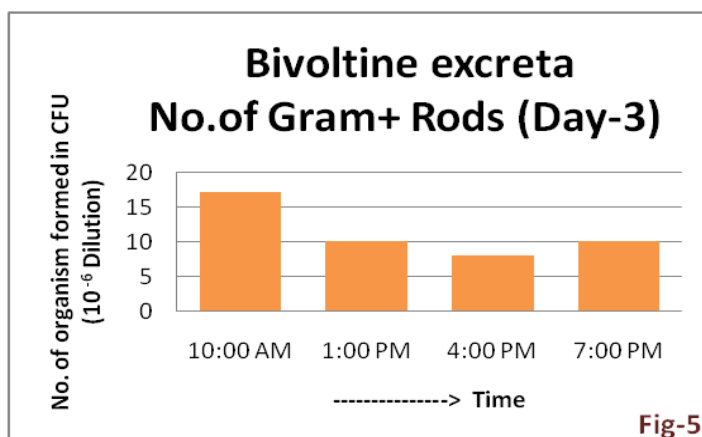


Fig-5: Excreta from Bivoltine Silkworm showing number of Gram+ve rods (Day-3)

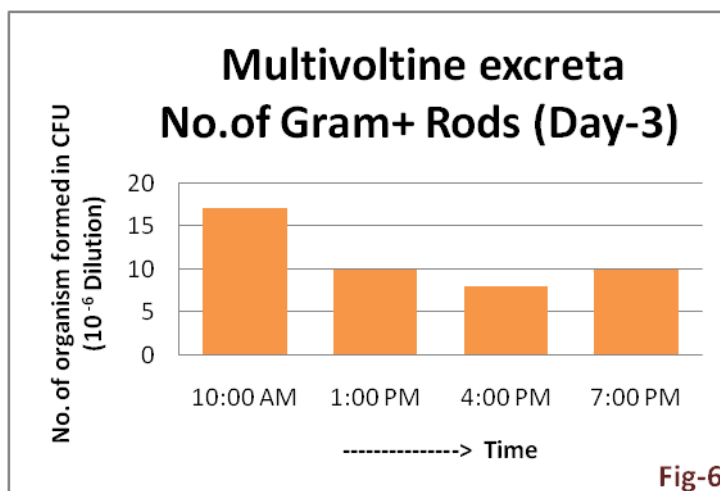


Fig-6: Excreta from Multivoltine Silkworm showing gram+ve rods (Day-3)

The nos. of gram +ve and gram -ve bacteria for both the breeds showed their occurrence at different times and here nearly opposite trends have been observed. CFU 10⁻⁶ varies in these two media and it is assumed that media preferences will be an important parameter for

isolation of cellulolytic bacteria present in the excrement of both breeds. For Growth amount media productivity comparisons study indicates suitability for isolation of gram +ve rod organisms (Fig 7).

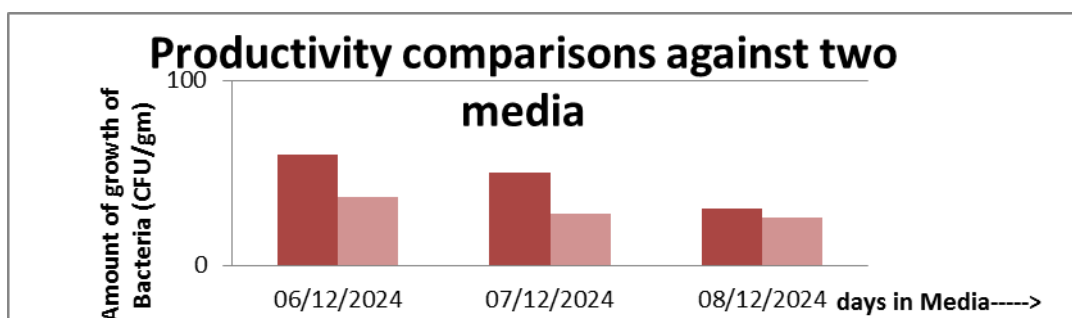


Fig-7: productivity comparisons of two non-selective media Nutrient agar and Soybean Casein Digest agar (SCDA).

