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ORIGINAL ARTICLE

Probiotic supplementation in systemic nickel allergy syndrome patients: study of its effects on lactic acid bacteria population and on clinical symptoms

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Keywords
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Abstract

Aims: The study aimed to evaluate the effects of probiotic *Lactobacillus reuteri* DSM 17938 strain supplementation in patients suffering from systemic nickel allergy syndrome, in terms of modulation of faecal LAB population linked to a reduction of GI and cutaneous symptoms and to an increase of patient's quality of life.

Methods and Results: A preliminary double-blind randomized placebo-controlled study was planned and a culture-independent method based on denaturing gradient gel electrophoresis (DGGE) analysis coupled to the 16S rRNA gene sequencing was applied to investigate on the dynamics of faecal LAB communities before and during a low-Ni diet, supplemented with the probiotic strain. Moreover, the severity and the frequency of GI and cutaneous reactions as well as patient's clinical condition perception (VAS scores) were estimated by statistical analysis. PCR-DGGE fingerprinting obtained using LAB-specific primers revealed significant shift in faecal community with an increase in biodiversity in patients supplemented with probiotic *Lact. reuteri* strain. In addition, GI reactions such as symptoms related to meals and type of stools significantly improved only in patients treated with *Lact. reuteri*, while severity and frequency of cutaneous symptoms (urticaria, itch and eczema) and recurrent abdominal pain (RAP) as well as VAS scores statistically decreased in both groups.

Conclusions: Our preliminary findings suggest that probiotic *Lact. reuteri* could be a useful supplementation during a low-Ni diet of patients with SNAS, to increase LAB population diversity, which could contribute to restore the intestinal homoeostasis conditions.

Significance and Impact of the Study: To date, no information is available on probiotics application and on their effects, in terms of intestinal microbiota modulation, on patients suffering from SNAS. Therefore, the identification of dominant LAB community and the study of its shift during the probiotic supplementation could enhance the knowledge of the SNAS syndrome.

Introduction

Probiotics are defined as 'live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host' (FAO/WHO 2001). Probiotic therapies have been proven to be effective in treating a variety of medical conditions, including antibiotic-associated diarrhoea and other chronic inflammatory conditions

(Soccol *et al.* 2010). The therapeutic potential of these treatments appears to be mediated through a number of mechanisms of action, including modulation of the immune response, competitive inhibition of invading microbiota in the gut, modification of pathogenic toxins and host products, and enhancement of epithelial barrier function (Oelschlaeger 2010; Chapman *et al.* 2011).

Up to now, a large number of probiotics have been promoted with health claims, based on several characteristics of selected strains, mainly belonging to *Lactobacillus* and *Bifidobacterium* genera (Soccol *et al.* 2010; Sanders *et al.* 2013). Within *Lactobacillus* genera, *Lactobacillus reuteri* is a commensal anaerobic probiotic species that resides in the human GI tract. In particular, the *Lact. reuteri* DSM 17938 strain originated from *Lact. reuteri* ATCC 55730 by curing of two plasmids harbouring antibiotic resistance genes (Rosander *et al.* 2008). Several authors have verified its safety in adults, children, infants and babies (Wolf *et al.* 1995, 1998; Karvonen *et al.* 2001; Alsheikh and Weizman 2003); its ability to *in situ* colonize GI mucosa, to modulate GI immune system, to reduce incidence of diarrhoea (Casas and Dobrogosz 2000; Maassen *et al.* 2000; Valeur *et al.* 2004) and duration of gastroenteritis (Shornikova *et al.* 1997a,b; Szajewska and Mrukowicz 2001; Rosenfeldt *et al.* 2002;). Clinical studies showed an improvement of atopic dermatitis, GI symptoms and intestinal permeability in patients treated with a mixture of *Lact. reuteri* (DSM 17938) and *Lactobacillus rhamnosus* strains (Rosenfeldt *et al.* 2003). Up to now, no information is available on the usefulness of *Lact. reuteri* supplementation on chronic and recurrent inflammatory skin disease, such as systemic nickel allergy Syndrome (SNAS). Nickel (Ni) is the most frequent cause of contact allergy in the industrialized world. It is estimated that about 17% of women and 3% of men are allergic to Ni, and the prevalence is even higher among dermatitis patients (Thyssen and Menné 2010). Ni is present in most of the dietary items and foods, which are considered a major Ni source for the general population. Ni content in food may vary considerably from place to place due to the difference in Ni content of the soil. Even if the major manifestation of Ni-allergy is allergic contact dermatitis (ACD), which affects 10–20% of the population (Schafer *et al.* 2001; Thyssen *et al.* 2007; Jacob *et al.* 2009), about 20–30% of Ni-ACD patients show the appearance of skin (urticaria/angioedema, flares, itching) and GI (meteorism, colic, diarrhoea) symptoms, after ingestion of Ni-containing foods (Braga *et al.* 2013). These clinical symptoms, related to the ingestion of Ni-foods, cause the clinical condition so-called systemic nickel allergy syndrome (Falagiani *et al.* 2008; Turi *et al.* 2008). The latter involves different organs and immunologic mechanisms that implicate Th2 as well as ACD's typical Th1 cytokines (Di Gioacchino *et al.* 2000; Minelli *et al.* 2010). Several studies have investigated the prevalence of contact Ni-allergy, addressing the relationship between nickel intake and onset of systemic symptoms. A recent epidemiological study, conducted on four allergy units in Sicily and evaluating 1·696 patients, revealed that 98 patients (5·78%) were confirmed SNAS positive, suggesting that this clinical entity may be an emergent allergic condition rather than an occasional finding. Moreover, evaluating the symptomatology, the most common symptoms were gastrointestinal (87 patients), cutaneous (51 patients) and systemic (37 patients), confirming that these signs and symptoms may be referred to nickel intake (Ricciardi *et al.* 2014). For this reason, patients with SNAS need to follow a lifelong Ni-poor diet, basically avoiding the majority of vegetables, which poses a potential risk for a deficit in essential elements. To minimize this, nutritionally balanced diets are now available. Moreover, Di Gioacchino *et al.* (2014) in a randomized, double-blind placebo-controlled trial demonstrated that the nickel oral hyposensitization allows patients affected by SNAS to safely re-introduce Ni-rich foods, with an improvement of gastrointestinal manifestations and consequently quality of life (Di Gioacchino *et al.* 2014).

