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LACTOBACILLUS SPOROGENES OR BACILLUS COAGULANS: MISIDENTIFICATION OR MISLABELLING?

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ABSTRACT: ***Probiotics are increasingly gaining scientific and commercial interest as functional foods in this era of self-care and complementary medicine. They are commonly considered as viable microorganisms that beneficially affect the host health when ingested. The microorganisms most frequently used as probiotic agents are lactobacilli, bifidobacteria and yeasts. Success of probiotics has led to development and marketing of a broad range of products based on probiotics. In this context, resolution of the taxonomy of bacterial species remains a key point to be clarified, since it is well known that different species belonging to the same genus may have different beneficial properties. From this point of view, Lactobacillus sporogenes, or, as it should be correctly classified, Bacillus coagulans, represents the archetypal misidentified probiotic and its annoveration among probiotics has often been matter of debate. In fact, since this bacterium shows characteristics of both genera Lactobacillus and Bacillus, its taxonomic position between the families Lactobacillaceae and Bacillaceae has often been discussed. The present review summarizes the current literature on salient features of L. sporogenes/B. coagulans as probiotic. Although there are characteristics that favour its use as probiotic, clinical evidences of its benefits are limited to few studies involving small patient population.***

KEYWORDS: *Bacillus coagulans*; Bacterial taxonomy; Probiotics; Quality control

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INTRODUCTION

The concept of probiotics rose about more than one hundred years ago, when Döderlein and, subsequently Metchnikoff proposed that bacteria producing lactic acid from sugars should have some beneficial effects (Metchnikoff 1907; Döderlein 1892). Originally defined as microorganisms promoting the growth of other microorganisms, their definition has been revised and changed in scope several times. Today they are considered as those viable microorganisms that when administered to man and animal, beneficially affects the host by improving the properties of the indigenous microflora (Lilly and Stillwell 1965; Fuller 1989; Guarner and Schaafsma 1998). More recently probiotics have been defined as mono- or mixed cultures of "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002).

Although known since a long time, only in the last two decades probiotics have started to receive major attention from researchers, and several studies have been carried out on the effects of probiotics microorganisms, using different formulae and with numerous purposes of preventing or treating diseases (Mercenier et al. 2002; Sartor 2005). According to definitions set above, a wide range of bacteria has been proposed as probiotic, as indicated in Table 1. However, only those classified as lactic acid bacteria have received major considerations in regard to food and nutrition, even if only for few of them clear evidences of probiotic activity have been shown (Dunne 1999; Saavedra 2001; Montrose 2005). Most of the wide variety of novel probiotic products developed and marketed in European countries in the last decade mainly contain lactobacilli, such as *L. acidophilus*, *L. casei*, *L. rhamnosus* for which several studies have evidenced some probiotical properties (Goossens et al. 2003; Szajewska & Mrukowicz 2005; Luyer et al. 2005). The dramatic increase in variety of probiotic products developed in the last years has catalyzed attention of researchers on the need to regulate probiotic marketing, particularly since several reports have shown poor reliability of marketed products (Hamilton-Miller et al 1996; Weese 2002; Fasoli et al. 2003; Temmerman et al. 2003; Coeuret et al. 2004; Drago et al. 2004). In fact, several studies performed worldwide have demonstrated the scarce quality control carried out on commercial probiotic product. In particular, many discrepancies between the effective content and the claims on the label have been found, together with misidentification. Among the latter, the most common refers to products labelled as containing *L. sporogenes*. This nomenclature is considered obsolete and misleading, since this species has been reclassified as *Bacillus coagulans*. Role of this bacterium as probiotic is based on few small-numbered studies and has been questioned by many authors.

This paper aims to summarize existing knowledge on use of *B. coagulans* as probiotic by reviewing English and Italian literature available in Pub Med by searching the terms "Lactobacillus sporogenes" and "Bacillus coagulans".

