6. Part VI – NARRATIVE

6.1 Data Relating to Safe Uses

6.1.1 History of Consumption of *Bacillus coagulans*

The available information suggests that lactic acid producing bacteria have been used in foods for centuries and these microorganisms are generally considered as harmless (Lee & Salminen, 1995). These bacteria are commonly used as starter cultures for fermentation in the dairy, meat and other food industries. Several strains selected for such uses have been previously associated or are endogenously found in humans. Such a selection ensures the safety of these bacteria in food. The inherent properties of these microorganisms have been utilized in the manufacturing of products such as cheese, yoghurts, fermented milk products, beverages, sausages, and olives. The available evidence also indicates that these bacteria can also improve the safety, shelf life, nutritional value, flavor and quality of the product. As discussed below, lactic acid bacteria can be used as cell factories for the production of food additives or enzyme preparations.

Traditionally, several lactic acid producing bacteria have been consumed in the diet. Several commercial preparations containing *Bacillus coagulans*, as described in GRN 399 (2011), GRN 526 (2014), GRN 597 (2015), GRN 601 (2015), GRN 660 (2016), and GRN 691 (2017) are being used commercially as a food ingredient. First mention of *B. coagulans* dates back to 1915 for coagulation of evaporated milk (Sarles & Hammer, 1932). For the past several decades, the role of lactic acid bacteria has been extensively studied in the intestinal microecology. These bacteria play an important role in maintaining digestive tract (Adams, 1999; Catanzaro & Green, 1997; Ouwehand et al., 2003; Soomro et al., 2001). *B. coagulans* was first isolated in 1932 (Horowitz-Wlassowa, 1932) and has been used in the production of food products. In a series of studies from Portugal during 1958 and 1959, the potential gastrointestinal effects of *B. coagulans* and other spore-forming bacteria have been investigated, including studies in children age 3 to 5 years (Guida et al., 1958; Guida & Guida, 1959). In a review article, Sanders *et al.* reported that among the 77 recognized *Bacillus* species, five species, including *B. coagulans,* have been evaluated for their functionality and sold worldwide for both human and animal uses. The available information suggests that *B. coagulans* has been in use for over 50 years (Sanders et al., 2003).

In Africa, dietary consumption of fermented foods has a long history (Okonko et al., 2006). In the Ibo ethnic group of Nigeria, *ugba* is a popular protein-rich solid, flavorful alkaline food, among other fermented foods. *B. coagulans* is one of the species identified in the preparation of *ugba.* It is produced by fermentation of African oil bean with *B. coagulans.* Consumption of *ugba* is known to result in the intake of *B. coagulans* vegetative form and its spores (Isu & Njoku, 1997). The available information indicate that a large proportion of the population (76%) has been reported to consume *ugba* as a snack (Onofiok et al., 1996). The presence of bacillus cells in *ugba* supports the intake of *B. coagulans*. The level of bacteria (*B. coagulans*) present in the *ugba* indicates that consumption of *B. coagulans* is greater than $1x10^9$ cfu/day. This provides support for the traditional use and consumption of *B. coagulans*.

In a recent review article (Cao et al., 2020), *B. coagulans* have been highlighted, as it has attracted great interest for its health effects with almost no safety concerns.

6.1.2 Current Uses and Regulatory Status

Bacillus coagulans strains have long been known to be safely consumed by the general human population. The available information shows that spore-forming bacteria, such as *B. coagulans* and *B. subtilis,* are used as dietary supplement for human consumption (Chou et al., 2013; Sanders et al., 2003). As a dietary supplement, *B. coagulans* is marketed for human consumption to improve and maintain ecological balance of the intestinal microflora. At present, across the world, *B. coagulans* has been sold as a supplement under different names such as Ganeden BC30, Nature's Plus, Sun Warrior product (SNZ 1969), Super Flora (SNZ 1969) GutFlor, Sporlac® (SNZ 1969), Sanvita, Ampilac, Bactolyte, Ba-Co-Flor, etc. Additionally, it is also marketed as a constituent with several other products. These formulations contain *B. coagulans* alone or in combination with lactobacilli or bifidobacteria, minerals, vitamins (particularly B complex), and prebiotics. The recommended dose of *B. coagulans* ranges from $3.6x10^8$ - $1.5x10^9$ cfu/capsule, two or three times *per* day for a healthy adult. (Catanzaro & Green, 1997) suggested a standard dose of *B. coagulans* at levels of $1.5x10^9$ cfu once or twice per day.

In the US, as dietary supplements[3], *Bacillus coagulans* has been in use before 1994[4]. As a supplement, *B. coagulans* and its preparations are marketed under the Dietary Supplement Health and Education Act (DSHEA, 1994). The available information from the National Institute of Health reveals: 4 products which contain "*Bacillus Coagulans*" in the product name; 5 ingredient name(s) which contain "Bacillus Coagulans"; and 1883 products which contain "Bacillus Coagulans" anywhere on the label (ODS/NLM, 2023). In a recent review article on the potential use of *B. coagulans* in the food industry, (Konuray & Erginkaya, 2018) reported that many food products such as Nutrition essentials; NutriCommit; Flora3; THORNE; Sunny Green Cleansing; Just Thrive; MegSporeBiotic; Sustenex; and Neolactoflorene containing *B. coagulans* have been sold in various countries.

In Europe, the European Food Safety Authority (EFSA) granted a Qualified Presumption of Safety (QPS) status for *B. coagulans* since 2008 (EFSA, 2012). The American Type Culture Collection (ATCC, 2020) has classified different strains of *B. coagulans* as Bio-safety Level 1, indicating that it is a well-characterized agent which does not cause disease in healthy humans. Health Canada has issued a no objection letter to Ganeden for products fortified with *Bacillus coagulans* GBI-30 6086 (Food Business News, 2017). Health Canada has also permitted the use of *Bacillus coagulans* in the production of a glucose isomerase enzyme. Food Standards Australia New Zealand (FSANZ) identified no safety concerns associated with *Bacillus coagulans* (FSANZ, 2019).

The Food Safety and Standards Authority of India (FSSAI, 2016), includes *B. coagulans* in the list of permitted components in food (FSSAI, 2016). In Japan, the Japanese Ministry of Health and Welfare (JPCRF, 2020) has approved the use of *B. coagulans* product (Lacbon) for improvement in symptoms caused by abnormalities in the intestinal flora or in dysbiosis (Majeed and Prakash, 1998). In Japan, Sankyo Corporation marketed the *B. coagulans* (SANK 70258)

[3]See:https://dsld.od.nih.gov/search/bacillus%20coagulans/bWFya2V0X3N0YXR1cz1hbGwvZW50cnlfZGF0ZT0yMDExLDIwMjMvc29ydD1tYXRjaC9wYWdlX3NpemU9MjAv

[4] NNFA List of Dietary supplements in Use Before October 15, 1994; available at: downloads.regulations.gov › FDA-2005-P-0259-0012

product under the trade name Lacbon. In India, *B. coagulans* is approved and has been marketed for the past four decades under the brand name Sporlac[5] by Sanzyme. In the past eight years (2011-2018), Sanzyme or Sanzyme Biologics has manufactured more than 6,000 tonnes of *B. coagulans* SNZ 1969 ($5x10^9$ spores/g) and has marketed it in various countries, including India, European countries, USA, Korea, Indonesia, etc., without reports of any significant adverse reports.

In the USA, FDA (2001) has approved the use of *B. coagulans* in the production of enzymes that are used for food production. As per 21 CFR 184.1372, *B. coagulans* (a nonpathogenic and nontoxigenic microorganism) is recognized as GRAS in the production of insoluble glucose isomerase enzyme. Additionally, FDA's Center for Veterinary Medicine has approved the use of *B. coagulans* as GRAS for veterinary purposes.

In addition to the use of *B. coagulans* in the production of enzymes, FDA has evaluated several GRAS notices on the use of *B. coagulans* in food. As of now, FDA has received eleven GRAS notices on *Bacillus coagulans* for its use in food products. In August 2011, the FDA received the first GRAS notice GRN 399 (2012), on the use of *B. coagulans* spore preparation in conventional foods, submitted by Ganeden Biotech Inc. The intended maximum use level of *B. coagulans* preparation was $2x10^9$ cfu/serving in multiple food categories. The estimated daily intake of *B. coagulans* spores from all uses was determined as $36.4x10^9$ cfu/day. Following its review, the FDA issued a "no questions" letter on July 31, 2012. Subsequently, the FDA received eight additional GRAS notices on the use of *B. coagulans* in foods and all these GRAS notices received no questions letter from the agency. The details of all these notices are provided in Table 5. In addition to the above mentioned nine GRAS notices, specifically on *B. coagulans,* FDA also received two GRAS notices GRN 240 (CFSAN, 2008) and GRN 378 (CFSAN, 2012a)) in which the use of *B. coagulans* along with other bacteria, to culture or ferment the food product has been proposed.