In this study, a culture-independent approach, based on PCR-DGGE analysis and 16S rRNA gene sequencing, was applied with the objective to evaluate the therapeutic efficacy of the probiotic *Lact. reuteri* DSM 17938 strain in patients suffering from SNAS, through (i) the characterization of faecal lactic acid bacteria community and (ii) the identification of its shifts before and during the intervention.

Material and methods

Patients' enrolment and exclusion criteria

This study was a preliminary randomized double-blind placebo-controlled trial with two parallel groups (experimental and control group). Twenty-two adult women, aged between 18 and 65 years, were assessed for eligibility and were enrolled at the Allergy and Clinical Immunology Unit of the University of Messina. The patients were recruited using the following criteria: age; positivity to a Ni patch test; presence of GI reactions, such as symptoms related to meals as dyspepsia, nausea and/or vomiting; type of stools (frequency and consistency); recurrent abdominal pain (RAP); and cutaneous reactions such as urticaria, itching and eczema after ingestion of Ni-containing food. Exclusion criteria included organic diseases (as established by medical history, complete blood count,

urinalysis, stool examination for occult blood, ova and parasites, blood chemistries, abdominal ultrasound, breath hydrogen testing and endoscopy, if needed), presence of other chronic disease such as coeliac disease, pregnancy and lactation, concomitant treatment with steroids and/or antihistamines, and participation in another study.

Patients were not allowed to use gastric acid inhibitors, laxatives, antidiarrhoeal medications or antibiotics for at least 1 month before the beginning of the study and systemic or topic antihistamines or corticosteroids during the study. Moreover, the consumption of probiotics or prebiotics for at least 2 weeks before the study was not allowed.

Study design

At baseline, the patients were randomized with a 1 : 1 ratio to receive active treatment (experimental group: E) or placebo treatment (control group: C) (Fig. 1). Patients' randomization was performed using a computer-generated random numbers to allocate participants. Sixteen patients (9 of 16 experimental group and 7 of 16 control group) completed the trial and six dropouts (two dropouts in experimental group and four in control group) were done. The study complied with the Declaration of Helsinki, as revised in 2000. Written informed consent was obtained from each patient and the study protocol was approved by the ethics committee of AOU G. Martino of Messina, Italy (Registration n.2009-013923-43). The study was divided in three times of intervention: at baseline (T0), after 2 weeks from the *Lact. reuteri* probiotic supplementation (T1) and 2 weeks after stop *Lact. reuteri* feeding (T2). Patients were asked to follow a low-Ni diet. Moreover, experimental group received 1 tablet (10^8 CFU ml^{-1}) per day of *Lact. reuteri* probiotic strain and the placebo group received 1 daily placebo tablet, both produced by BioGaia, AB (Stockholm, Sweden) and sold by NOOS (Roma, Italy).

Faecal samples collection

Faecal samples, from experimental and placebo patients, were collected, in duplicate, at T0, T1 and T2 sampling time using sterile plastic containers. Collection was performed every Thursday (±1 day), to avoid major influences of changing dietary habits during the weekend, and samples were immediately transported under refrigerated conditions (4°C) to the Laboratory of Microbiology of the Department of Agri-Food Science and Environmental Systems Management (DiGeSA) of the University of Catania, Italy, then frozen at −80°C until use.