Table 1. Microorganisms used as probiotics in humans and animals

Lactobacilus spp.	Bifdobacterium spp.	Lactic acid bacteria	Non lactic acid bacteria
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis*	*Bacillus cereus*
L. amylovorus	*B. animalis*	*Enterococcus faecium*	*Escherichia coli*
L. casei	*B. bifidum*	*Leuconostoc mesenteroides*	*Saccharomyces cerevisiae*
L. crispatus	*B. breve*	*Sporolactobacillus inulinus*	*Saccharomyces boulardii*
L. gallinarum	*B. infantis*		
L. gasseri	*B. longum*		
L. johnsonii			
L. paracasei			
L. plantarum			
L. reuteri			
L. rhamnosus			

Table 2. Main characteristics of B. coagulans in respect to the genera Bacillus and Lactobacillus

Property	B. coagulans	Bacillus	Lactobacillus
Catalase	+	+	-
Oxidase	-	+	-
Nitrate reduction	-	+ a	-
Spores	+	+	-
Motility	+	+	-/+
Production of lactic acid	+	-	+
Meso-diaminopimelic acid	+	+	-/+

GENERAL CHARACTERISTICS

The species *L. sporogenes* was originally isolated and described in 1933 by Horowitz-Wlassowa and Nowotelnow and subsequently reclassified as *Bacillus sporogenes*. More recently, it has been evidenced that *B. sporogenes* shares the same characters of *B. coagulans*, and therefore it has been moved into *B. coagulans* group. Accordingly to the 8th edition of Bergey's Manual of Determinative Bacteriology, spore-bearing rods producing lactic acid, facultative or aerobic and catalase positive are to be classified within the genus *Bacillus*. Several studies on *B. coagulans* have reported different cells morphologies, spore surfaces and sporangia, leading to creation of many synonyms (Claus and Berkeley 1998, Nakamura 2000). The phenotypic heterogeneity of the species makes a satisfactory description of the species for practical use rather difficult (De Clerck et al. 2004). This diversity has been confirmed by genotypic assays on several strains from different sources. For example, a considerable variability within *B. coagulans* species has been shown both by 16S rDNA sequence comparison and total DNA-DNA relatedness analysis allowing to define some common genomic traits of this species (De Clerck et al. 2004).

Even if some commercial products are still labelled as "*L. sporogenes*", it is well known that *L. sporogenes* is to be renamed as *B. coagulans*. However, as indicated in Table 2, *B. coagulans* differs from the other bacteria of the genus *Bacillus* for position of endospore in the cellular body (terminal in *B. coagulans*, centrally or subterminally located in other bacilli), lack of cytochrome-*c* oxidase and for the incapability to reduce nitrate to nitrite.

In the vegetative form, *B. coagulans* cells appear as Gram-positive, mobile rods, occurring singly or, rarely, in short chains of variable lengths. They optimally grow at a temperature range of 35-50°C and at pH values comprised between 5.5 and 6.5. Metabolically, they are facultative anaerobes and produce acids but no gas from fermentation of maltose, mannitol, raffinose, sucrose and trehalose. These characteristics favour growth of *B. coagulans* in acid foods and it has been often reported to spoil milk products, vegetables or fruits because of production of high amount of lactic acid (Anderson 1984; Cosentino et al. 1997; Roman-Blanco et al. 1999; DeClerck et al. 2004). By contrast, production of lactic acid and of other products such as thermostable enzymes may be exploited at industrial level (Payot et al. 1999; Batra et al 2002; Yoon et al. 2002).

Spores of *B. coagulans* are ellipsoidal bodies located at one of the cellular poles, resistant to heat and adverse environmental conditions, and able to germinate also in presence of diluted HCl or NaOH solutions.

MARKETED *B. COAGULANS/L. SPOROGENES*-BASED PRODUCTS

Several products containing *B. coagulans* are now available on the market, and typing "Lactobacillus sporogenes products" in any net search engine leads to hundreds of sites promoting a large number of products. Most of them report the old nomenclature of *L. sporogenes*, and rarely there are indications about the real

taxonomy of the bacterium. Formulations include *L. sporogenes* alone or combined with lactobacilli or bifidobacteria, vitamins (particularly B complex), minerals, hormones and prebiotics. Indications for the use of *L. sporogenes*, cover all the usual range of probiotics, such as lactose intolerance, gastrointestinal infections, dyspepsia, hypercholesterolemia, non-specific vaginitis, urinary tract infections. It is also suggested as adjuvant to antibiotic therapy and as enhancer of immune response. A few of all these applications are supported by clinical studies, as discussed below.