Mitsubishi Chemical Foods Corporation determined that the use of *B. coagulans* SANK 70258 as an ingredient in various food preparations at a maximum level of $2x10^9$ cfu/serving is GRAS (GRN No. 000691; Mitsubishi, 2017). The intended level of usage of *B. coagulans* spore preparation was calculated to yield an exposure of $36.4x10^9$ cfu/day. The green malt isolate, *B. coagulans* SANK 70258, is a Gram-positive, spore-forming, lactic-acid producing bacterium. *B. coagulans* SANK 70258 spore preparation was reported as nonpathogenic, nontoxigenic, nonmutagenic, and nongenotoxic and did not induce acute, subchronic, chronic, or reproductive toxicity in rats. *B. coagulans* SANK 70258 did not show treatment-related adverse events in human studies at levels up to $4x10^8$ cfu/day for eight weeks studied through a randomized, controlled clinical trial. FDA issued no questions regarding the GRAS notice (CFSAN, 2017b).

Recently, Advanced Enzyme Technologies. Ltd., affirmed the use of *B. coagulans* strain LBSC as an ingredient in in conventional foods also at a maximum level of $2x10^9$ cfu/serving is GRAS (GRN No. 000949; CFSAN, 2020). The safety of *B. coagulans* strain LBSC was shown by genomic analysis of the strain, a record of safe ingestion of numerous strains of *B. coagulans*, toxicity studies and research in humans of several strains of *B. coagulans*, concluding that the expected exposure to *B. coagulans* LBSC spore preparation is without significant risk of harm.

[5] As mentioned earlier, *B. coagulans* SNZ 1969 is marketed in India under the name Sporlac. In the US, Sabinsa Corporation who markets their product *Bacillus coagulans* in the brand name 'LACTOSPORE' holds the trademark 'Sporlac'.

Table 7. FDA Evaluated GRAS Notices on Use of *Bacillus coagulans* in Food

GRN No.	Substance	Date of closure	FDA's Letter
949	*Bacillus coagulans* LBSC (DSM 17654)	June 3, 2020	FDA has no questions
725	Inactivated *Bacillus coagulans* GBI-30, 6086	Feb 12, 2018	FDA has no questions
691	*Bacillus coagulans* SANK 70258 spore preparation	Aug 28, 2017	FDA has no questions
670	Inactivated *Bacillus coagulans* GBI-30, 6086	Mar 15, 2017	FDA has no questions
660	*Bacillus coagulans* GBI-30, 6086	Jan 13, 2017	FDA has no questions
601	*Bacillus coagulans* SBC37-01 spore preparation	Apr 28, 2016	FDA has no questions
597	*Bacillus coagulans* SNZ 1969 spores preparation	Feb 29, 2016	FDA has no questions
526	*Bacillus coagulans* strain Unique IS2 spores preparation	Mar 23, 2015	FDA has no questions
399	Preparation of *Bacillus coagulans* strain GBI-30, 6086 spores	Jul 31, 2012	FDA has no questions
378	Cultured [dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources] fermented by [*Streptococcus thermophilus, Bacillus coagulans, Lactobacillus acidophilus, Lactobacillus paracasei subsp. paracasei, Lactobacillus plantarum, Lactobacillus sakei, Lactobacillus bulgaricus* and *Proprionibacterium freudenreichii subsp. shermanii* or mixtures of these strains]	Mar 26, 2012	FDA has no questions
240	Corn, cane, or beet sugar cultured with *Lactobacillus paracasei* subsp. *paracasei*, *Bacillus coagulans* LA-1, or *Propionibacterium freudenreichii* subsp. *shermanii*, or mixtures of these microorganisms	Oct 24, 2008	FDA has no questions

Adapted from FDA GRAS Inventory website

Among the nine notices to FDA, the GRAS notices on *B. coagulans* LBSC (GRN 949) and *B. coagulans* SNZ 1969 spore preparations (GRN 691) were recently submitted for its uses in several conventional foods. Following completion of its review, on June 3, 2020 and February 29, 2016, the FDA issued no question letter on the use of *B. coagulans* LBSC and *B. coagulans* SNZ 1969 spore preparation in foods, respectively. The proposed use of *B. coagulans* in both these GRAS notices was as an ingredient in conventional foods at levels up to $2x10^9$ cfu per serving and these notices are incorporated herein as reference. Furthermore, genotypic analysis (nucleotide identity) revealed >99% similarity of *B. coagulans* BCP92, subject of this GRAS, with *B. coagulans* SNZ-1969, *B. coagulans* LSBC-1, *B. coagulans* Unique IS-2, *B. coagulans* GBI-30 and *B. coagulans* ATCC-7050. Hence with regard to safety related studies, in addition to a comprehensive literature search, the safety studies conducted with GRN 597 and GRN 949 are being referenced as evidence for safety for *B. coagulans* BCP92.

6.2 Studies Linked to Safety

In recent years, the safety of different strains of *B. coagulans* has been extensively investigated in pre-clinical and clinical studies (see below). The animal toxicity studies of *B. coagulans* include, acute, subchronic and chronic oral toxicity, and one-generation reproduction toxicity. In human clinical studies, in addition to efficacy, relevant safety endpoints were also included in some studies. The whole genome sequence of *B. coagulans* BCP92 has been screened for the presence of antibiotic resistance, toxins and or virulence related genes. Also, the phenotypic

and genotypic studies have clearly established that *B. coagulans* BCP92 is closely related to the well-studied *B. coagulans* SNZ 1969 strain.

The safety of the proposed use of *B. coagulans* BCP92 as a food ingredient was evaluated based on a review of the totality of the available evidence on identification of the microorganism using conventional phenotypic analysis in combination with genotypic analysis, antibiotic resistance of the strain and potential production of virulence factors. The potential for toxicity as evaluated in pre-clinical studies, and potential for adverse effects as evaluated in clinical studies for different *B. coagulans* strains is classified as Biosafety Level 1 (BSL-1) organism. This can be considered as corroborative weight of evidence to call out *B. coagulans* BCP92, subject of this GRAS, as an organism that is not known to cause disease in healthy human adults. The safety-in-use assessment of *B. coagulans* strains listed and data provided in nine GRAS notices (Table 7) on the use of different strains of *B. coagulans* in conventional foods supports the safety of use of *B. coagulans* BCP92. Additionally, more recent information pertinent to the safety of *B. coagulans* identified from searches of the publicly available literature, and a review of data and information on the phenotypic and genotypic analysis of *B. coagulans* BCP92 elaborated in section 2.1.3 was evaluated. The totality of available evidence, in combination with information on the established history of use of the *B. coagulans*, was relied upon to conclude the safety-in-use.

6.2.1 Safety Based on Genome Sequencing

Whole genome sequencing, in correlation with phenotypic properties, is commonly used to assess the safety of bacterial strains. In the case of *B. coagulans* LBSC (DSM 17654), no adverse genes or sequences were identified based on whole genome assembly (Saroj & Gupta, 2020). Another study, based on both complete genome analysis and phenotypic studies, had proved the safety of *B. coagulans* strain 13002 (Wu et al., 2022). A similar molecular approach was used for *B. coagulans* BCP92 to satisfy the requirements and support the safety assessment of the strain.

In an unpublished report (Pellucid, 2022 & detailed in section 2.1.4), a bioinformatics safety assessment of *B. coagulans* BCP92 was carried out based on the whole genome sequence. Quick identification of genes coding for putative antimicrobial proteins within BCP92 draft genome was performed using the Comprehensive Antibiotic Resistance Database (CARD) database. Standalone (local) blastp (protein-protein) analysis was performed with query protein sequences of the annotated BCP92 genome against the reference CARD 3.2.5 database consisting of 4661 protein sequences of resistance genes. Total 151 hits were found in the blastp search with percent identity below 60%(90% cut-off), hence was considered absence of antibiotic resistance genes in the genome. Further analysis was performed using the Resistance Gene Identifier (RGI) v. 6.0.0 tool. The tools "AMR Panel" and "AmpliSeq™ AMR Report" from the web-based One Codex platform were also used to detect a broad set of genes associated with 17 classes of antibiotics resistance or to assess the presence of 478 antimicrobial resistance genes across 28 antibiotic classes, respectively. Only the BLAST results showing a cut-off E-value below $1e^{-5}$, an identity >80%, and a coverage >90%were considered for all three categories. Antibiotic resistance genes were found to be absent in the different methods listed above.

Furthermore, safety assessment was performed on *Bacillus coagulans* BCP92 by analyzing genes related to virulence factors in the Whole Genome Sequence (WGS) data. Putative virulence factors were identified by local BLASTP against the Virulence Factor Database (VFDB). Genes involved in the production of various toxins were also searched in the annotated genome sequence of *B. coagulans* BCP92. Only the BLAST results showing a cut-off E-value below $1e^{-5}$, an identity

>80%, and a coverage >90% were considered for all three categories. Virulence factor genes/proteins were downloaded from the Virulence Factor Database (VFDB). The total number of sequences in the core database was 4195. A BLAST homology search between the protein sequences of *B. coagulans* BCP92 and virulence factor proteins revealed no significant hits (total 412 hits <80% identity).