Clinical outcomes

During each time of faecal sampling, the patients were subjected to clinical evaluation. In particular, the impact of symptoms on the quality of life of each patient was evaluated indicating a score on a visual analogue scale (VAS) where 0 was equal to no symptoms and 10 to extreme malaise. The frequency of gastrointestinal symptoms was assessed in three steps: (i) <3 times per month; (ii) 3–6 times per month; (iii) >6 times per month, and it was taken into consideration if they were related to meals or not. Bowel habits, stool frequency and consistency according to the Bristol Stool scale side effects were also evaluated. The Bristol Stool Scale classifies form and consistency of stools into seven categories (from separate hard lump to entirely liquid stools).

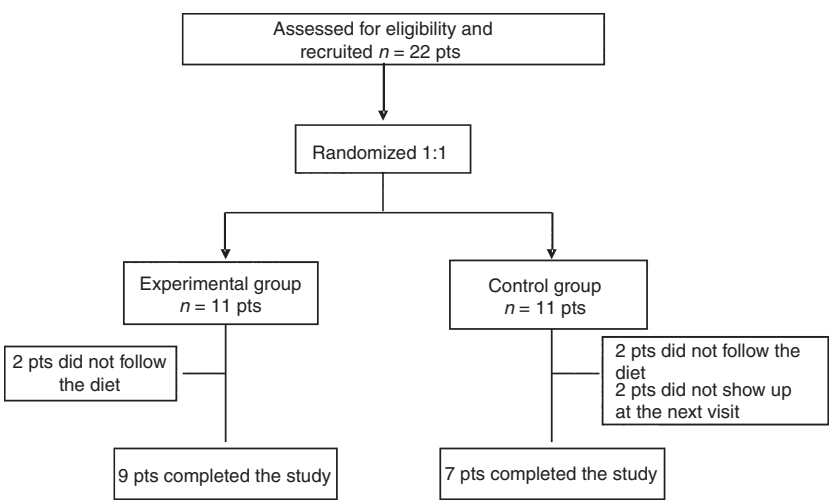

Figure 1 Flow diagram: enrolment and follow-up diagram of patients included in the study.

DNA isolation

The commercial QIAgen Kit QIAamp® DNA Stool Mini Kit (QIAgen, Hilden, Germany) was used for the DNA extraction from faecal samples. Faecal samples were gently defrosted and pretreated before DNA extraction following the protocol reported by Nylund *et al.* (2010). Lysate fraction obtained from the first homogenization step was removed before performing the second round of bead beating. The repeated bead beating (RBB) step was performed with lysis buffer ASL (Stool lysis buffer; QIAgen, Hilden, Germany) of the kit, and after the RBB, treatment the samples were centrifuged at +4°C for 5 min at full speed and the supernatant was collected. DNA was extracted by following the kit manufacturer's instructions, purified and stored at −20°C.

PCR amplification

To investigate the lactic acid bacteria communities, PCR products were generated using the group-specific primers Lac1 (AGCAGTAGGGAATCTTCCA) and Lac2 (ATT TCACCGCTACACATG) used to amplify a 340-bp fragment of the V3 region of the 16S rRNA gene. A 40-bp GC clamp was attached to the reverse primer Lac2 to obtain PCR fragments suitable for DGGE analysis (Walter *et al.* 2001). PCR amplification was performed with the KIT 5-PRIME MasterMix (Eppendorf, Italy). Reaction mixtures consisted of 75 mmol l^{-1} Tris-HCl (pH 8·4), 50 mmol l^{-1} KCl, 1·5 mmol l^{-1} Mg^{2+}, 10 mmol l^{-1} each of the four deoxynucleoside triphosphates (dNTP), 1·255 U ml^{-1} of *Taq* polymerase, 10 pMol of each primer and 1 μl of appropriately diluted template DNA in a final volume of 50 μl. The thermocycle program consisted of the following time and temperature profile: 94°C for 2 min; 35 cycles of 94°C for 30 s, 61°C for 1 min, 68°C for 1 min; and 68°C for 7 min.

DGGE of PCR amplicons

DGGE analysis of PCR amplicons was based on the protocol described earlier (Muyzer *et al.* 1993) using the DCode System (Bio-Rad Laboratories, Hercules, CA). Polyacrylamide gels (dimensions, 200 × 200 × 1 mm) consisted of 8% (v⁄v) polyacrylamide (37·5 : 1 acrylamide–bisacrylamide) in 0·5× Tris-acetate-EDTA buffer (TAE). Denaturing acrylamide of 100% was defined as 7 mol l^{-1} urea and 40% formamide. The gels were poured from the top using a gradient maker and a pump (Econopump; Bio-Rad) set at speed of 4·5 ml min^{-1}, and gradients 30–60% were used for the separation of the generated amplicons. Electrophoresis was performed for 4 h and 30 min at voltage of 130 V in a 0·5× TAE buffer at a constant temperature of 60°C. Gels were stained with silver nitrate according to the method of Sanguinetti *et al.* (1994).

