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Protective effect of *Streptococcus thermophilus* CCFM218 against house dust mite allergy in a mouse model

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ABSTRACT

House dust mite allergy accounts for a majority of severe allergic asthma cases, and there is a need for new prevention and treatment strategies. Specific probiotics have shown beneficial effects in the treatment of allergic diseases, owning to their inherent immunomodulatory properties. To obtain new and effective anti-allergy probiotics, cytokine profiles of 25 lactic acid bacteria strains were measured by *in vitro* co-culture with mice spleen cells. Of the various strains, strong IL-10-inducing *Streptococcus thermophilus* CCFM218 (ST218) significantly suppressed IL-4 secretion *in vitro* and was postulated to have a better anti-allergy effect *in vivo*. To determine the anti-allergy property of ST218, its protective effect on allergic response was evaluated in a mouse allergy model together with *Lactobacillus rhamnosus* GG. In contrast to LGG, ST218 had a better suppressive effect on allergic response *in vivo*, characterized by increased specific IgG2a and IL-10 levels in serum, regulatory T cells in the mesenteric lymph nodes and a reduction in serum Th2 cytokine IL-4. It indicated that ST218 was an excellent anti-allergy strain that can be favorable to use in the treatment or prevention of allergic diseases.

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1. Introduction

Allergic asthma is a chronic inflammatory disease characterized by airway obstruction in response to allergen exposure (Mukherjee & Zhang, 2011). House dust mite (HDM) allergens are among the most common and widespread in most environments, and up to 85% of asthmatics are typically HDM-allergic (Gregory & Lloyd, 2011). The classic symptoms of HDM-allergic asthma, i.e., wheezing and coughing, generally cause discomfort, whereas an active asthma reaction may pose a threat to life without timely treatment (Martínez & Ferguson, 2009). Recurrent attacks are a major characteristic of allergic asthma, and have a serious effect on the quality of life in patients with this condition. At present, roughly 10–15% of individuals in Western populations have asthma; about 25% of whom experience weekly symptoms and 15% daily symptoms (Kandane-Rathnayake et al., 2009). Until recently, no effective treatment is available for HDM allergy. The primary management strategy is still to avoid contact with HDM allergens, but there is considerable uncertainty due to the widespread existence of HDM allergens in the environment.

HDM-allergic asthma represents an imbalance in the Th1/Th2 responses characterized by increased levels of Th2 cytokines, i.e., IL-4 and IL-5 and allergen-specific IgE (Jacquet, 2011). Probiotics have demonstrated the capacity to counter-regulate the Th2 responses and balance the Th1/Th2 responses, and may thus offer a potentially effective strategy for the prevention or treatment of HDM allergy. The results of clinical trials indicated that *Lactobacillus rhamnosus* GG reduced the incidence of atopic eczema in at-risk children during the first two years of life (Kalliomaki, Salminen, Poussa, Arvilommi, & Isolauri, 2003), and *Lactobacillus fermentum* has also been shown to be beneficial in attenuating the extent and severity of atopic dermatitis in young children (Weston, Halbert, Richmond, & Prescott, 2005). The mechanisms behind these beneficial effects are associated with the induction of regulatory T cells (Tregs) and the production of cytokines TGF-β and IL-10, which play essential roles in the suppression of Th2-polarized allergic response (Hansen et al., 2000; Jeon et al., 2012).

However, not all candidate probiotics have proven to be equally efficient in regulation of the Th1/Th2 balance to inhibit allergic response (Fujiwara, Inoue, Wakabayashi, & Fujii, 2004). Forsythe, Inman, and Bienenstock (2007) demonstrated that the application

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of *Lactobacillus salivarius* had no beneficial effect on airway inflammation in OVA-sensitized mice. In another study, early probiotic supplementation with *Lactobacillus acidophilus* LAVRI-A1 did not reduce the risk of atopic dermatitis in high-risk infants, and to a certain extent it even increased allergen sensitization in infants receiving supplements (Taylor, Dunstan, & Prescott, 2007). Moreover, *Lactobacillus plantarum* WCFS1 treatment was shown to augment the Th2 phenotype in a mouse peanut allergy model associated with increased levels of specific IgE and Th2 cytokines IL-4 and IL-5 (Meijerink et al., 2012).