In summary, genes known to confer resistance to antibiotics relevant to human or veterinary importance were not found in the genomic sequences of *B. coagulans* BCP92. Neither were toxin genes or hazardous virulence factors found in the genome. Antibiotic-resistance in a strain can develop due to random mutation or genetic acquisition, and appropriate selective evolutionary pressure (e.g., sub-lethal concentrations of antibiotics present in culture media). Hence *B. coagulans* BCP92 strain is maintained in pure culture, kept frozen and without the presence of antibiotics. As such, it is unlikely that additional antibiotic resistance has been acquired by the *B. coagulans* BCP92.

6.2.1.1 Absence of Biogenic Amines

Biogenic amines (BA) are amino acid derivatives, and are formed during fermentation and decomposition of protein. These biogenic amines include histamine, tyramine, cadaverine, putrecine, and related metabolites. They may cause adverse effects and could be involved in several pathogenic syndromes. To rule out the presence of BA, PATRIC (RAST) annotation of *B. coagulans* assembled genome was performed. The presence of genes involved in the creation of bioamines such as Histamine, Tyramine, and Putrescine were examined. Sequences of genes encoding biogenic amines especially amino acid decarboxylases, were downloaded from Uniprot database.

Standalone Blastp (protein-protein) analysis was performed with query protein sequences of the annotated *B. coagulans* BCP92 genome against the custom database of biogenic amines protein sequences. Only the BLAST results showing a cut-off E-value below $1e^{-5}$, an identity >80%, and a coverage >90% were considered for all three categories. Standalone Blastp search against the custom database of biogenic amines protein sequences from Uniprot generated 109 hits with either absence of harmful biogenic amines and/or low percent identity. In conclusion, *B. coagulans* BCP92 did not contain any sequences/genes related to biogenic amines, thus confirming the safety of the strain through the genome-based approach.

6.2.1.2 Presence of CRISPR-Associated Genes

Coding sequences for Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated genes (Cas) were identified using manual searches through the functional annotation generated from NCBI PGAP and RAST toolkit. The presence of a CRISPR system indicates an advantage in promoting genome stability by acting as a barrier to entry of foreign DNA elements.

B. coagulans BCP92 genome harbors 33 spacers, 34 repeats organized in at least 1 CRISPR arrays with the same direct repeat sequence. The exact number of arrays cannot be determined due to the fragmented nature of the *B. coagulans* BCP92 genomic assembly that includes small contigs containing partial, truncated CRISPR arrays. Additionally, this array is linked to several unidirectionally arranged Cas genes (cas1, cas2, cas3, cas4, cas5, cas7, and cas8). Such genomic association is typical of Class I-C, characterized by the lack of cas6, encoding for an endoribonuclease employed by most Type-I systems for pre-crRNA processing and by the

presence of a single protein encoded by the Cas8c gene. The comparative genomic analysis revealed that eight strains of *Bacillus coagulans* possess cas operons of type I-B (2–6, 36D1, H-1, P-38, and XZL9), type I-C (CSIL1, DSM1, H-1, MA-13), or type IV (DSM1), mostly located in the vicinity of CRISPR arrays as observed by Aulitto et al. (2022).

The CRISPR-Cas system is considered a defense mechanism against mobile genetic elements. This system can protect against re-invasion by capturing and integrating foreign nucleic acid fragments from the initial invasion. The CRISPR locus, with associated Cas-genes, equally provides the host strain with the potential to defend itself against any incoming extra-chromosomal DNA molecules. This is an indication of stability of the genome of *B. coagulans* BCP92 and a very low possibility of the strain to acquire antimicrobial resistant genes since resistance genes are mostly introduced by mobile genetic elements.

6.2.1.3 Safety and Stability of *B. coagulans* BCP92

Genes involved in the production of lipopeptides (*fenA*, *fenB*, *fenD*, *fenE*, *srfAA*, *srfAB*, *srfAC*, *lchA*, *lchAB*, *lchAC*) were manually searched through NCBI PGAP annotation. Genes coding for surfactins, cyclic lipopeptides, fengycin and lychenisin were not found in *B. coagulans* BCP92 genome. Genes involved in the production of toxin were also screened. The genome of strain *B. coagulans* BCP92 did not harbor the genes encoding for haemolysin BL (Hbl), the non-haemolytic enterotoxin (Nhe), enterotoxin (cytotoxin K) and emetic toxin gene (cereulide - ces). Similar results were observed for *B. coagulans* GBI 30, 6086 as determined by(Salvetti et al., 2016).

A number of Mobile Genetic Elements (MGE), such as prophage, transposase and other mobile elements were identified within the genomes of the *Bacillus coagulans* BCP92. Only one intact prophage region was predicted within the genome of the strain. The intact prophage was found on contig 1 (genomic coordinate: 77335-130113 [52.7Kb]). The putative intact prophage region identified, lacked essential genes such as topoisomerase, replisome (Primase) and DNA polymerase required for replication. The results were further confirmed with Phage-web and Virosorter2 as it predicts partial phage in the contig 1 region. These observations suggests that this prophage sequence is defective and non-functional.

Total 21 transposase genes were identified by ISEScan, from which two (IS256 & IS1380) are complete and others (19) are partial transposases. NCBI PGAP annotation identified total 31 transposases (Insertion sequences), from which only 4 were complete sequences (IS1182, IS110, IS5 & IS256). IslandViewer-4 and its two prediction algorithms, islandPath-DIMOB and SIGI-HMM allowed the identification of 28 mobile elements (transposase and integrase). None of the annotated virulence genes, antibiotic resistance genes, or pathogenicity-related genes were found in the predicted genomic islands (GIs). The risk of horizontal dissemination/gene transfer is thus absent or very low; As a consequence, the microbial strain *B. coagulans* BCP92 can be considered safe.

6.2.2 Specific Antibiotic Susceptibility MIC Studies

In an unpublished *in vitro* study, the antibiotic susceptibility of *B. coagulans* BCP92 to several commonly used antibiotics was analyzed and the details have been discussed in Section 2.1.3.5. The antibiotic susceptibility was evaluated by measuring the minimum inhibitory concentration (MIC) values. In order to distinguish resistant from susceptible strains, the EFSA Panel has suggested microbiological cut-off values (EFSA 2012a). The MICs for *B. coagulans*

BCP92 (subject of this GRAS) and *B. coagulans* SNZ 1969 were compared with MIC cut-off values reported by EFSA (2012a) in Table 8. The findings from antibiotic susceptibility assay showed that none of the MIC-values for *B. coagulans* BCP92 exceeded the EFSA MIC cut-off values.

Table 8: Comparison of Minimum Inhibitory Concentrations (MIC) values of various antibiotics for *B. coagulans* BCP92 (Pellucid Lifesciences) and *B. coagulans* SNZ 1969 with EFSA Values (adapted from GRN 864)

Antibiotic Studied	MIC (µg/ml) *B.coagulans* BCP92	MIC (µg/ml) *B.coagulans* SNZ 1969	EFSA MIC Cut-off (µg/ml)
Gentamicin	0.05	<0.5	4
Streptomycin	0.5	<0.5	8
Tetracycline	0.1	<0.12	8
Erythromycin	0.01	<0.016	4
Clindamycin	0.05	<0.03	4
Chloramphenicol	2	2	8
Vancomycin	0.1	<0.25	4
Kanamycin	1	-	8

(Hong et al., 2008) described unpublished work from another laboratory in which 33 isolates of *Bacillus* strains were tested and over half showed resistance to clindamycin. Similarly, (Sorokulova et al., 2008) also reported clindamycin MIC above the EFSA break-point for other *Bacillus* strains such as *B. licheniformis*. (Hong et al., 2008) speculated that clindamycin resistance may be an intrinsic characteristic of *Bacillus* species. Despite these observations, the strain *B. coagulans* BCP92 does not show resistance to clindamycin.

6.2.3 Specific Bile Salt and Acid Tolerance Studies

In order to check the tolerance of *B. coagulans* BCP92, its capacity to survive in varying concentrations of bile salts was investigated. The details of the study are elaborated in Section 2.1.3. The number of viable cells enumerated revealed that *B. coagulans* BCP92 was stable in acidic pH and at a bile salt concentration of 0.3% (w/v). These results correspond to the data obtained by other researchers with *B. coagulans* strains including *B. coagulans* SNZ 1969 (GRN 864, CFSAN, 2019).

6.2.4 Animal Toxicity Studies

6.2.4.1 Acute & Sub-Acute Animal Toxicity Study with *B. coagulans* BCP92

In an acute toxicity study in female Wistar rats (unpublished), conducted as per OECD guidelines, oral administration of *B. coagulans* BCP92 (subject of this GRAS) toxicity was evaluated at a dose level of 2000 mg/kg body weight (bw), with a suspension corresponding to 400 x 10^9 cfu/g. Animals were observed at least twice daily for any clinical signs and mortality throughout the 15 days of observation period. No abnormalities were detected in clinical signs like appearance, respiration, motor activity, tremors, convulsions, corneal reflexes, lacrimation, miosis, mydriasis, salivation, diarrhea and other discharge. Individual body weights of all animals were recorded at the start of treatment, day 8 and day 15. No abnormal changes in body weight or any other clinical signs were noted in all animals. No macroscopic abnormalities were obtained at

termination. The data from this study indicate that the LD_{50} of *B. coagulans* BCP92 (subject of this GRAS) is greater than 2000 mg/kg bw (8×10^{11} cfu/kg bw).