Cloning in plasmid inserts and sequencing of 16S rRNA gene

The 16S rRNA gene clone libraries were constructed from experimental and control faecal samples collected at T0 and T2 intervention time. Amplicons derived from PCR of the 16S rRNA gene using primer pairs Lac1-Lac2 were purified and cloned in *Escherichia coli* JM109 using the pGEM-T plasmid vector system (Promega, Madison, WI) in accordance with the manufacturer's instructions. Transformants were screened using blue/white selection on Luria agar containing Xgal/IPTG and 100 mg ml^{-1} ampicillin. Recombinant colonies were transferred to fresh culture plates and incubated overnight. Plasmid DNA was extracted from each of the positive clones using the QIAprep Spin Miniprep Kit (QIAgen) and re-suspended in water before sequencing. Clones containing 16S rRNA gene inserts were amplified and its mobility was compared to the rDNA-derived patterns of faecal samples by DGGE (data not shown). Clones that produced a single DGGE amplicon with a melting position identical to that one of the dominant bands in the faecal samples DNA patterns were sequenced by Biodiversity Spa (Brescia, Italy) company. To determine the closest known relatives of the clones, partial 16S rRNA gene sequences were compared to those in the GenBank database (http://ncbi.nlm.nih.gov/BLAST/) and the Ribosomal Database Project (http://rdp.cme.msu.edu/index.jsp) using BLAST program. Sequences with a percentage similarity of 98% or greater were considered to belong to the same species.

References strains

Several reference strains, reported in Table 1, were used in this study to identify the dominant LAB species revealed in the DGGE profiles of faecal samples. Reference stains and the pure culture of *Lact. reuteri* DSM 17938 (Noos S.r.l., Rome, Italy) were cultured using the medium MRS broth (Oxoid, Basingtoke, UK). Incubation was performed at 37°C for 24–48 h under anaerobic condition using an Anaerogen Kit (Oxoid). Overnight cultures were subjected to DNA extraction as described by Gala *et al.* (2008) and PCR amplification was carried out as described above.

Statistical analysis

To reveal differences among clinical symptoms, statistical analysis was performed with SPSS for Windows (ver.

17.0); differences between categorical groups were analysed by Fisher's exact test and differences between two unpaired groups were analysed by the Mann–Whitney *U*-test. Differences between K paired groups were analysed by Friedman test (not dichotomous variables) or Cochrane test (dichotomous variables). The significance level was set at $P = 0{\cdot}05$.

Results

Detection of LAB population dynamics through PCR-DGGE

DNA isolation and subsequent PCR-DGGE analysis of the V3 regions of the 16S rRNA gene were successful for all faecal samples. The applicability of the PCR-DGGE method to monitoring LAB population was first tested using DNA from several *Lactobacillus* species as template in a competitive PCR. Fragments from all species were found in the PCR-DGGE profile, but in addition, some extra fragments appeared above the single-stranded DNA (data not shown). In Figs 2, 3 and 4 are reported amplicons of experimental and control samples. In detail, profiles of experimental samples from E1 to E4 (Fig. 2 lines 1–6 and 8–13) and E5 to E9 (Fig. 3 lines 4–12 and 14–19) exhibited a complex LAB profile with fluctuations in both positions and number of amplicons throughout the intervention. Differently, analysis of faecal samples from control group (C1–C4 Fig. 4 lines 2–16; C6 Fig. 2 lines 14–16; C7 Fig. 3 lines 1–3) exhibited a quite stable profile with few shifts during the intervention. It is interesting to highlight that all experimental samples showed the presence of *Lact. reuteri,* up to 2 weeks of probiotic supplementation, which remained dominant until the end the intervention.

Table 1 Reference strains used in this study

Strains	Code number
Lactobacillus acidophilus	DSM 20079
Lactobacillus brevis	DSM 20054
Lactobacillus buchneri	DSM 20057
Lactobacillus casei	DSM 20011
Lactobacillus delbruekii subsp. lactis	DSM 20072
Lactobacillus fermentum	DSM 20052
Lactobacillus hilgardii	DSM 20176
Lactobacillus lactis	DSM 20481
Lactobacillus mindensis	DSM 14500
Lactobacillus paracasei subsp. *paracasei*	DSM 5622
Lactobacillus pentosus	DSM 20314
Lactobacillus plantarum	DSM 20246
Lactobacillus reuteri	DSM 17938
Lactobacillus rhamnosus	DSM 20021
Leuconostoc mesenteroides	DSM 20346
Leuconostoc pseudomesenteroides	DSM 20193

Identification of LAB community by cloning and sequencing analysis

To identify the origin of the bands in the LAB DGGE patterns, the migration of the V3 regions of the 16S rDNA amplicons of the all pure *Lactobacillus* genus reference strains was compared with the LAB profiles generated from faecal samples from experimental and control groups. Moreover, amplicons from experimental E1 and E3 samples at T1 time, from E4 at T2 time and from control sample C6 at T2 time, were subjected to cloning and sequencing analysis to identify the dominant species. Results are reported in Table 2 and illustrated in Fig. 2. In detail, *Lactobacillus plantarum*, corresponded to amplicons *a* and *c,* was detected both in experimental and control samples except in E3 sample. Amplicons *b* and *d,*