CHARACTERISTICS OF *B. COAGULANS* AS PROBIOTIC

In 2001, the joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of probiotics in food recognized the need for guidelines to set out a systematic approach for the evaluation of probiotics leading to the substantiation of health claim. As a consequence, a consensus panel on selection criteria for probiotics was developed in which base requirements for probiotics were stated (FAO/WHO, 2002). They include a correct identification at strain level of the microorganism, as well as *in vitro* tests to determine physiologic and functional health characteristic of the strain and *in vivo* trials to substantiate efficacy in humans or in animals.

Genus/species/strain

Probiotics effects are known to be strain specific. Therefore, proper identification becomes important to associate a specific effect with a particular strain. Initial studies on a candidate probiotic should include testing of phenotype and genotype-stability, and nomenclature of bacteria should be updated to the current names according to approved list of bacterial names, easily available in the net (http://www.bacterio.net). Moreover, guidelines stated explicitly that protracted use of older or misleading nomenclature is not acceptable on product labels. From this point of view, most products containing *B. coagulans* but labelled as *L. sporogenes*, should change their labels, neither seems to be valid maintenance of the old nomenclature in honor of the original discoverers, as stated by some manufacturers.

In vitro tests

Acid and Bile stability

To exert their beneficial effects probiotics must resist to the acidity of the stomach, lysozyme and bile acids (Tuomola et al. 2001). Few data on acid and bile stability of *B. coagulans* are available (Cavazzoni and Adami 1993; Hyronimus et al. 2000). Among the strains tested by Hyronimus and co-workers (two collection strains and one, named BCl_4, isolated from cattle faeces), none was able to survive at pH of 2.5 and 3.0. By contrast, *B. coagulans* CNCMI-1061, used as probiotics in chickens, was shown to survive in the vegetative form at a rate of 50%, thus underlining strain differences among *B. coagulans* species (Cavezzoni and Adami 1993*). B. coagulans* BCl_4 has been shown to be weakly tolerant to bile (growth delayed of at least 40 min in presence of bile in respect to control), while strains from bacterial collection were classified as sensitive to bile (growth delayed of more than 60 min) (Hyronimus et al. 2000).

Data on resistance of *B. coagulans* spores to acidic environment are not available, although spores of bacilli are usually recognized as resistant to adverse environment. For some species of bacilli, survival in acidic media simulating pH of the stomach has been demonstrated (Ciffo et al. 1987; Clavel et al. 2004). Thus, analogously, spores of *B. coagulans* could likely survive at gastric pH and reach the intestine, where sporulation could occur (Anon 2002).

Adhesivity

Adhesion properties are considered an important issue, and particularly, ability to adhere to intestinal mucosa is one of the essential selection criteria for probiotics, since adhesion to intestinal mucosa represents the first step in colonization process (Tuomola et al. 2001). Moreover, stable adhesion to colonic mucosa seems associated to shortening of diarrhoea, immunogenic effects, competitive exclusion and other effects (Salminen et al. 1996; Saavedra et al. 1994).

Though not supported by *in vitro* studies, *B. coagulans* seems to be characterized by the inability to adhere to intestinal epithelium in piglets, where it is considered a transient colonizer lost one week after administration (Adami and Cavazzoni 1999).

Miscellaneous characteristics

Although resistance to acid environment and bile acids and adhesion to intestinal cells are considered essential prerequisite for probiotics, other properties should be equally considered, such as antimicrobial activity against potentially pathogenic bacteria and viability and stability during processing and storage.