In a sub-acute toxicity study, *Bacillus coagulans* BCP92 was administered orally for 28 days in Wistar Rats as per OECD guidelines. In this study, six groups of experimental rats received 250, 500 or 1000 mg/kg bw/day of a *B. coagulans* BCP92 spore preparation (400×10^9 cfu/g) for 28 consecutive days. The control animals received only water. Clinical signs/symptoms were observed daily, mortality and morbidity were checked twice a day and body weight, food intake were measured weekly. Detailed urinalysis, hematological examinations, clinical biochemistry examinations were performed at the end of treatment period. Four groups of rats were sacrificed after 28 days and the remaining two groups were retained as recovery groups and sacrificed after 42 days for gross pathology and histopathology. The results from this study indicate that there were no treatment related changes in any of the parameters studied i.e., clinical signs, body weight, food intake, urinalysis, hematological examinations, clinical biochemistry, gross pathology and histopathology after 28 days of repeated administration with *B. coagulans* BCP92. Based on the results of the study it was concluded that for *Bacillus coagulans* BCP92, a NOAEL was achieved at 1000 mg/kg/day (4×10^{11} cfu/kg bw/day) for 28 days (highest dose tested).

6.2.5 Toxicity Studies with other strains of *B. coagulans*

In the published literature, several animal toxicity studies are available in which potential adverse effects of different strains of *B. coagulans* have been investigated. A summary of these studies is presented in Table 9.

Recently, safety assessment of an isolated strain of *Bacillus coagulans* with potential commercial applications was investigated (strain SKB LAB-19) (Chaudhari et al., 2022b). SKB LAB-19 was screened for enzyme production, antimicrobial properties, pH/bile salt tolerance, temperature stability, antidiarrheal activity in Swiss albino mice and Wistar rats; and acute oral toxicity in mice. SKB LAB-19 was found to be safe and displayed promising results to reverse *E. coli* and castor oil induced diarrhoea. Preliminary results suggested that SKB LAB-19 is safe and has the potential to be used in humans.

In animal toxicity studies, *B. coagulans* SNZ 1969 was assessed for its safety through acute and sub-acute toxicity studies in Wistar rats (Metlakunta & Soman, 2020). In the acute oral toxicity study, rats were gavaged with 2000 mg/kg bw of a *B. coagulans* SNZ 1969 spore preparation (final cell concentration of 5×10^{11} cfu/g). In the sub-acute (28-day) repeated-dose study of oral toxicity, groups of Wistar rats were administered 0, 50, 500, or 1000 mg/kg bw/day of a *B. coagulans* SNZ 1969 spore preparation (final cell concentration of 5×10^{11} cfu/g). There were no treatment related changes in any of the studied parameters, clinical signs, body weights, feed intake, urinalysis, hematological parameters, clinical biochemistries, gross pathology, and histopathology. The LD50 for *B. coagulans* SNZ 1969 was determined to be >2000 mg/kg bw (10^{12} cfu/kg bw) and the NOAEL in the 28-day study was 1000 mg/kg bw/day (5×10^{11} cfu/kg bw/day) for 28 days, the highest dose tested.

Acute and sub-acute oral toxicity tests of *B. coagulans* Unique IS-2 (MTCC-5260) were conducted in Sprague Dawley rats (Sudha et al., 2011a). The 36 rats (18/sex) in the acute study were gavaged with *B. coagulans* at 0, 1.6, or 3.2×10^{10} spores/kg bw and observed for 14 days. The sub-acute (28-day) study included 48 Sprague Dawley rats, 24 rats/sex, which received gavage doses of 0, 6.5×10^8, 3.25×10^9, or 6.5×10^9 cfu/kg bw/day. There was no mortality and no treatment-related changes in clinical signs, body weight, feed intake, urinalysis, hematology,

clinical chemistries, gross pathology, or histopathology exhibited by experimental rats at both time intervals. In these studies, the LD50 for *B. coagulans* Unique IS-2 (MTCC-5260) was > $3.2x10^{10}$ spores/kg bw and the sub-acute NOAEL was $6.5\ x10^9$ cfu/kg bw/day, the highest dose tested.

In another acute toxicity study, *B. coagulans* GBI-30, 6086 was administered to Wistar rats at a dose level of 5 g/kg bw ($5.2\ x\ 10^{11}$ cfu/kg bw) which did not show any mortality or adverse effects. The results suggest that the LD_{50} of *B. coagulans* was greater than 5 g/kg bw ($5.2\ x\ 10^{11}$ cfu/kg bw) (Endres et al., 2009). In another acute oral toxicity, *B. coagulans* Unique IS2 was investigated in Sprague Dawley (SD) rats (6/sex/group) at three doses 0 (control, vehicle), 3250 and 6500 mg/kg bw ($5\ x\ 10^9$ cfu/g). The findings of this study after 14 days of observation show that the LD_{50} of *B. coagulans* is greater than 6500 mg/kg bw ($0.33\ x\ 10^{11}$ cfu/kg bw) (Sudha RM, 2011a). The available acute oral toxicity studies suggest that the oral LD_{50} for different strains of *B. coagulans* is greater than $5\ x\ 10^{11}$-$1\ x\ 10^{12}$ cfu/kg bw.

6.2.6. Repeat-Dose Animal Toxicity Studies

Cavazzoni et al. (1998) investigated the effects of a newly isolated strain of *B. coagulans* during the first seven weeks of life in chickens. In this study, 75 male Ross strain broiler chickens were randomly assigned to three treatment groups: Group C- received the standard diet without any additive; Group A- received the antibiotic virginiamycin (10 ppm) contained in the daily diet; and Group P- received *B. coagulans* daily at a dose level of $1.6x10^{10}$ cfu/kg/day (1000 ppm) for the first seven days of life, then fed $4.0x10^9$ cfu/kg/day (250 ppm) during days 8-49. The investigators noted that *B. coagulans* became integrated in the enteric microflora and did not interfere with other bacterial groups in this animal model. *B. coagulans* was found to be transient, without any adhesion to the intestinal epithelium. Presence of *B. coagulans* was detected in the feces after one week treatment.

In a short-term oral toxicity study, Sudha et al. (2011a) investigated the potential adverse effects of *B. coagulans* Unique IS-2 strain in rats. In this study, Sprague Dawley rats were orally (gavage) fed with 0, 130, 650, 1300 mg *B. coagulans* Unique IS2 preparation/kg bw/day for 14 consecutive days and follow up was done for 28 days. The *B. coagulans* Unique IS2 preparation contained $5\ x\ 10^9$ cfu/g. No treatment-related changes were observed in clinical signs, body weights, feed intake, urine parameters, hematological examinations, clinical chemistry, gross pathology and histopathology. Based on the results of this study, the investigators concluded that *B. coagulans* Unique IS2 was clinically well tolerated at doses up to 1300 mg or $6.5x10^9$ cfu/kg bw/day, when administered orally to Sprague Dawley rats for 14 consecutive days. The No Observed Adverse Effect Level (NOAEL) for *B. coagulans* Unique IS2 was determined as 1300 mg ($6.5x10^9$ cfu)/kg bw/day, the highest dose tested (Sudha et al., 2011a).

In short-term repeat-dose studies, dogs (n=2), rabbits (n=3) and guinea pigs (n=15) were orally administered maximum ingestible single daily doses of 10 g/kg bw, 30 g/kg bw and 50 g/kg bw of *B. coagulans* powder preparation, respectively, for 7 days (Losada & Olleros, 2002). During the course of the treatment as well as for 10 days subsequent to the withdrawal of treatment, no adverse effects were noted. In a long-term repeat-dose study, male rats were fed a preparation containing $5x10^9$ spores of *B. coagulans*/g at levels of 0.3, 3 and 5 g/kg bw/day for 15 months. No differences in body weight gains between treated groups and the control group were noted. As compared to the control group, no significant differences in organ weights were noted in the treated groups. Additional details of these investigations were not available (Sankyo, 1968; Majeed &

Prakash, 1998; Anonymous, 2002). Although details of these early experiments are not available, these studies indicate that *B. coagulans* preparation is non-toxic.

