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Research paper

# Evaluation of newly isolated probiotics in the protection against experimental intestinal trichinellosis

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ABSTRACT

The potential use of probiotics in controlling enteric infections has generated tremendous interest in the last decade. The protective efficacy of seven oral doses of two newly isolated Egyptian probiotic strains; *Lactobacillus acidophilus P110* (*L. acidophilus*) and *Lactobacillus plantarum P164* (*L. plantarum*) versus *Lactobacillus casei ATCC 7469* (*L. casei*) – against experimental intestinal trichinellosis – was assessed via parasitological, immunological and histopathological parameters, after verifying their in vivo safety and intestinal colonization.

Parasitologically, the highest adult count reduction was observed in *L. plantarum*-fed infected sub-subgroup (56.98, 65.42 and 69.02%) – on the 5th, 12th and 17th days post infection (P.I.), respectively. Lesser percentage reductions were recorded in both the *L. casei*-fed infected sub-subgroup (36.19, 23.68 and 31.58%) and *L. acidophilus*-fed infected sub-subgroup (36.50, 11.8 and 7.61%) at the same intervals. On the 28th day post challenge, the highest larval count reduction was in *L. plantarum*-fed infected sub-subgroup (87.92%). While lower percentage yet still significant were observed in the *L. casei*-fed infected (74.88%) and *L. acidophilus*-fed infected sub-subgroups (60.98%).

Immunologically, serum IFN-γ levels in the probiotic-fed non infected sub-subgroups were higher than those in the probiotic-fed infected sub-subgroups. Both showed higher levels of IFN-γ than the non probiotic-fed sub-subgroups. Histopathologically, intestinal sections of the probiotic-fed infected sub-subgroups showed amelioration of the inflammation and damage resulting from *Trichinella spiralis* (*T. spiralis*) infection. Results indicate that, through mechanical and immunological mechanisms, *L. plantarum* showed parasitological and histopathological protective superiority with respect to both *L. casei* and *L. acidophilus* against murine*T. spiralis infection.*



## 1. Introduction

Trichinellosis is meat borne parasitic disease which is emerging and re-emerging in many countries and is caused by larval stages of nematodes of the genus *Trichinella* (Pozio, 2007). In the international ranking of food-borne parasites, *Trichinella spiralis* was ranked among the top ten (FAO/WHO, 2014). Humans, swine and equine are considered the most important hosts from the medical and veterinary points of view (Pozio, 2007). Consumption of undercooked pork meat and pork products is considered the most important source of human infection (Appleton et al., 2012).

*T. spiralis* is the most pathogenic and prevalent species causing trichinellosis in man (Appleton et al., 2012). During its life cycle, *T. spiralis* causes both intestinal and muscular inflammations. Traumatic injury is caused by the large diameter of both adult worms and newborn larvae leading to epithelial cells death (Appleton et al., 2012).

Benzimidazole derivatives (mostly albendazole and mebendazole) are the commonly used drugs for treating human intestinal trichinellosis. Taking into consideration that these medications are active against adult worms in the gut and are ineffective against the larvae embedded in the tissues, there are serious concern about the currently used therapeutic agents (Reporting and Surveillance Guidelines, 2014). In this respect, preventing infection can be considered a promising tool against trichinellosis.

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Lately, significant efforts to design effective vaccines against *T. spiralis* infection were exerted. The main obstacle seems to be related to the complicated antigenic components of the parasite (Yang et al., 2010). As a result of the growing evidence of the poor effect of drugs against larvae and the difficulties in producing vaccines, the need for new alternatives to control trichinellosis arose (Bautista-Garfias et al., 2002).

A strong emphasis has been given to the applications of probiotics. The World Health Organization (WHO) defined probiotics as live microorganisms [M2] which, when consumed in adequate amounts, confer a health benefit on the host (WHO, 2002). A microbial strain has to fulfill a number of specific criteria to be regarded as a probiotic. These should include safety, performance and technological aspects (Gibson and Fuller, 2000). During the last decade, probiotics acted as means for controlling not only intestinal parasitic infections but also some non-gut infections (Travers et al., 2011). Probiotics can prevent enteric infections by three major strain-specific mechanisms relying on modulation of intestinal environment, immune modulation and secretion of active molecules (Travers et al., 2011).

*Lactobacilli* are the most commonly used probiotics (Mombelli and Gismondo, 2000). Their inherent biological features enable them to predominate and overcome the potential pathogens infecting human's digestive tract (Pacifico et al., 2014). *Lactobacillus casei* is the most popularly used probiotic in the protection against *T. spiralis* infection. Several strains of *L. casei* have proven their efficacy against trichinellosis—such as *L. casei ATCC 7469*, *L. casei ATCC 469* and *L. casei* Shirota strains (Randazzo and Costamagna, 2005; Martinez-Gomez et al., 2009, 2011). In previous studies, it has been demonstrated that single or multiple administrations of *L. casei* in mice before *T. spiralis* infection induced protection (Bautista-Garfias et al., 2002; Martinez-Gomez et al., 2009).