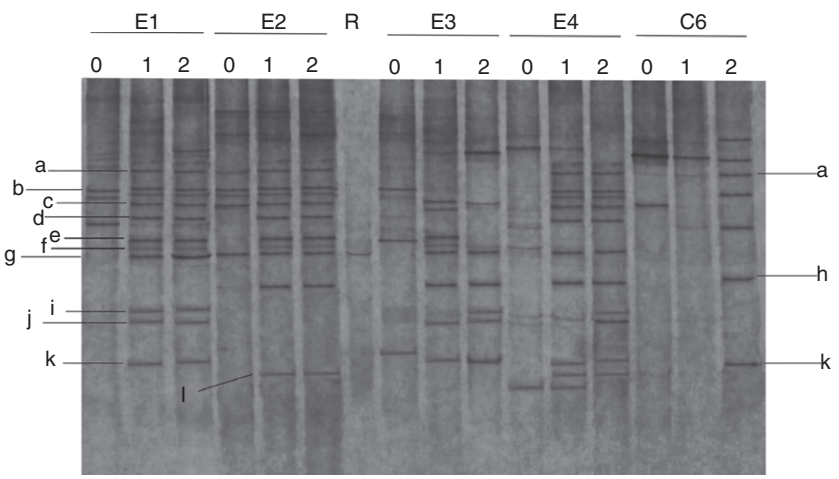

Figure 2 DGGE profiles of faecal samples from control and experimental groups (C and E groups) taken at three different time T0, T1 and T2 over a period of treatment. R, *Lactobacillus reuteri* DSM 17938 (Noos, srl).

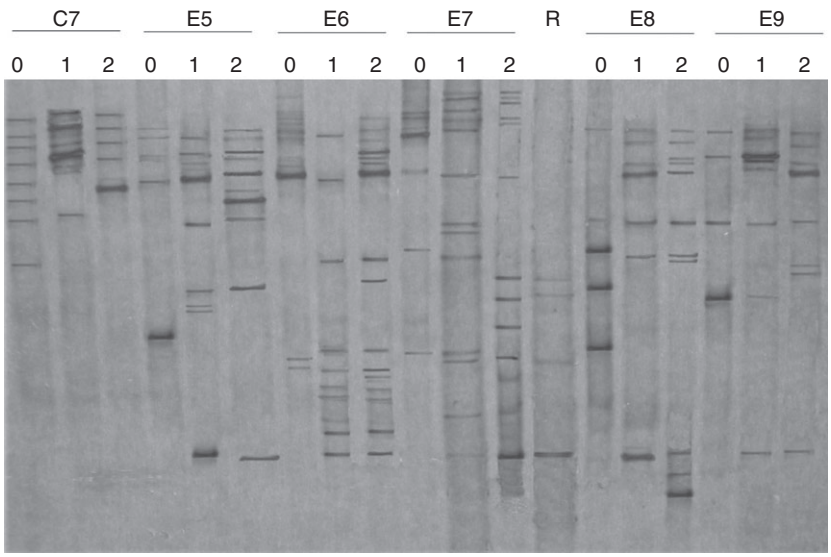

Figure 3 DGGE profiles of faecal samples from control and experimental groups (C and E groups) taken at three different time T0, T1 and T2 over a period of treatment. R, *Lactobacillus reuteri* DSM 17938 (Noos, srl).

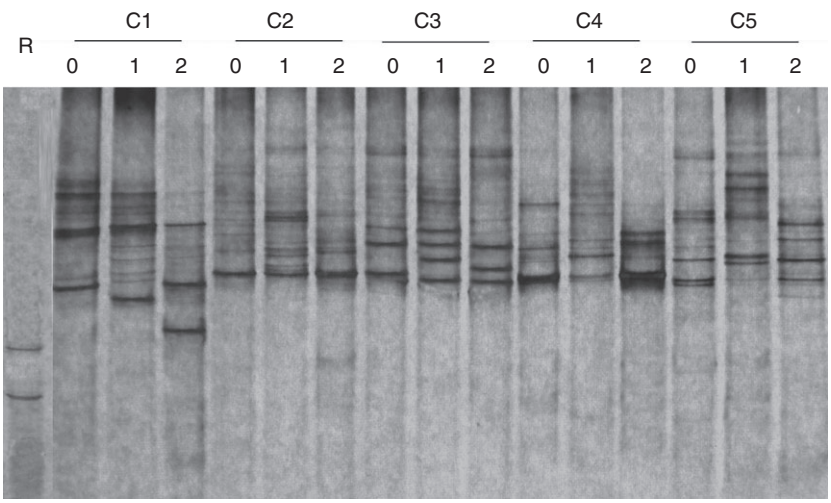

Figure 4 DGGE profiles of faecal samples from control group (C) taken at three different time T0, T1 and T2 over a period of placebo treatment. R, *Lactobacillus reuteri* DSM 17938 (Noos, srl).

