Research Article

ANTIBIOTIC SENSITIVITY PATTERN OF THE ISOLATED LACTIC ACID BACTERIA FROM SILKWORM (*BOMBYX MORI* L.) GUT

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ABSTRACT

Laboratory study was undertaken at the Department of Agricultural Microbiology, TNAU at Coimbatore to find out the sensitive pattern of lactic acid bacteria isolated from silkworm (*Bombyx mori* L.) gut. About 21 Lactic Acid Bacteria (LAB) isolates were isolated from silkworm gut of bivoltine double hybrid {(CSR6 x CSR26) X (CSR2 x CSR27). Susceptibility of the LAB isolates to antibiotics were performed by disc diffusion method. Overnight grown culture of each LAB isolate was spread evenly on the surface of MRS agar plate and allowed to dry. Antibiotic discs were then placed on LAB inoculated plates and incubated at 37°C for 24 hr. In this study, various antibiotics were supplied in the form of dodeca discs (Hi Media, India) which included Cefuroxime (30mcg), Cefaclor (30mcg), Ceftriaxone (30mcg), Cefalexin (30mcg), Ceftazidime (30mcg), Ceftizoxime (30mcg), Cefadroxil (30mcg), Ampicillin (30mcg), Cefaperazone (75mcg), Cefixime (5mcg) and Cefazolin (30mcg). The inhibition zone diameters of all antibiotics were between 0 and 30 mm for LAB strains isolated and tested after incubation at 37°C for 24 hr. Results were expressed as sensitive (S, ≥ 21 mm), intermediate (I, 16-20mm) and resistant (R, ≤ 15 mm). From total isolates, about 8 LAB isolates (SWGL 1, SWGL 4, SWGL 7, SWGL 9, SWGL 12, SWGL13, SWGL 16 and SWGL 17) were resistant to Cefixime, Cefaperazone, Cefadroxil and Ceftazidime. Six nos. of LAB strains were highly resistant to Cefaclor (SWGL 2, SWGL 3, SWGL 5 and SWGL 6), seven nos. to Ampicillin (SWGL 1, SWGL 3, SWGL 7, SWGL 9, SWGL 14, SWGL 16, SWGL 20 and SWGL 21) and two were intermediate to Ceftriaxone (SWGL 5 and SWGL 12). Three LAB isolates (SWGL 10, SWGL 14 and SWGL 15) were found sensitive to all three antibiotics (Cefazolin, Cefuroxime and Cefalexin). These isolates found to be sensitive to most of the antibiotics tested support for transferring antibiotic resistance genes to pathogenic microbes. They may also be incorporated as a feed for growth and development of silkworm.

INTRODUCTION

In sericulture the productivity and quality largely depends on the healthiness, growth of the silkworm larvae and the suitable environmental conditions. Growth and development of larvae depends on the physiological processes that take place in the silkworm. Therefore, improvement of silk quality means improvement of the feed nutrition and upkeep of the larval health, since silkworm is highly susceptible to infection caused by major pathogenic groups. Broad spectrum antibiotics *viz.,* penicillin, streptomycin, tetracycline and chloramphenicol were already tried on silkworm and found successful (Venkatesh and Srivastava, 2010). Antibiotics in silkworms are approved for four different purposes: disease treatment, disease prevention, disease control and for health maintenance or growth promotion (Phillips *et al.,* 2004). Beneficial effects of antibiotics by modulation of gut microflora and influence on mucosal immunity or through altering enzymatic activities has been extensively studied in humans, animals and many insects (Yeung *et al.,* 2002). Several prophylactic and curative measures are aimed for managing the disease taking into account the ecofriendly nature and cost effectiveness.

Antibiotics are widely used in sericulture industry as a component of bed disinfectants and as therapeutic applications against bacterial diseases (Subramanian *et al*., 2009). When antibiotics were administered to the silkworm, there is shift in the nitrogen metabolism in favour of increasing the body weight and increased output of silk. Rahmathulla *et al.* (2003) observed that antibiotics administration with different concentrations significantly improved the rearing and cocoon parameters like larval duration, larval weight, growth index, single cocoon weight, single shell weight, shell ratio, average filament length, non breakable filament length, raw silk recovery percentage, denier, reelability and neatness, better performances were recorded with the increase of antibiotics concentration. Oral administration of antibiotics along with mulberry leaves to healthy silkworm boost the growth, fecundity and silk contents (Tayade *et al.,* 1988) as well as reduces the incidence of diseases (Rai and Devaiah, 1988). During antibiotic treatment, all bacteria in the human/animal body are exposed to selective pressure of the antibiotic. Consequently, the intestinal microflora is highly exposed, especially during oral therapy. When LAB live in a biotope (human or animal intestine, bovine udder) and are regularly challenged by antibiotics it results in the selection of naturally resistant strains carrying an important genetic pool that might be capable of transferring antibiotic resistance genes to other strains present in the human intestine. The ability to transfer antibiotic resistance genes must be considered as an important parameter for the selection of the probiotic strains. The aim of this study was to study the antibiotic sensitivity pattern of the isolated lactic acid bacteria as they may be incorporated in food or feed as probiotic for silkworm.