In an unpublished repeat dose toxicity study, conducted as per Guidelines for the Testing of Chemicals No. 407 and in compliance with OECD Principles of Good Laboratory Practices, the effects of *B. coagulans* SNZ 1969 were investigated in rats following oral administration (Lavanya, 2015; as reported In GRN 864). In this study, highly concentrated, i.e., undiluted *B. coagulans* cell mass of potency $1.84x10^{11}$ cfu/g was orally administered (gavage) to Wistar rats (6 animals/sex/group) at doses of 0, 250 (46×10^9 cfu), 500 (92×10^9 cfu) and 1000 (184×10^9 cfu) mg/kg bw/day for 28 consecutive days. The highest dose treated animals received a dose of $1.84x10^{11}$ cfu *B. coagulans*/kg bw/day. Following treatment, no mortality was observed at any dose level throughout the treatment period. No treatment related clinical signs or symptoms were observed in any of the animals throughout the study. Detailed clinical examination revealed no evidence of treatment related changes in animals throughout the treatment period. Ophthalmological examination revealed no test item related changes in both the eyes at the end of treatment period from group G1 and group G4. No treatment related changes in body weight were noticed in both males and females throughout the treatment period as compared to the control group of animals. Individual animal feed consumption of the test groups of both the sexes was comparable. All hematology parameters in animals of different test groups of both the sexes were comparable to their respective control groups. Changes observed in hematology parameters were comparable to baseline values and biologically insignificant and could not be correlated to any of the other toxicological findings. Changes observed in clinical chemistry parameters were comparable to baseline values; hence, these changes were considered to be insignificant and could not be correlated with the effect of test item administration. Urine parameters in the test groups of both sexes were comparable and revealed no significance as compared to the respective control animals. Absolute and relative organs weights in both sexes at all dose groups were found to be comparable to the control group. No treatment related gross pathological lesions were observed in any of the animals euthanized at the end of the treatment period. Histopathological examination revealed no evidence of treatment related changes in the organs/tissues evaluated. Histopathological examination was normal in control and treated animals at comparable levels. Under conditions of this study, it is determined that the No-Observed Adverse Effect Level (NOAEL) of the test item is 1000 mg/kg bw/day ($1.84x10^{11}$ cfu/kg bw/day).

In a subchronic toxicity study, Endres et al. (2009) investigated the effects of *B. coagulans* in Wister Crl:(WI) BR rats (10/sex/group) following oral administration at doses of 0, 100, 300 and 1000 mg/kg bw/day ($1.36x10^{11}$ cfu/g) for 90 consecutive days. The highest dose received by the animals was $1.36x10^{11}$ cfu *B. coagulans*/kg bw/day. The study was conducted as per OECD guidelines. The highest dose treated animals received a dose of $1.36x10^{11}$ cfu *B. coagulans*/kg bw/day. No deaths or treatment-related clinical changes were observed throughout the study period in any of the groups. Appearance and behavior of the animals were similar for all groups. No toxicologically significant differences between the treatment and control groups with respect to feed consumption, water consumption, sensory reactivity, general and behavioral conditions, hematological and clinical chemistry evaluations was noted. At termination, no treatment-related macroscopic or microscopic changes in the organs were noted. The NOAEL for both males and females was determined as >1000 mg ($1.36x10^{11}$ cfu)/kg bw/day, the highest dose tested (Endres et al., 2009). In addition to the animal toxicity studies, Endres et al. (2009) also investigated the potential genotoxic effects of *B. coagulans* (GBI-30, 6086) in *in vitro* bacterial reverse mutation

test, *in vitro* mammalian chromosomal aberration test, and *in vivo* mammalian micronucleus test. In these studies, *B. coagulans* did not reveal mutagenic, clastogenic, or genotoxic effects.

In a subchronic toxicity study conducted in accordance with OECD guidelines, Akagawa et al., (2016) evaluated the toxicological profiles of *B. coagulans* strain SANK 70258 in rats. In this repeat-dose oral gavage study, *B. coagulans* ($5.09x10^{11}$ cfu/g) was administered to 6-week old Crl:CD (SD) rats (10/sex/group) for 90 consecutive days at dose levels of 0, 500, 1000, and 2000 mg/kg bw/day. All standard safety related parameters as per OECD guidelines were investigated. According to the results, no deaths occurred in either males or females, and no treatment-related changes were observed in any of the clinical signs including a detailed observation with functional observational battery (FOB), functional test, motor activity, body weight, food consumption, ophthalmoscopy, urinalysis, hematology, blood chemistry, organ weight, necropsy or histopathology. Some hematological and clinical chemistry parameters did show significant changes in the treatment group as compared to the control group. However, these changes were not considered as treatment related as the occurrence was sporadic, there was no dose-response relationship, and values were within the historical control ranges. In this study there were no increases in WBC, neutrophil or eosinophil, or any histopathologic changes indicative of inflammation in any organ/tissue including the digestive track at the maximum dose, suggesting that the *B. coagulans* did not infect rats. The NOAEL was determined as 2000 mg/kg bw/day, the highest dose tested, in males and females. This dose is equivalent to a daily intake of $1.02x10^{12}$ cfu/kg bw/day. The investigators concluded that *B. coagulans* is harmless and can be used for human consumption.

In a subsequent one year study, Endres et al. (2011) investigated the long term effects of *B. coagulans* consumption in rats. This was a combined study to investigate chronic oral toxicity along with one-generation reproductive toxicity. In this feeding study, Wistar rats (20/sex/group) were maintained on a diet containing *B. coagulans* preparation at levels of 0, 10000, 20000 and 33300 mg/kg feed for 52 to 53 weeks. The equivalent dose level was approximately 0, 600, 1200 and 2000 mg/kg bw/day, respectively. No mortality was noted in the treatment groups. Clinical observations did not reveal any toxic signs related to the test article. No *B. coagulans* treatment-related changes in body weight, body weight gain, or feed consumption were noted during the study. Blood samples drawn at 3 weeks and 3, 6 or 12 months did not reveal any toxicological relevant changes in hematology, clinical chemistry or urinalysis. Statistically significant changes noted were either not dose-related, or were well within the historical background range or not correlated with other hematological or histopathological changes. At termination, macroscopic and microscopic examinations did not reveal any treatment-related lesions. The NOEL in male and female rats was determined as 1948 and 2525 mg/kg bw/day, respectively, the highest dose tested (Endres et al., 2011). The test article contained $6.88x10^{10}$ cfu/g; therefore, the NOEL is equivalent to $1.34x10^{11}$ cfu/kg bw/day and $1.74x10^{11}$ cfu/kg bw/day for male and female rats, respectively.

In the one-generation reproductive toxicity study by Endres et al. (2011), Wistar rats divided in to four groups (males- 10/group; females- 20/group) were fed a diet containing *B. coagulans* preparation at a dose level of 0, 600, 1200 and 2000 mg/kg bw/day. For this study, male rats were fed the diet for 70 days before mating and during the three-week mating period, while female rats were fed for ten weeks prior to mating, during the three-week mating period, throughout pregnancy and lactation and up to weaning of the F1 offspring (up to weaning; postnatal day 21). No mortality was reported in the parental generation. Pregnancy outcome, reproductive performance and live births were unaffected by the treatment. There were no signs

of treatment-related toxicity on the F0 (parental) generation (male or female). The NOEL for the parental group (reproductive performance) male and female rats was established as 2372 and 3558 mg/kg bw/day, respectively. The NOEL for the F1 offspring was determined as 3558 mg/kg bw/day. The test article contained $6.88x10^{10}$ cfu/g; therefore, the NOEL for the F1 offspring is equivalent to $2.45x10^{11}$ cfu/kg bw/day (Endres et al., 2011).

In summary, the safety of multiple strains of *B. coagulans* has been examined in a variety of animal toxicity studies. The *B. coagulans* strains tested in these studies are very similar to the subject of the present GRAS assessment. The data from studies with *B. coagulans* BCP92 (subject of this GRAS) and from different strains can be used to support the safety of *B. coagulans* BCP92 spores' preparation. Studies of several strains of *B. coagulans* indicate that the organism is not toxic as evidenced from acute toxicity, subchronic toxicity, chronic toxicity (one year) and reproductive toxicity studies. The longest duration oral toxicity study of *B. coagulans* was a one-year repeat dose toxicity study of *B. coagulans* GBI-30, 6086.

Table 9. Animal Toxicity Studies with Different Strains of *Bacillus coagulans* (Adapted from GRN 864)

Reference	Strain of *B. coagulans*	Study type	Study design (animal, # per group)	Duration	Dose	Results
Sudha et al. (2011a)	Unique IS2	Acute toxicity	OECD Guideline 401, SD rats, 6/sex/dose	Single, oral dose (gavage implied)	0, 3250, and 6500 mg/kg bw/day of test article of $5x10^9$ cfu/g daily	No treatment related effects were observed at any dose, including no findings of body weight changes, clinical signs, or gross pathological changes
Endres et al. (2009)	GBI-30, 6086	Acute eye irritation study	New Zealand white rabbits	Single application with observation at 1, 24, 48, and 72 h	0.1 g of undiluted cell mass at $1.93x10^{11}$ cfu/g applied to one eye, second eye served as control, without washing after application	Slight to moderate conjunctival irritant effects observed that were fully reversible after 72 h. No corneal involvement or adverse signs in iris. Would not be classified as eye irritant
Endres et al. (2009)	GBI-30, 6086	Acute skin irritation study	New Zealand white rabbits	Single application of 4 h duration with observation at 1, 24, 48, and 72 h. OECD Guideline 404 compliant	0.5 g of undiluted cell mass at $1.93x10^{11}$ cfu/g moistened with water and applied to 6 cm^2 intact skin, then animals were wrapped	Test results demonstrate that article is not irritating the skin. Slight erythema after 1 h exposure, but all findings were minor and fully reversible

						biologically significant
Endres et al. (2011)	GBI-30, 6086	Combined chronic/one generation reproductive study	OECD Guideline 452, HsdBrlHan Wistar rats	1 year, 1 generation	0, 600, 1300 or 2000 mg/kg bw/day of test article of $6.88x10^{10}$ cfu/g	NOEL for parental male and female rats: 2372 and 3558 mg/kg bw/day (mean value), respectively NOEL for reproductive performance of male and female rats: 2372 and 3558 mg/kg bw/day (mean value), respectively NOEL for F1 offspring: 3558 mg/kg bw/day (mean value)

6.2.7. Outcome of *B. coagulans* in the Human GI Tract

Following oral ingestion, spores of *B. coagulans* passes through the stomach and reach the duodenum, where it germinates and multiplies rapidly (Losada & Olleros, 2002). Upon oral ingestions, spores take 4 hours to travel to the duodenum or small intestine and start germination. After reaching the intestine, it continues to germinate and becomes metabolically active as a part of facultative anaerobes (capable of producing energy through aerobic respiration and then switching back to anaerobic respiration depending on the amounts of oxygen and fermentable material in the duodenum) and produces lactic acid fermentation products. *B. coagulans* has been reported to stay temporarily in the human intestinal tract (Majeed and Prakash, 1998). *B. coagulans* are excreted via feces for seven days following discontinuation of administration of *B. coagulans* (Majeed and Prakash, 1998). The evidence suggests that *B. coagulans* improves gastrointestinal ecology by replenishing the quality of desirable obligatory bacteria and antagonizing pathogenic microorganism (Anonymous, 2002).