After 3 days study of different samples (excreta) collected from bivoltine and multivoltine breeds, it was noticed that Nutrient Agar Medium shows better productivity than Soybean Casein Digest Media to isolate gram +ve bacilli strains.

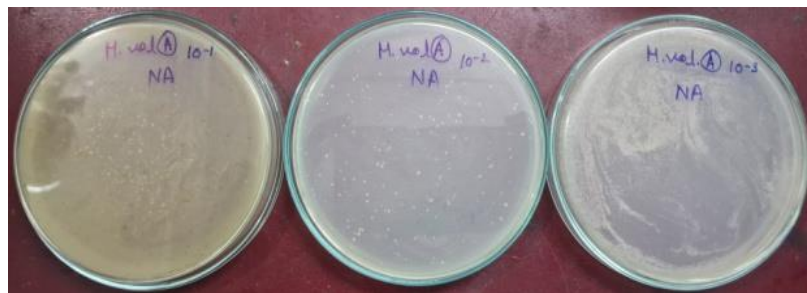
Huge microbial growth have been detected in each sample and in each case 0.1 gm sample is weighed and prepared sample in 10 ml. Peptone NaCl water and therefore diluted the sample up to 10⁻⁷ dilution to isolate their colonies. Then the growth load is calculated as nearly 1x 10⁸ CFU / ml of sample observed in each case.

From each 10^{-8} dilution we picked up different shapes oriented colonies with morphological variations and these are represented in Excel Sheets.

As it is found in at many +ve rods and gram -ve rods are present in the various types of samples, we have whispered that there is high probability for the existence of maximum isolates of Bacillus and Pseudomonas cells. (Culture plate 1- Culture plate 4)



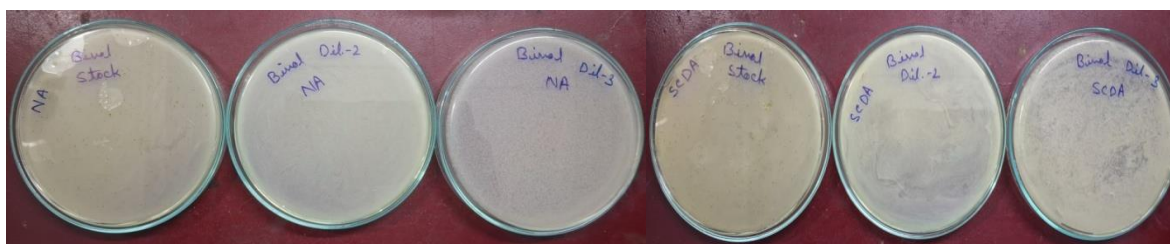
CP1: Different colony appearing.



CP 2: Culture media nutrient Agar (Multivoltine 1gm, 10ml broth) in serial dilution.



CP3: Culture media soybean casein digest) and then serial dilution and plated.



CP4: Result of Bivoltine excreta sample (0.4 gm in 10 ml) and then serial dilution and plating.

CONCLUSION

The progress of this work serves as a favorable starting point for the biotechnological utilization of sericultural waste. Future phases will focus on the biochemical, physiological, and genetic aspects of these strains for the safe treatment of cellulose-rich agricultural crops. Selective culture practices will be maintained for the screening and sorting of high-quality bacterial strains to develop soil conditioners.

REFERENCES

1. Kumar R and Vatsa DK. Isolation and screening of cellulolytic Bacteria from yak (*Bos grunniens*) Excreta from Lahaul Valley Region for pre-treatment of paddy straw in Himachal Pradesh. *International Journal of Bio-Research and stress management*, 2024; 15(1): 01-07.
2. Li H, Xu X, Zhang M, et al. Accelerated degradation of cellulose in silkworm excrement by the interaction of horsefly larvae and cellulose degrading bacteria. *EcoToxicology and environmental safety*, 2019; 170: 418-426.
3. Li H, Zhang M, Zhang Y, et al. Characterization of Cellulose - Degrading Bacteria Isolated from Silkworm Excrement and optimization of its cellulose production. *Polymers*, 2023; 15(20): 41-62.
4. Li Q, Liao S, Wei J, et al. Isolation of *Bacillus subtilis* strain SEM-2 from Silkworm excrement and characterization of antagonistic effect against *Fusarium* spp. *Canadian journal of microbiology*, 2020; 66(6): 401-412.