originated from *Lactobacillus acidophilus* species, were detected in all experimental samples at T1 and T2 time, except in E3 sample, which exhibited this species at baseline and disappeared throughout the intervention. Amplicons *e* and *f* were identified from clones 7A and 8A and showed 99% identity with *Lactobacillus paracasei* species. *Lact. reuteri*, which corresponded to bands *g* (Fig. 2), was detected only in all experimental samples starting from the probiotic supplementation, except in E2 sample, in which appears starting from baseline. Clones 13A and 16A (band *h*) were identified as *Lactobacillus crispatus* and were detected both in experimental, at T1 and T2 time, and in control samples, only at the end of intervention. Amplicons *i* and *j* originated from *Lactobacillus casei* were detected only in experimental samples during the probiotic supplementation, and amplicon *k*, which corresponded to *Lactobacillus delbrueckii* species, was revealed both in experimental and control groups (Fig. 2). The amplicon *l* was identified as *Lactobacillus gasseri*, which was detected both in experimental and control samples (Fig. 2).

Clinical outcomes

Evaluating clinical parameters, no statistical differences at baseline (T0) between experimental and placebo groups were revealed concerning age and positivity to Nickel (data not shown), presence of urticaria (4 of 9 *vs* 4 of 7), itch (7 of 9 *vs* 6 of 7), eczema (7 of 9 *vs* 3 of 7), gastrointestinal symptoms related to meals (4/9 *vs* 4/7), RAP and type of stools. Moreover, a significant improvement of all cutaneous symptoms was markedly detected after 2 weeks

of probiotic treatment, as illustrated in Fig. 5a,b. In particular, skin symptoms such as urticaria, presence of itch and presence of eczema (Fig. 5a–c) statistically decreased in both groups (experimental and control) at the end of the intervention. On the contrary, evaluating gastrointestinal symptoms, the presence of symptoms related to meals was statistically reduced only in patients treated with *Lact. reuteri* (Cochrane Test $P = 0{\cdot}049$) (Fig. 6a) as well as type of stools (Friedman test $P = 0{\cdot}004$) (Fig. 6c), and recurrent abdominal pains (RAP) even improved in both groups showed a more pronounced reduction (Friedman test experimental group $P = 0{\cdot}009$; placebo group $P = 0{\cdot}036$) (Fig. 6B) in the experimental group. Finally, concerning the visual analogue scale (VAS) data, a significant improvement in patient's quality of life was observed in both groups (Friedman test $P = 0{\cdot}002$) (Fig. 7).

Table 2 Clone retrieved by dominant fragments in DGGE patterns of the LAB intestinal community

Sample	Clone	Fragment	Length (bp)	Species	% Similarity
E2	1A	a	330	*Lactobacillus lantarum*	98
	2A	b	323	*Lactobacillus acidophilus*	98
	3A	c	330	*Lactobacillus plantarum*	98
	4A	d	323	*Lact. acidophilus*	99
	5A	l	328	*Lactobacillus gasseri*	100
	6A	g	338	*Lactobacillus reuteri*	100
E3	7A	e	332	*Lactobacillus paracasei*	99
	8A	f	332	*Lact. paracasei*	99
	9A	g	338	*Lact. reuteri*	100
	10A	k	327	*Lactobacillus delbruekii*	98
E4	11A	i	335	*Lactobacillus casei*	98
	12A	j	335	*Lact. casei*	98
	13A	h	311	*Lactobacillus crispatus*	100
	14A	g	338	*Lact. reuteri*	100
C6	15A	d	323	*Lact. acidophilus*	99
	16A	h	311	*Lact. crispatus*	100
	17A	l	328	*Lact. gasseri*	100

Discussion

In the present study, for the first time, the effectiveness of the probiotic administration coupled to a low-Ni diet in patients suffering from systemic nickel allergy syndrome was revealed. A number of diseases, such as autoimmune, allergic and metabolic diseases (Hawrelak and Myers 2004; Prakash *et al.* 2011a,b), have been associated with alterations of the gut microbiota and certain bacteria, considered 'good,' such as *Bifidobacteria* and *Lactobacilli*, are shown to be correlated with a decrease in the occurrence of a number of disorders, suggesting that the targeted increase of these beneficial bacteria could decrease the incidence and severity of prominent diseases (Prakash *et al.* 2011a,b). Hence, the gut microbiota has gained importance in disease aetiology and pathology with emerging evidence demonstrating its key role in maintenance of health body, including protective, structural and metabolic roles (Cani 2009; Cani and Delzenne 2009). Nevertheless, several factors could alter the balance of gut microbiota in terms of alteration of its composition, change in bacterial metabolic activity and/or a shift in local distribution of the community. In this regard, it is noteworthy that probiotics could exert beneficial effects