Several metabolites produced by probiotics have shown antimicrobial effects, including organic acids, fatty acids, hydrogen peroxide and bacteriocins or proteinaceous compounds (Ouwenhand 1998; Nes and Johnsborg 2004). However, occurrence of production, efficacy in vivo and their effects on indigenous microflora remain uncertain. *B. coagulans* is assumed to inhibit bacterial pathogens, but its mechanism of action is far to be elucidated. Although an activity in reducing the density of vancomycin resistant enterococci intestinal colonization in mouse has been reported (Donskey et al 2001), other authors have shown that *B. coagulans* is unable to produce non-volatile substances with inhibitory activity on vancomycin resistant enterococci (Wilson and Perini 1988). As occurred for other species, production of bacterial inhibitory substances by *B. coagulans* seems to be strictly strain-dependent, since a plasmid-encoded bacteriocin-like inhibitory substance, named coagulin, is produced by *B. coagulans* I_4, a strain isolated from cattle faeces (Hyronimus et al.1998). Because of its spectrum of activity encompassing other *B. coagulans* strains, enterococci, *Listeria spp* and other unrelated species, it has been proposed as an alternative to nisin (Hyronimus et al.1998).

Probiotic bacteria selected for commercial use should retain the characteristics for which they were originally selected. Resistance to technological processes ensures viability and activity of bacteria

in delivery vehicles. Since probiotics are often marketed in lyophilized form, microorganisms should survive industrial processing and remaining alive during storage. Spores are well known to be more resistant than vegetative cells to harsh environmental conditions. This characteristic allows spores to survive industrial manufacturing and ensures a long-term viability that more labile lactobacilli cannot do (Sanders et al. 2001). Available data on *B. coagulans* are mainly referred to strain CNCM I-1061, which has been proved to remain unchanged in its spore content after 5 years of storage (Adami and Cavazzoni 1993).

IN VIVO STUDIES ON *B. COAGULANS*

Studies published on journals indexed in PubMed and supporting the role of *B. coagulans* as probiotic in animals and in humans are rather scarce, especially when compared to literature on the use of *Lactobacillus* species as probiotics.

Animal studies

Studies on the effects of *B. coagulans* administered to animals have been essentially limited to those performed by an Italian group. In their studies, the effects of administration of *B. coagulans* CNCM I-1061 on the growth performance and composition of intestinal microflora were evaluated (Cavazzoni et al. 1998; Adami and Cavazzoni 1999). Data obtained in these studies indicated that addition of *B. coagulans* to chicken diet significantly improved chicken performance as compared with chicken receiving no additive or antibiotic as a growth-promoting prophylactic activity, with highest mean body weights and daily weight gains for birds treated with bacillus (Cavazzoni et al 1998). When compared with standard diet or to Zn-bacitracin diet, inclusion of *B. coagulans* in diet of piglets significantly reduced mortality and improved daily weight gain and feed conversion ratio (Adami and Cavazzoni, 1999). In the same study analysis of fecal flora evidenced an increase of proportions of aerobic and anaerobic spore forming bacteria and decreased anaerobic cocci, coliforms and bacteroides in *B. coagulans*-treated animals.

Donskey et al used a mouse model of vancomycin resistant enterococci stool colonization to test the hypothesis that oral administration of a *B. coagulans* strain would decrease the density of colonization (Donskey et al. 2001): results indicated that of three enterococal strains (two harbouring *vanB* and one *vanA* genes) only one van B was significantly affected by *B. coagulans* treatment, thus suggesting a strain dependent activity. However, as suggested by the same authors, the short treatment duration and the very few enterococal strains evaluated might have notably affected results of the study.

Human studies

We found only three clinical studies reporting evaluation the probiotic activity of *B. coagulans* in humans. Two of them report the same data from the same open label study (Mohan JC et al. 1990a and Mohan JC et al. 1990b). In this study, a small group of patients with hyperlipidemia (n=17) was treated with *B. coagulans*, here named *L. sporogenes,* for three months (360 million spores/day) and assessed for serum lipid levels. Administration of *B. coagulans* was associated to a significant reduction of total serum cholesterol, LDL-cholesterol and total cholesterol to HDL –cholesterol and LDL-cholesterol to HDL-cholesterol ratios and to a marginal increase in HDL-cholesterol. These data may be considered as obtained from an interesting pilot study, but can not considered conclusive for a role of *B. coagulans* in controlling serum lipids in hyperlipidemic patients (Sanders et al. 2003).