Hence, it is necessary to establish an effective *in vitro* screening model with fair predictive value to select potential anti-allergy probiotics. It has been determined that Th2 cytokine IL-4 is an important inflammatory mediator in type I allergic diseases. Previous study suggested that lactic acid bacteria (LAB) capable of inducing IL-10 production to inhibit IL-4 secretion *in vitro* may have a potential anti-allergy effect *in vivo* (Niers et al., 2005). In this study, the *in vitro* immunomodulatory properties of 25 LAB strains were evaluated in a co-culture assay with mice spleen cells. *Streptococcus thermophilus* CCFM218 (ST218) had a relatively high IL-10 level and low IL-4 level *in vitro* compared with other strains, and was postulated to have an anti-allergy effect *in vivo*. To determine the anti-allergy property of ST218, its protective effect on allergic response was evaluated in a mouse allergy model together with LGG which had a relatively low IL-10 level and high IL-4 concentration *in vitro*.

2. Materials and methods

2.1. Bacterial strains

The bacterial strains used in this study and their origins are shown in Table 1. *Lactobacillus* and *Streptococcus* strains were anaerobically cultured overnight to stationary phase in MRS medium at 37 °C, and *Bifidobacterium* strains were grown anaerobically in MRS supplemented with 0.05% L-cysteine-hydrochloride (Sigma, USA). For immune cell stimulation, the bacterial strains were grown to stationary phase, washed and re-suspended at approximately 1×10^8 cfu mL^{-1} in 0.1 M sterile phosphate-buffered saline (PBS, pH 7.4). For the *in vivo* experiments, selected bacterial strains were cultured overnight, washed and re-suspended in 10% sterile skim milk. The bacteria suspension was turned into bacterial powder by rapid freeze-drying and stored at −20 °C. The bacterial strains were recovered in sterile skim milk at 37 °C for 30 min and adjusted to approximately 1×10^{10} cfu mL^{-1} before the animal experiment. A bacterial count was performed before the cell and animal experiments.

2.2. In vitro stimulation of spleen cells with LAB strains

Fresh spleens were collected and pooled from normal BALB/c mice ($n = 3$) under sterile conditions, and LAB stimulation of the spleen cells was performed as mentioned previously (Ongol et al., 2008) with a slight modification. Briefly, the spleen cells were re-suspended in RPMI 1640 medium (Hyclone, USA) supplemented with penicillin, streptomycin and 10% fetal bovine serum and counted. Cell culture was performed *in vitro* by plating single-cell suspensions at a concentration of 2×10^5 cells per well (200 μL) in 96-well plates (NUNC, USA) in triplicate and stimulating the spleen cells with 2×10^6 cfu of the bacteria suspension per well (20 μL) at 37 °C with 5% CO_2. After incubation for 72 h, the supernatants were collected and stored at −80 °C until further analysis. The levels of IL-10, IL-12 and IL-4 were measured by ELISA according to the manufacturer's instructions (Rapidbio Lab, Langka Trade Co., Ltd., Shanghai, China).

2.3. Animal experiment

2.3.1. Animal

Four-week-old female BALB/c mice were purchased from Shanghai SLAC Laboratory Animals Co., Ltd. (China). Mice were housed at the Animal Center of Jiangnan University, and handled

Table 1
Effect of 25 LAB strains on cytokine production *in vitro*.