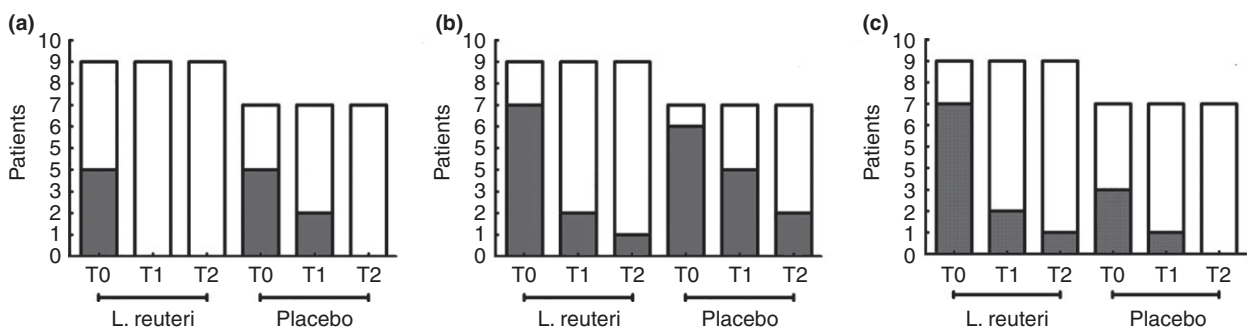

Figure 5 Cutaneous symptoms in patients treated with *Lactobacillus reuteri* and in placebo patients a: Urticaria reduction at T0, T1 and T2 in patients treated with *Lact. reuteri* ($P = 0{\cdot}018$) and in placebo patients ($P = 0{\cdot}049$); □ absence of urticaria, ■ presence of urticaria; b: Itch reduction at T0, T1 and T2 in patients treated with *Lact. reuteri* ($P = 0{\cdot}006$) and in placebo patients ($P = 0{\cdot}049$); □ absence of itch, ■ presence of itch; c: Eczema reduction at T0, T1 and T2 in patients treated with *Lact. reuteri* ($P = 0{\cdot}018$) and in placebo patients ($P = 0{\cdot}049$), □ absence of SCD, ■ presence of SCD.

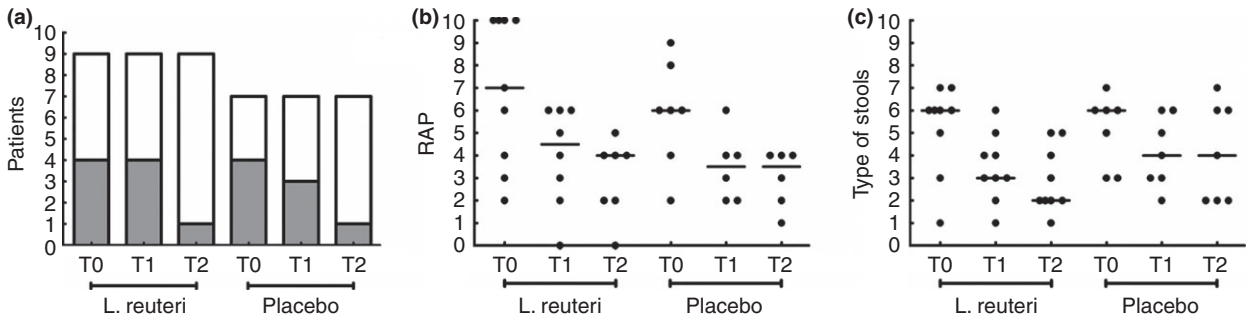

Figure 6 Gastrointestinal symptoms in patients treated with *Lactobacillusreuteri* and in placebo patients a: Symptoms related to meals reduction at T0, T1 and T2 in patients treated with *Lact. reuteri* ($P = 0{\cdot}049$) but not in placebo patients ($P = 0{\cdot}097$); ■ symptoms related to meals; b: RAP reduction at T0, T1 and T2 in patients treated with *Lact. reuteri* ($P = 0{\cdot}009$) and in placebo patients ($P = 0{\cdot}036$). Lines represents median. c: Type of stools changing at T0, T1 and T2 in patients treated with *Lact. reuteri* ($P = 0{\cdot}004$) but not in placebo patients ($P = 0{\cdot}522$).