MATERIALS AND METHODS

Stock cultures of the isolates were stored in 40% glycerol at –20 °C. The organisms were subcultured three times prior to use in sterile de Man, Rogosa and Sharpe (MRS) broth using 1% inoculum and 24 hr incubation at 37 °C.

Antibiotic susceptibility test

Antibacterial susceptibility testing in the clinical laboratory is the most performed test using antibiotic strips. The method was originally standardized according to the International Organization for Standardization (ISO) and quality assurance guidelines of World Health Organization (WHO). The activated cultures were swabbed on to the MRS agar plates. In this study, various antibiotics were supplied in the form of dodeca discs (Hi Media, India) which included Cefuroxime (30mcg), Cefaclor (30mcg), Ceftriaxone (30mcg), Cefalexin (30mcg), Ceftazidime (30mcg), Ceftizoxime (30mcg), Cefadroxil (30mcg), Ampicillin (30mcg), Cefaperazone (75mcg), Cefixime (5mcg) and Cefazolin (30mcg). The zones of inhibition were measured after incubation at 37°C for 24 h.

Table 1. Antibiotic sensitivity of the lactic acid bacteria from silkworm gut

Antibiotic Isolate	SWGL1	SWGL2	SWGL3	SWGL4	SWGL5	SWGL6	SWGL7	SWGL8	SWGL9	SWGL10	SWGL11	SWGL12	SWGL13	SWGL14	SWGL15	SWGL16	SWGL17	SWGL18	SWGL19	SWGL20	SWGL21
Ampicillin (30 mg)	R	S	R	S	S	S	R	S	R	S	S	S	S	R	S	R	S	S	S	R	R
Cefaclor (30 mg)	S	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cefadroxil (30 mg)	R	S	S	R	S	S	R	S	R	S	S	R	R	S	S	R	R	S	S	S	S
Cefalexin (30 mg)	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	R
Cefaperazone (75 mg)	R	S	S	R	S	S	R	S	R	S	S	R	R	S	S	R	R	S	S	S	S
Cefazolin (30 mg)	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	R
Cefixime (5 mg)	R	S	S	R	S	S	R	S	R	S	S	R	R	S	S	R	R	S	S	S	S
Ceftazidime (30 mg)	R	S	S	R	S	S	R	S	R	S	S	R	R	S	S	R	R	S	S	S	S
Ceftizoxime (30 mg)	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	R
Ceftriaxone (30 mg)	S	S	S	S	I	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S
Cefuroxime (30mg)	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	R

RESULTS AND DISCUSSION

Results were expressed as sensitive (S, ≥ 21 mm), intermediate (I, 16-20mm) and resistant (R, ≤ 15 mm) shown in Table 1. From total isolates, about 8 LAB isolates (SWGL 1, SWGL 4, SWGL 7, SWGL 9, SWGL 12, SWGL13, SWGL 16 and SWGL 17) were resistant to Cefixime, Cefaperazone, Cefadroxil and Ceftazidime. Six nos. of LAB strains were highly resistant to Cefaclor (SWGL 2, SWGL 3, SWGL 5 and SWGL 6), seven nos. to Ampicillin (SWGL 1, SWGL 3, SWGL 7, SWGL 9, SWGL 14, SWGL 16, SWGL 20 and SWGL 21) (plate 1) and two were intermediate to Ceftriaxone (SWGL 5 and SWGL 12). Three LAB isolates (SWGL 10, SWGL 14 and SWGL 15) were found sensitive to all three antibiotics (Cefazolin, Cefuroxime and Cefalexin).