The effects of daily administration of *B. Coagulans* spores ($2.5x10^8$/day) for 10 days to a subject on the growth and proliferation of *B. coagulans* in the GI tract were investigated (Cao et al., 2020). On the eighth day of administration, the total number of *B. coagulans* remaining in the intestine was $2.5x10^5$ cfu. On day six, after discontinuation of the treatment, less than ten *B. coagulans* spores were recovered in the feces. The study was repeated, with increasing the dose to $8x10^8$ spores/day of *B. coagulans* for four days. No *B. coagulans* spores were found in the feces before the administration. By the second day of administration, $3.8x10^5$ *B. coagulans* spores were found in the feces. On day three after the discontinuation of *B. coagulans* ingestion, there were $1.1x10^5$ spores in the feces, while on day eight no *B. coagulans* spores were noted in the feces. The results of this study suggest that *B. coagulans* is transiently maintained in the intestinal tract.

6.2.8. Human Clinical Studies

Some of the relevant findings from human clinical studies with different strains of *B. coagulans* are summarized in Table 9 and were summarized upon literature review and/or adapted from GRN 597, GRN 864 and GRN 949.

In a meta-analysis of clinical trials, Doron et al. (2008) reported that certain microorganisms are used in prevention of antibiotic associated diarrhea. Among them *B. coagulans* are most effective and safe. In another review article, Johnston et al. (2007) described use of microorganisms and prevention of antibiotic associated diarrhea in children together with adverse effects. In this assessment of 10 clinical trials, Lactobacilli spp., Bifidobacterium spp., Streptococcus spp., or *Saccharomyces boulardii* alone or in combination, *Lactobacillus* GG, *B. coagulans, Saccharomyces boulardii* were administrated at $0.5x10^{10}$ to $4x10^{10}$ cfu/day. *Lactobacillus* GG, *B. coagulans, Saccharomyces boulardii* at $0.5x10^{10}$ to $4x10^{10}$ cfu/day were found as the most promising microorganisms.

In a recent double blind, placebo controlled, multi-centered trial, Majeed et al. (2016) evaluated the safety and efficacy of *B. coagulans* MTCC 5856 in diarrhea predominant IBS patients. In this study, 30 newly diagnosed diarrhea predominant IBS patients were enrolled. Along with standard care of treatment, 18 patients in group one received placebo while in group two 18 patients received a *B. coagulans* MTCC 5856 tablet containing $2x10^9$ cfu/day as active for 90 days. Laboratory parameters, anthropometric and vital signs were within the normal clinical range during the 90 days of supplementation in the placebo and *B. coagulans* MTCC 5856 group. No statistically significant changes in clinical chemistry or vital signs were noted. No serious adverse events were reported. One reported adverse event was determined to be unrelated to the study product. Five dropouts (1 treatment, 4 control) were due to personal reasons. Significantly reduced discomfort (bloating, vomiting, diarrhea, stool frequency, abdominal pain) with treatment was noted. The investigators concluded that the *B. coagulans* MTCC 5856, at a dose of $2x10^9$ cfu/day, was found to be safe for 90 days of supplementation.

Mohan et al. (1990a; 1990b) investigated the effects of *B. coagulans* on serum lipid levels in hypercholesterolemic patients in two open label clinical studies. *B. coagulans* spore ($3.6x10^8$ cfu/day) was administered to 17 patients suffering from type II hyperlipidemia for 12 weeks. No adverse effect of the treatment was noted. The *B. coagulans* used in this study was *B. coagulans* SNZ 1969.

Iino et al. (1997a, 1997b), in two separate studies, investigated the effects of *B. coagulans* on the gut microbiota. In the first study, the effects of *B. coagulans* on stool color, stool shape, stool frequency, defecation feeling and stool odor in 28 adult healthy Japanese women were studied (Iino et al., 1997a). The subjects ingested one sachet (containing lactose and $1x10^8$ *B. coagulans* cells/g) per day for two weeks. Improvements in stool properties (color, shape), along with increases in defecation frequency, was noted. No tolerance data was reported and there were no reports of adverse events. In the second study, Iino et al. (1997b) studied the effects of *B. coagulans* on intestinal flora, decayed products and stool property. In this study, 18 healthy adult women were divided in three groups to receive $0.2x10^8$, $1.0x10^8$ and $2.0x10^8$ cells of *B. coagulans* per day for two weeks. No subject complained of gas generation, diarrhea or continuous abdominal pain problems due to ingestion of *B. coagulans*. No adverse effects of *B. coagulans* were reported. The *B. coagulans* strain used in these investigations was the mother strain of *B. coagulans* SNZ 1969.

Ara et al. (2002) investigated the effects of *B. coagulans* in 23 female volunteers aged 20 to 40 years old. These subjects had a tendency for constipation as a result of changes in the intestinal environment. The study was conducted for 12 weeks. Monitoring of the subjects was done four weeks before administration, four weeks during administration of placebo and four weeks during administration of *B. coagulans* ($1x10^8$ cfu/day). The subjects were asked to keep a diary on defecation frequency and fecal characteristics (fecal shape, color and odor) and skin characteristics (number of comedowns). The skin was analyzed by counting the number of skin eruptions every two weeks. Stool defecation frequency was greater on administration of *B. coagulans* compared to before administration. No reports of adverse effects or intolerance of the supplements were noted. The *B. coagulans* strain SANK 70258 was used in these investigations (which is the mother strain of *B. coagulans* SNZ 1969).

Ara et al. (2002) also evaluated the effects of *B. coagulans* powder in 20 healthy adults (16 males and 4 females) with a tendency for constipation, on dermal characteristics as a result of the changes in the intestinal environment. The study duration was six weeks. Monitoring of the subjects was done two weeks prior, two weeks during administration of *B. coagulans* ($1x10^8$ cfu/day) and two weeks after treatment. Stool samples were collected before administration, 14 days after the start of administration and 14 days after the end of administration. For decomposition products, specimens were analyzed. Defecation frequency and fecal characteristics were examined and recorded from the volunteers. No reports of adverse effects or intolerance of the supplements were noted while consuming the *B. coagulans* powder. The *B. coagulans* strain SANK 70258 was used in this study.

In yet another randomized, placebo controlled, double-blind trial, Kajimoto et al. (2005) investigated the effects of a strain of *B. coagulans* SANK 70258 in subjects suffering from seasonal allergic rhinitis. In this study, 55 volunteers (aged 20-65 years - healthy men and women) with the history of Japanese cedar pollinosis were allocated. The subjects (n=29) randomly received either test food containing 4×10^8 viable *B. coagulans* cells/day or placebo (n=26) for eight weeks. The subjects were monitored for safety related parameters such as hematology (9 commonly measured parameters) and clinical chemistry (25 commonly analyzed parameters), as well as for improvements from allergy. Gastrointestinal symptoms and skin symptoms were recorded for any adverse effects. The observation from physical examination, hematology and clinical chemistry parameters did not reveal any adverse effects in either of the groups. After intake of the test food, no adverse reactions were noted. The results from this study suggest that *B. coagulans* at a dose of 4×10^8 cfu/day was safe for consumption by humans for eight weeks. Again, the *B. coagulans* SANK 70258 was used in this study.

In another randomized, double-blind trial, Cui et al. (2004) investigated the effects of *B. coagulans* in subjects with acute and chronic diarrhea. In this study, 204 subjects were divided into two groups. The control group (n=101) (51 with acute diarrhea and 50 with chronic diarrhea) received tablets containing Golden Bifid *(Bifidobacterium longum)* at a dose of 1×10^8 cfu three times daily for 3-7 days (acute diarrhea) and 14-21 days (chronic diarrhea), while the treatment group (n=103) (51 with acute diarrhea and 52 with chronic diarrhea) received *B. coagulans* at a dose of 1×10^8 cfu, three times daily for 3-7 days (acute diarrhea) and 14-21 days (chronic diarrhea). No adverse effects were noted in either of the groups. It was concluded that the *B. coagulans* species safety is similar to Golden Bifid tablets.