A more recent trial has examined the efficacy of a fructo-oligosaccharide (FOS)-*B. coagulans* preparation in the prevention of diarrhoea due to antibiotics in childhood (La Rosa et al 2003). In this multicentre, randomized, double blind vs. placebo study, 98 patients were evaluated: occurrence and duration of diarrhoea were significantly reduced in children receiving FOS-*B. coagulans*. Also these authors still indicate *B. coagulans* as *L. sporogenes* which is described here as a naturally encapsulated sporogen *Lactobacillus*. Unfortunately, the study did not include a group treated with either *B. coagulans* or FOS alone, thus not allowing to discriminate between the activities of the two active principles of the formulation.

Safety

Unlike probiotic species, such as lactobacilli, bacteria belonging to the genus *Bacillus* are not considered normal inhabitants of the gastrointestinal tract. Strains of *Bacillus* should be evaluated comprehensively for safety since infections due to consumption of probiotic containing *Bacillus subtilis* have been reported (Sanders 2003). A monograph on *L. sporogenes* by unknown authors, reports data on absence of toxicity and side effects following consumption of *L. sporogenes* probiotics (Anon. 2002). However the authors do not cite any reference about it.

Although with the exceptions of *Bacillus cereus* and *Bacillus anthracis*, *Bacillus* species are generally regarded as non-pathogenic, the relevance of other *Bacillus* species as food poisoning organisms and etiological agents in non-gastrointestinal infections in animals and in man is being increasingly recognized (Banerjee et al. 1988; Rowan et al. 2003).

From this point of view, evaluation from an independent panel of experts of the safety of *B. coagulans* for human consumption as occurred for lactobacilli seems absolutely required, before considering this bacterium as safe. For this reason the use of the wrong nomenclature of *L. sporogenes* becomes once more questionable, since it seems to try to get advantage from the old tradition of safety of lactobacilli to remedy to the lack of safety reports on *B. coagulans*.

SHOULD L. SPOROGENES/B. COAGULANS HAVE A FUTURE?

On a taxonomic basis *L. sporogenes* should not have a future, since it does not exist as a lactobacillus species. The answer for *B. coagulans* seems to be more complex. Without doubts, it presents two important advantages over other probiotic strains: first it is rather stable in suboptimal conditions and during production and storage processes, thus assuring extended shelf life; second, it require low costs for production.

On the other hand, till now, limited solid scientific evidences have been accumulated on probiotic activity of *B. coagulans*, which probably need to be deeply investigated, before being classified as probiotic. One should advocate the fact that this issue is shared with other microbial strains, whose probiotic properties are often assumed from other strains belonging to the same species. However, it has been well recognized from many authors that each strain may largely differ from other of the same species in ability to exert some probiotic activity, and studies performed on one strain should not be valid for the other ones.

CONCLUSIONS

Some evidence is suggestive for germination of *Bacillus* spore in the gastrointestinal tract (Hoa et al. 2000; Casula and Cutting 2002), but it is still on debate about the bacterial form (spores or vegetative cells) responsible for probiotic activity. In any case, the administration of spores as feed additives represents a peculiar characteristic of *Bacillus* probiotics which could offer some advantages, such as low cost of production processes, ease of preparation, resistance to production process and extended shelf-life over a wide range of temperatures.

However, evidences supporting the probiotic activity of *B. coagulans* are very sparse and additional well-designed studies involving high numbers of subjects are needed before reaching any conclusion on the effects of *B. coagulans* administration. In any case, the use of the term *Lactobacillus sporogenes* seems to aim to deliberately confound consumers, trying to benefit from association with the extensive literature on the safety and health benefits of the genus Lactobacillus.

In conclusion, it is becoming more and more evident that development of probiotics products largely depends on their quality. Recognition of product inequality and lack of regulatory guidelines have led to development of FAO/WHO guidelines with the aim of ensuring product safety and reliability and a level playing field for all companies producing probiotic products. However the first step should be the correct labelling and identification of marketed probiotics.

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