in the human GI tract, and in recent years, there has been increasing clinical evidence indicating their beneficial effects in the treatment of gastrointestinal and allergic diseases (Prakash *et al.* 2011a,b; Sanders *et al.* 2013). The identification of dominant LAB community and the study of its shift during the probiotic supplementation could enhance the knowledge of the SNAS. In this preliminary trial, the intestinal LAB community of SNAS patients was investigated using a culture-independent approach and the effect of the probiotic *Lact. reuteri* DSM 17938 (NOOS S.r.l., Italy) both on LAB dynamics and on clinical parameters was studied. *Lact. reuteri* DSM 17938 is able to produce a broad spectrum of antimicrobial substance (Wolf *et al.* 1995; Casas and Dobrogosz 2000) against pathogens of the GI tract, including *Helicobacter pylori*; to compete with pathogens for binding sites and substrates; and to stabilize the mucosal barrier with a decrease in intestinal permeability and stimulation of intestinal immune responses (El-Ziney and Debevere 1998; Casas and Dobrogosz 2000; Maassen *et al.* 2000). Its beneficial effects in the balance of gastrointestinal microbiota have been shown by many clinical trials, which demonstrated that the strain is able to improve symptoms of acute dermatitis (Shornikova *et al.* 1997a,b; Rosenfeldt *et al.* 2003; Valeur *et al.* 2004). Moreover, a study conducted on healthy patients showed that *Lact. reuteri* is also able to influence the consistency of the faeces, reducing the number of watery stools daily (Karvonen *et al.* 2001).

Based on our preliminary data, in the present study the beneficial effects of *Lact. reuteri* administration on the intestinal LAB modulation have been proved. While LAB diversity was relatively low both in control and experimental patients at baseline, probably due to the microbial imbalance, different trend was observed during the probiotic intervention. In fact, while control group showed a quite stable LAB community, the experimental group

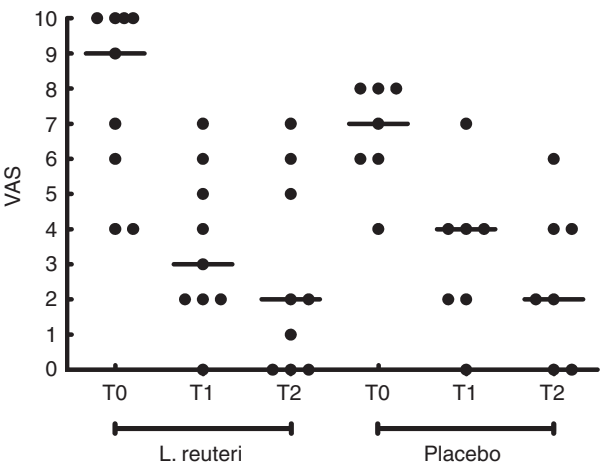

Figure 7 VAS reduction at T0, T1 and T2 in patients treated with *Lact. reuteri* ($P = 0{\cdot}002$) and in placebo patients ($P = 0{\cdot}002$). Lines represent median.

achieved a significant increase in LAB biodiversity and dynamics. Exposure to probiotic bacteria during the course of the study is, therefore, a more likely explanation for the increase in *Lact. casei*, *Lact. paracasei*, *Lact. plantarum*, *Lact. acidophilus* and *Lact. delbrueckii*. Moreover, during the probiotic intake, the presence of *Lact. reuteri* was achieved only in experimental group, indicating the bacterium capability to colonize and survive throughout the human GI tract, as reported by Rosander *et al.* (2008). It is interesting to highlight that most of the clones (12 of 16) was detected in the experimental samples during the low-Ni diet and only 4 of 16 clones were revealed in control samples, indicating that the association diet–probiotic supplementation could increase the *Lactobacillus* ability to colonize the GI tract. In addition, gastrointestinal symptoms related to meals were statistically reduced in patients treated with

Lact. reuteri and the type of stools significantly changed during the intervention, in agreement with several studies, which revealed that the probiotic *Lact. reuteri* is able to reduce the duration of diarrhoeal symptoms in subject who received the probiotic supplementation *vs* placebo (Shornikova *et al.* 1997a,b; Szajewska *et al.* 2006). Moreover, concerning cutaneous symptoms and RAP, despite their decrease was statistically significant in both groups, it is interesting to highlight that the improvement was more pronounced in experimental group than in placebo one.

In conclusion, the approach applied in the present research allowed us to identify the dominant LAB species in patients suffering from SNAS and to assert that the *Lact. reuteri* DSM 17938 strain was capable to survive passage through the human GI tract, significantly influencing both the LAB dynamics and the clinical symptoms.

Our preliminary findings suggest that the low-Ni diet is essential to improve cutaneous and patient's quality of life of patients with SNAS; however, its association with probiotics allowed to decrease rapidly the frequency and the severity of symptoms related to meals as well as the type of stool, probably due to the increase of intestinal LAB diversity. Moreover, despite the decrease of cutaneous symptoms and RAP was statistically significant in both groups, it is interesting to highlight that the improvement was more pronounced in experimental group than in placebo one.

Further investigations using a prolonged observation period will be necessary for clarify the therapeutic effect of probiotics and for in-depth understand and exploit the microbial ecosystem of patients suffering from SNAS.

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Conflicts of Interest

None of authors have any conflict of interests to report.

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