Astegiano et al. (2006) evaluated the effect of a dietary mixture ("IBS Active") containing L-tryptophan, inulin, angelica, vegetal charcoal, vitamin PP, group B vitamins (B1, B2, B6) and

microorganisms *(Bacillus coagulans, Lactobacillus acidophilus, Streptococcus thermophilus*) in patients suffering with irritable bowel disease. In this study, treatment group (n=37; 11 men and 27 women; mean age 44.3±5.1 years) received "IBS Active" over a period of 5 to 8 months, while the control group (n=28; 6 men and 22 women; mean age 48.6±3.7 years) were instructed to continue their customary therapy for 6 months (range, 5-7). Evaluation on subjects was done for abdominal pain and/or distension, constipation, diarrhea, and alternating constipation and diarrhea. No adverse events were reported.

In a prospective, randomized double-blind, placebo-controlled trail, Kalman et al. (2009) investigated the effect of *B. coagulans* on gastrointestinal symptoms in adults with post-prandial intestinal gas-related symptoms (abdominal pain, distention, flatulence) but no gastrointestinal (GI) diagnoses to explain the symptoms. In this study, 61 adult volunteers aged 36.5±12.6 years (weight 75.4±17.3 kg) received either *B. coagulans* GBI-30 (n=30) or placebo (n=31) for four weeks. In the treatment group, the subjects received one capsule containing $2.0x10^9$ cfu *B. coagulans*/day for four weeks. The subjects were evaluated every two weeks. During each visit, the participants were evaluated with a series of questionnaires in addition to hemodynamics (standard biochemical safety testing) and adverse event monitoring. In the publication, the details of the hemodynamic or biochemical parameters were not mentioned. The investigators reported no adverse events in the group that received *B. coagulans* GBI-30.

In a randomized, double-blind, placebo-controlled trial, (Dolin, 2009) investigated the effects of the *B. coagulans* preparation on symptoms of diarrhea-predominant irritable bowel syndrome. In this study, 55 volunteers (including patients with diarrhea-predominant irritable bowel syndrome-IBS-D) were divided into two groups. 26 volunteers (7 male, 19 female) received *B. coagulans* (GBI-30, 6086) and 29 volunteers (6 male, 23 female) received placebo once a day for 8 weeks. The patients were advised to take the capsules containing *B. coagulans* or placebo daily for 8 weeks. Adverse events reported for the most part was mild to moderate and self-limiting. Five subjects receiving *B. coagulans* and six receiving placebos reported six adverse effects. In the placebo group, headache as severe side effects were reported. The results reported suggest that in patients with IBS-D, *B. coagulans* (GBI-30, 6086) is safe.

In summary, in over 20 published clinical studies, the effects of *B. coagulans* has been investigated. In these studies, there were no reports of serious adverse effects or observed safety concerns. The daily intake of *B. coagulans* was up to approximately $20x10^9$ cfu/day and the period of intervention ranged from a few days to approximately 13 weeks. In some studies, reports of mild to moderate gastrointestinal symptoms with intake of *B. coagulans* were noted. However, the effects were generally self-limiting and reversible. Overall, findings from these studies, in both healthy and compromised individuals, did not reveal any evidence of pathogenicity or toxicity following ingestion of *B. coagulans*. In these studies, *B. coagulans* was generally well tolerated.

Table 10. Human Clinical Studies with Different Strains of *Bacillus coagulans* (literature review and adapted from GRN 864; GRN 597 & GRN 949)

***B. coagulans* strain**	**Study type**	**Study population (age and sex, # randomized [completed])**	**Duration of intake**	**Dose cfu/day**	**Findings**	**Reference**
B. coagulans SNZ1969	randomized, placebo-controlled,	92 patients with constipation-predominant irritable	60 days	500 million (twice daily)	One adverse event	Soman et al. (2022a)

	double-blind study	bowel syndrome (IBS-C) and diarrhea predominant IBS-D			unrelated to the study treatments was reported in IBS-D group	
B. coagulans SNZ1969	randomized, placebo-controlled, double-blind study 2 groups	30 individuals, aged 18-60 years with GI discomfort	30 days	$2x10^9$	Safe and effective in reducing GI discomfort, especially dyspepsia. Minimal AEs unrelated to *B. coagulans* SNZ1969 reported	Soman et al. (2022b)
B. coagulans LBSC (DSM17654)	randomized, double-blind, placebo-controlled trial 2 groups	40 IBS patients (Rome IV criteria) aged 18- 65 years	80 days	$2x10^9$	No intervention-associated AEs and no SAEs were reported. Vital, biochemical and hematological parameters were within normal range. Upper GI endoscopy revealed no clinical changes of GI mucosa on *B. coagulans* LBSC supplementation.	Gupta et al. (2021)
B. coagulans Unique IS2	randomized, double-blind, placebo-controlled trial	108 IBS patients (78M, 30F) aged 20-60 years (median = 45 years)	8 weeks	$2x10^9$	Hematology of both the arms remained normal. No significant changes were reported in pro-inflammatory cytokines. *B. coagulans* was well tolerated with no SAE	Madempudi et al. (2019a)

B. coagulans GBI	randomized, single-blind crossover trial 2 groups	29 apparently healthy athletes who consumed a diet high in whey protein	2 weeks	10^9	Reporting of findings was generally inadequate, and no mention was made of any adverse effects or AEs.	Jager et al. (2018)
B. coagulans (*Weizmannia coagulans*) MTCC 5856	randomized, double-blind, placebo-controlled trial	56 healthy female participants	10 weeks	lactospor in cream %2	No adverse events or skin irritation was observed in any participants during the study.	Majeed et al. (2023)
B. coagulans MTCC 5856	RCT 2 groups	40 IBS patients, 6M & 34F, mean age = 42.1±10.1 years	90 days	$2x10^9$	No statistically significant changes in the vitals were observed from the baseline to final visit. No serious AEs or significant AEs were noticed in this study. There was only one AE reported, fever and weakness, in the placebo group	Majeed et al. (2018)
B. coagulans MTCC 5856	RCT 2 groups	Patients with diarrhea predominant IBS (at least 75% loose or mushy stools) 36.2 ± 11.07 y (treatment), 35.4 ± 10.75 y (control); M/F n=18 [17] treatment, n=18 [14] control	90 days	$2x10^9$	No statistically significant changes in clinical chemistry or vital signs; no serious adverse events; 1 reported adverse event determined to be unrelated to study product. Five dropouts (1 treatment, 4 control) due to personal reasons. Significantly reduced discomfort (bloating, vomiting, diarrhea, stool frequency, abdominal pain) with treatment	Majeed et al. (2016)

Endres et al. (2009)	GBI-30, 6086	Acute oral toxicity	OECD Guideline 423, with 5 animals/sex/dose, Wistar Crl:(WI) BR rats	Single oral gavage dose	0 or 5000 mg/kg bw ($1.04x10^{11}$ cfu/g) in 1% methylcellulose in water (control received vehicle only)	The single dose produced no treatment-related signs of toxicity and no body weight changes in the 14-day post-dose observational period. Gross pathological examination revealed no remarkable differences between treated and control animals
Gu et al. (2015)	CGMCC 9951	Short-term repeat dose toxicity	KM mice, females: n=6/group; males: n=5/group	28 day	0, $1x10^{6}$, $1x10^{8}$, $1x10^{10}$ spores/kg bw/day	Investigators concluded safe dose as 1×10^{10} spores/kg bw/day
Sudha et al. (2011a)	Unique IS2	Short-term repeat dose toxicity	OECD Guideline 407, SD rats, 6 animals/sex/dose	28 day	0, 130, 650, 1300 mg/kg bw/day orally "fed" from stock test article of $5x10^{9}$ cfu/g	Authors derived a NOAEL of 1300 mg/kg bw/day based on no findings of toxicity at this dose. All observations noted were considered to be non-significant
Endres et al. (2009)	GBI-30, 6086	Sub-chronic 13-week oral toxicity	OECD Guideline 408 as well as Red Book guidelines followed with 10 animals/sex/dose, Wistar Crl:(WI) BR rats	13 weeks	0, 100, 300, and 1000 mg/kg bw/day by oral gavage suspension in 1% methylcellulose in water from test article stock of $1.36x10^{11}$ cfu/g	NOAEL considered by authors to be >1000 mg/kg bw/day. Effects observed included some decreases in water consumption, hematology and clinical chemistry alterations, lower absolute brain weights in high dose males, lower relative kidney and adrenal weights in some females, and mean body weight in high dose males. All observations were determined by authors to be reflective of individual variability, within historical control ranges, or not