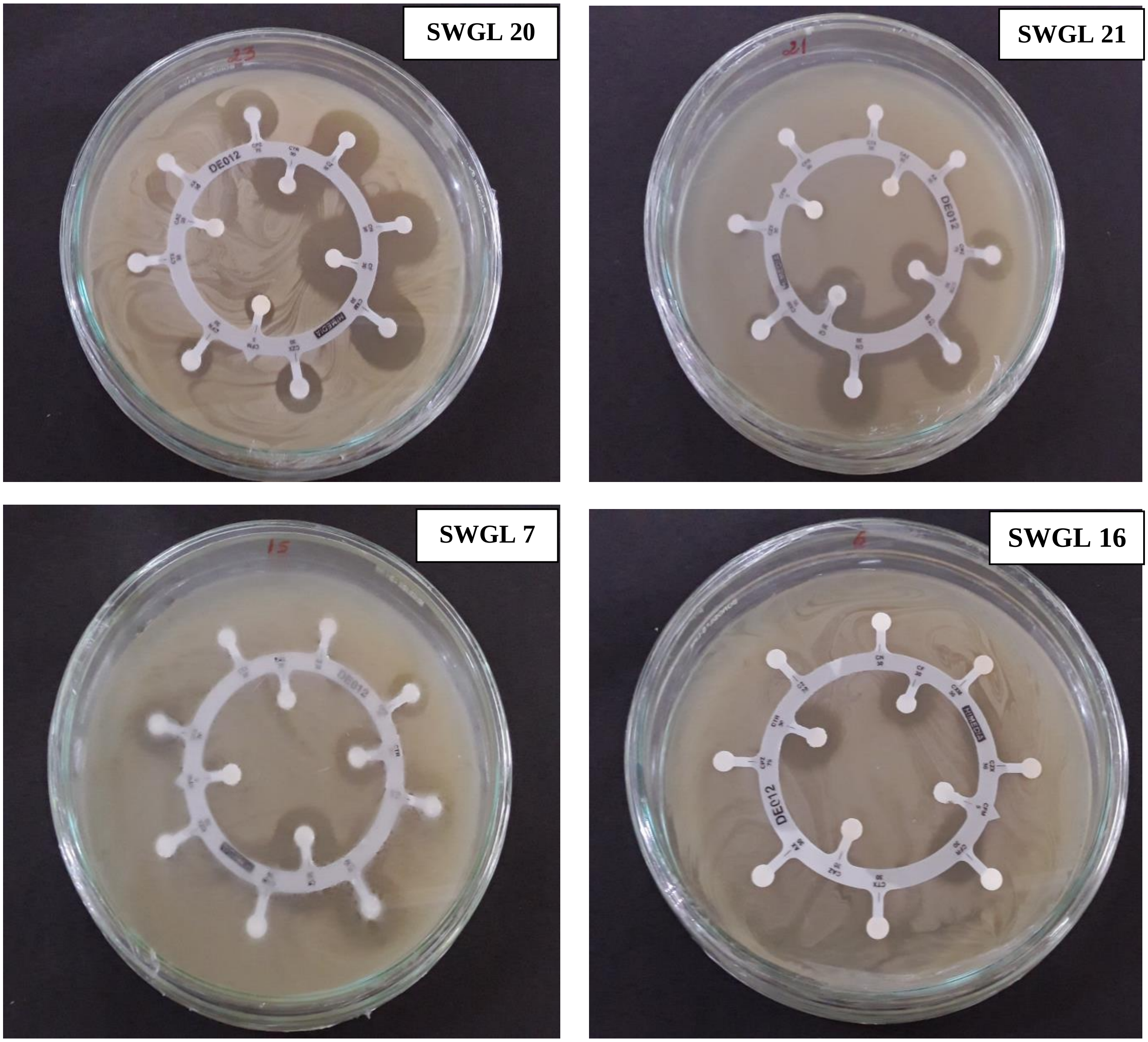

Plate 1. Antibiotic sensitivity of the selected isolates (Zone of inhibition seen around discs)

All the bacterial products anticipated for use as feed additives must be examined to establish the susceptibility of the component strains to appropriate range of antimicrobials of human or animal importance (Vankerckhoven *et al.*, 2008). Susceptibility against antibiotics is the most essential probiotic characteristic. In present study, antibiotics susceptibility tested for all strains exhibited sensitivity to all conventional antibiotics tested. High per cent of antibiotic resistance showed to Ampicillin (57.14%) by most of the isolated strains SWGB 3, SWGB 7, SWGB 10, SWGB 14, SWGB 16 and SWGB 21. Resende *et al.* (2014) reported that strain AAS2 can be considered as an efficacious probiotic candidate due to the lack resistant to all the antibiotics tested. Studies on *Enterococcal* isolates from foods such as raw meat and fermented milk have shown multiple antibiotics resistant to *Enterococci*, which nevertheless were mostly susceptible to the clinically relevant antibiotics, Ampicillin and Vancomycin (Klein *et al*., 1998).

Large numbers of probiotic bacteria are consumed to maintain and restore the microbial balance in the intestines. It must be kept in mind that they have a potential to transfer antibiotic resistance to pathogenic bacteria. For these and other applications the safety aspects of these bacteria are of concern, including the presence of potentially transferable antibiotic resistance. Resistance to a given antibiotic can be intrinsic to a bacterial species or genus (inherent or natural resistance) that results in an ability of the microorganism to thrive in the presence of an antimicrobial agent due to an inherent characteristic of the organism. Intrinsic resistance is not horizontally transferable and thus has no risk in non-pathogenic bacteria.

CONCLUSION

Antibiotics enhance feed consumption and growth by stimulating metabolic processes within the silkworm as well as reduce the occurrence of diseases which causes immense loss to sericulture industry. The present findings suggest that the silkworm gut microflora could be an excellent source of antibiotics properties which help to promote the health of silkworm. The strains SWGL 1, SWGL 3, SWGL 7, SWGL 9, SWGL 14, SWGL 16, SWGL 20 and SWGL 21 could be used as alternative source of antibiotics against pathogens.

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