Species	Strain	IL-10	IL-12	IL-4	IL-10/IL-4
L. acidophilus[1]	CCFM6	678 ± 153^{bcd}	39 ± 2^{bcdef}	252 ± 13^{cdef}	2.70 ± 0.74^{a}
L. acidophilus[2]	CCFM137	851 ± 38^{e}	48 ± 1^{g}	211 ± 24^{bcde}	4.01 ± 0.26^{bc}
L. casei[3]	Lc2W	767 ± 29^{cde}	34 ± 2^{bcde}	224 ± 10^{bcdef}	3.17 ± 0.30^{ab}
L. casei[3]	CCFM9	739 ± 26^{bcde}	41 ± 1^{ef}	200 ± 16^{bcd}	3.29 ± 0.62^{ab}
L. casei[1]	CCFM236	685 ± 30^{bcde}	38 ± 2^{bcdef}	254 ± 27^{def}	2.65 ± 0.13^{a}
L. gasseri[1]	CCFM15	737 ± 207^{bcde}	39 ± 5^{cdef}	232 ± 26^{bcdef}	2.74 ± 0.55^{a}
L. helveticus[4]	CCFM310	698 ± 13^{bcde}	38 ± 3^{bcdef}	271 ± 5^{f}	2.63 ± 0.09^{a}
L. plantarum[1]	CCFM231	711 ± 537^{bcde}	36 ± 2^{bcdef}	238 ± 9^{bcdef}	2.93 ± 0.11^{a}
L. plantarum[3]	CCFM10	708 ± 9^{bcde}	40 ± 1^{def}	243 ± 13^{bcdef}	2.63 ± 0.19^{a}
L. plantarum[2]	CCFM184	634 ± 57^{bc}	41 ± 3^{ef}	231 ± 6^{bcdef}	2.70 ± 0.14^{a}
L. plantarum[1]	CCFM47	594 ± 12^{b}	32 ± 1^{b}	209 ± 8^{bcde}	2.84 ± 0.16^{a}
L. plantarum[2]	CCFM169	685 ± 69^{bcde}	32 ± 1^{bc}	259 ± 22^{ef}	2.66 ± 0.49^{a}
L. plantarum[2]	CCFM168	748 ± 15^{bcde}	39 ± 1^{cdef}	263 ± 7^{ef}	3.11 ± 0.40^{ab}
L. bulgaricus[3]	CCFM4	660 ± 21^{bc}	42 ± 3^{fg}	242 ± 41^{bcdef}	2.70 ± 0.45^{a}
B. lactis[3]	Bb12	632 ± 22^{bc}	37 ± 3^{bcdef}	248 ± 64^{bcdef}	2.64 ± 0.77^{a}
B. breve[5]	Bb2	662 ± 97^{bc}	37 ± 2^{bcdef}	255 ± 59^{def}	2.62 ± 0.22^{a}
L. reuteri[4]	CCFM13	635 ± 21^{bc}	43 ± 1^{fg}	248 ± 24^{bcdef}	2.54 ± 0.02^{a}
L. reuteri[4]	CCFM14	792 ± 16^{cde}	38 ± 2^{bcdef}	232 ± 10^{bcdef}	3.35 ± 0.31^{ab}
S. thermophilus[1]	CCFM218	832 ± 79de	40 ± 2^{def}	198 ± 16^{bc}	4.22 ± 0.74^{c}
S. thermophilus[1]	CCFM3	742 ± 24^{bcde}	41 ± 1^{ef}	258 ± 13^{ef}	3.00 ± 0.09^{a}
S. thermophilus[2]	CCFM147	714 ± 13^{bcde}	36 ± 2^{bcdef}	247 ± 6^{bcdef}	2.93 ± 0.09^{a}
L. rhamnosus[3]	LGG	700 ± 7^{bcde}	34 ± 1^{bcde}	226 ± 23^{bcdef}	2.78 ± 0.31^{a}
L. rhamnosus[1]	CCFM237	648 ± 79^{bc}	33 ± 2^{bcd}	226 ± 10^{bcdef}	2.87 ± 0.48^{a}
L. brevis[6]	CCFM12	720 ± 49^{bcde}	33 ± 3^{bcd}	211 ± 20^{bcde}	3.43 ± 0.56^{bc}
L. delbrueckii[1]	CCFM29	701 ± 46^{bcde}	39 ± 2^{bcdef}	296 ± 27^{f}	3.26 ± 0.22^{ab}
Control		365 ± 72^{a}	25 ± 2^{a}	105 ± 12^{a}	3.43 ± 0.27^{bc}

Different numbers within the species column represented different origins: 1, traditional product; 2, Inner Mongolia Agricultural University; 3, Commensal strain; 4, China Center of industrial Culture Collection; 5, China General Microbiological Culture Collection Center; 6, Nanjing Agricultural University.
The mean values (standard deviations) within the same column followed by different superscript letters differ significantly ($P < 0.05$).

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