Currently, researchers are interested in isolating and testing new probiotic strains. On this basis, two new strains; *Lactobacillus acidophilus P110* and *Lactobacillus plantarum P164* have been isolated from faeces of healthy breast-fed Egyptian infants and were identified as promising probiotics (Khalil et al., 2007). Accordingly, the current study was designed to evaluate, for the first time, the possible protective efficacy of these newly isolated Egyptian strains versus *L. casei* against experimental intestinal trichinellosis.

## 2. Materials and methods

### 2.1. Animals

Male Swiss strain albino mice six to eight weeks old, weighing 20–30 g each, were used in this study. Mice were purchased and bred in the animal house of the Medical Parasitology department, Faculty of Medicine, Alexandria University. Each ten animals of each subgroup were housed in a clean polypropylene cage with perforated cover in a well-ventilated room (25 ± 2 °C), with a relative humidity of (43 ± 3%) and were maintained on 12:12 h light:dark cycle at the animal house. Animals were fed with standard pellet food and water under strict hygienic conditions. Bedding was changed every day. Mice were allowed to adapt to the laboratory environment for one week before the experiment. Rules of the ethical committee of Alexandria University were followed as regards to animal housing, sacrifice and experimentation.

### 2.2. Probiotic strains

*L. casei ATCC 7469* was purchased from ATCC (American type culture collection), while *L. acidophilus P110* and *L. plantarum P164* were isolated from faeces of healthy breast-fed Egyptian infants born in Alexandria according to Khalil et al. (2007). Prior to use, tested strains were subcultured (1%, v/v) in MRS broth and incubated aerobically at 37 °C overnight.

### 2.3. Prestudy evaluation of the probiotic strains (Fig. 1)

For evaluating the in vivo safety and colonization of the tested *Lactobacillus* strains, 40 mice were divided into four equal groups each group of 10 mice were orally fed with *L. casei*, *L. acidophilus*, *L. plantarum* and broth. Feeding was gently performed via a tuberculin syringe fitted with a ball tip, curved, blunt sterile needle. Mice swallow as the feeding tube approaches the pharynx, facilitating entry into the esophagus (Cunliffe-Beamer, 1983). Seven daily oral doses of each probiotic were orally fed to mice. The daily dose of a probiotic per mouse was 1.0 ml/kg with a concentration of $1.9 \times 10^9$ CFU/ml (Randazzo and Costamagna, 2005). While mice of the broth-fed group received the same daily volume of broth as the probiotic-fed groups .

#### 2.3.1. Safety

Both physical and biochemical safety measures were observed. Physical safety was assessed by monitoring the mortality rate, general health, changes in behavior, as well as body weight per mouse in each group during the seven days of feeding and on day one after feeding was stopped (Songisepp, 2012).

Biochemical safety was assessed on day one after feeding was stopped. Mice in each group were anesthetized by applying a local anaesthetic cream on their hyperextended neck for 30 min. Then, a 25 G needle was inserted in a caudocephalic direction into the jugular veins. Blood was withdrawn into heparinized Wassermann tubes slowly in order to avoid the collapse of these small veins (Parasuraman et al., 2010). Serum of each mouse was separated, and biochemical blood analyzer (Hitachi 7180, Hitachi, Japan) was used to measure the following biomarkers; aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum urea, serum creatinine as well as total serum cholesterol by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum urea, serum creatinine as well as total serum cholesterol (Chorawala et al., 2013).

#### 2.3.2. Lactobacillus *colonization*

Mice were euthanized after blood collection on day one after probiotic feeding was stopped. Both qualitative and quantitative assessments of bacterial colonization were performed in the jejuna of sacrificed mice of the three probiotic-fed groups. One cm segments were taken from the jejunum. Sections were stained with Gram stain and examined under oil immersion lens to visualize the bacterial colonization (Gephart, 1981). For quantitation, another cm segment was processed and the mean of *Lactobacilli* per oil field was calculated as described by Jiang et al. (2001).

### 2.4. Parasite

*T. spiralis* strain was maintained in the Medical Parasitology Department, Faculty of Medicine, Alexandria University, Egypt by serial passage in Swiss strain albino mice as described by Wassom et al. (1988).

### 2.5. Experimental study design (Fig. 2)

Three hundred and seventy mice were allocated into two main groups: Non infected group (I) and Infected group (II). Group I consisted of 170 mice and was further subdivided into three subgroups; Ia: (10 non infected non fed mice), Ib: (150 non infected probiotic-fed mice) and Ic: (10 non infected broth-fed mice). Mice in non-fed non infected subgroup (Ia) were sacrificed to record the level of