B. coagulans[a] + inulin + *B*-carotene	Crossover 2 groups	Patients with type 2 diabetes 52.9 ±8.1 y; M/F n=51 [48]	6 weeks	$0.3x10^8$	No serious adverse reactions were reported	Asemi et al. (2016)
B. coagulans + FOS	RCT 3 groups	Children with chronic abdominal pain (>2 months with recurrence at least once per week) 7.44 ± 2.44 y (*B. coagulans* + FOS), 7.06 ± 2.38 y (peppermint oil), 7.42 ± 2.49 y (control); M/F n=40 [29] *B. coagulans* + FOS; n=40 [34] peppermint oil; n=40 [25] control	1 month	$0.5x10^9$	No adverse reactions or intolerance observed	Asgarshirazi et al. (2015)
B. coagulans[a]	RCT 3 groups	Diabetic patients 51.3 ± 10.4 y (synbiotic), 52.0 ± 7.2 y (bread with *B.coagulans*), 53.4 ± 7.5 y (control); M/F (81% F) n=27 [25] synbiotic, n=27 [25] (bread with *B.*coagulans), n=27 [26] control	8 weeks	$0.3x10^9$	No side effects were reported following the consumption of the bread with *B. coagulans*	Bahmani et al. (2016; Tajadadi-Ebrahimi et al. (2014); Shakeri et al. (2014)
B. coagulans GIB-30, 6086	Crossover 2 groups	Healthy men and women 65-80 y; M/F n=42 [36]	28 days	$1x10^9$	No mention of adverse effects	Nyangale et al. (2015)
B. coagulans lilac-01 + okara powder	RCT 2 groups	Healthy Japanese volunteers with a tendency for constipation 50.6 y; M/F n=148 treatment, 149 control (okara powder) [268]	2 weeks	$0.1x10^9$	No mention of adverse effects	Minamida et al. (2015)
B. coagulans[a] + FOS	RCT 2 groups	Adults with irritable bowel syndrome 39.8 ± 12.7 y; M/F n=41 [23] treatment, n=44 [33] control	12 weeks	$0.45x10^9$ FOS – 300 mg/d	17 (41%) patients in the treatment group discontinued the study; 12 (27%) due to vomiting and 5 (12%) due to diarrhea. 11 (25%) patients in the control group discontinued the study; 5 (11%) due to constipation, 3 (7%) due to	Rogha et al. (2014)

					urticarial, and 3 due to bloating (7%). No other side effects were observed	
B. coagulans GIB-30, 6086	RCT 2 groups	HIV infected persons receiving cART Median age 49 y (treatment), 51 y (control); M/F n=12 [10] treatment, n=12 [7] control	90 days	$2x10^9$	No serious adverse events were reported. Only mild gastrointestinal symptoms were reported during the study; in the microorganism group, 3/10 reported bloating. In the placebo group, 1/7 reported increased diarrhea	Yang et al. (2014)
B. coagulans Unique IS-2 (MTCC-5260)	RCT 2 groups	Women with bacterial vaginosis 32.5 ± 3 y (treatment), 33 ± 3 y (control); F n=20/group	90 days	$4x10^9$	No mention of adverse effects	Sudha et al. (2012a)
B. coagulans Unique IS-2 (MTCC-5260)	Phase I trial 1 group	Patients with acute diarrhea 35.44 ± 8.76 y; M/F n=28 treatment	10 days	$4x10^9$	Significant reductions in counts of RBC and WBC and serum creatinine levels was observed, however values were within the normal range; no other changes in safety parameters were observed	Sudha & Bhonagiri (2012b)
B. coagulans Unique IS-2 (MTCC-5260)	Open label 3 groups	Men and women with hyperlipidemia 42-53 y; M/F n=10/group	60 days	$10x10^9$ $20x10^9$	No mention of adverse effects	Sudha et al. (2011b)
B. coagulans GIB-30, 6086	Open label 1 group	Healthy subjects; 27 y; M/F n=10 (10)	28 days	$0.5x10^9$	No serious adverse events were reported throughout the study	Kimmel et al. (2010)
B. coagulans GIB-30, 6086	RCT 2 groups	Patients with symptoms of rheumatoid arthritis 62.5 y; M/F n=23 [22] treatment, n=22 [22] control	60 days	$2x10^9$	No serious adverse reactions reported throughout the study. Treatment group reported 4 adverse events including	Mandel et al. (2010)

					shingles, poison ivy, a cold and leg edema (all deemed unrelated to study treatment). One subject in the treatment developed an URI and discontinued treatment. Control group reported 3 adverse events including GI reflux, URI, and urinary tract infection	
B. coagulans GIB-30, 6086	Crossover 2 groups	Healthy adults; 44 y; M/F n=10 [9]	30 days	$2x10^9$	No serious adverse events were reported throughout the study	Baron et al. (2009)
B. coagulans GIB-30, 6086	RCT 2 groups	Patients with diarrhea prominent IBS 52.3 ± 11 y (treatment), 44.0 ± 17.9 y (control); M/F n=26 [26] treatment, n=29 [26] placebo	8 weeks	$2x10^9$	Adverse events were, for the most part, mild to moderate, and were generally self-limiting. Five patients who received treatment reported 6 adverse events; six patients who received placebo reported six adverse events. One severe adverse event (headache) was reported in the placebo group	Dolin et al. (2009)
B. coagulans GIB-30, 6086	RCT 2 groups	IBS-abdominal pain and bloating patients 48.36 y; M/F n=50 [22/group]	8 weeks	~$0.8x10^9$	No treatment related adverse events or serious adverse events reported during the study period. Four adverse events reported in the placebo group and two in the treatment group were unrelated to the treatments	Hun (2009)
B. coagulans GIB-30, 6086	RCT 2 groups	Patients with self-reported post-meal	4 weeks	$2x10^9$	"… the *Bacillus coagulans*-based	Kalman et al. (2009)

		intestinal gas-related symptoms 34.8 ± 12.5 y (treatment), 38.2 ± 12.6 y (control); M/F n=30 treatment, n=31 control			product was effective and safe…"	
B. coagulans[a]	RCT 2 groups	Patients with acute or chronic diarrhea 18-65 y; M/F n=103 treatment, n=101 control	3-7 day (acute diarrhea) 14-21 day (chronic diarrhea)	$0.3x10^9$	Body weight, body temperature, respiratory rate, heart rate, blood pressure, blood routine, and liver and renal functions were within normal limits. No treatment related adverse effects	Cui et al. (2004)
B. coagulans[a]	Open label 1 group	Patients with primary hyperlipidemia 45.6 y; M/F n=20 [17]	12 weeks	$0.36x10^9$	No adverse effects were noted except constipation in one patient	Mohan et al. (1990 a,b)

[a] Referred in the paper as *Lactobacillus sporogenes*, a previous name of *B. coagulans*
Abbreviations: cART = combination antiretroviral treatment; CFU = colony forming units; d = day; F = female; FOS = fructo-oligosaccharides; g = gram; GI = gastrointestinal; M = male; mo = month; RBC = red blood cell; URI = upper respiratory infection; WBC = white blood cell

6.3 Reports of Infection and *B. coagulans*

It is well recognized that lactic acid-producing bacteria are non-pathogenic to humans (Fooks & Gibson, 2002; Doron & Gorbach, 2006). Lactic acid bacteria that occur naturally have an excellent safety profile. In spite of their widespread uses, no major safety issues or health risks to humans have been noted (Holzapfel et al., 1995; Salminen et al., 1996). As compared to most of the common *Lactobacillus* and *Bifidobacterium* species, commonly sold at health food stores and/or used in the production of cultured dairy products, *B. coagulans* has a longer safe history of use. The available information from the published studies did not reveal any significant pathogenic or opportunistic illness caused following administration of *B. coagulans*.

Based on the University of Maryland Cancer Center records, Banerjee et al., (1988) reported that 18 febrile patients experienced 24 episodes of *Bacillus* bacteremia from January 1978 to June 1986. In one episode, the cause was identified as related to *B. coagulans*. Twelve of the 24 episodes of *Bacillus* bacteremia were considered possible infections. Of the twelve patients, 4 had clinically documented sites of infection at the time of the bacteremic episodes, but specific microbiologic documentation of the offending pathogen(s) was not obtained. The remaining eight patients did not have a clear cause for the *Bacillus* bacteremia, nor had a clinical site of infection. Therefore, *B. coagulans* is likely only an opportunistic bacterium and as such, indicates that *B. coagulans* may only be opportunistic in a highly immuno-compromised population, and would not be defined as virulent. No information, in the published literature was found indicating that *B. coagulans* causes infection following oral ingestion.

6.4 Expert Panel Review, Summary and Discussion

At the request of Pellucid Lifesciences, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[6], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to evaluate the Generally Recognized As Safe (GRAS) status of a standardized *Bacillus coagulans* BCP92 spore preparation for use as a food ingredient in conventional foods at maximum addition levels up to $2x10^9$ cfu per serving.

A comprehensive search of the scientific literature for safety and toxicity information on different strains of *B. coagulans,* was conducted through June 2023, and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Pellucid Lifesciences and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on August 07, 2023, and unanimously agreed to the decision described herein.

B. coagulans BCP92 proposed for use in conventional foods by Pellucid Lifesciences is a gram-positive, catalase-positive, rod-shaped, slightly acidophilic, thermotolerant, aerobic to microaerophilic, highly resilient bacteria. *B. coagulans* BCP92 preparation is a pale brownish powder with a characteristic odor. The specifications of the product containing *B. coagulans* BCP92 spores has been fully developed by Pellucid Lifesciences. The bacterial strain is an isolate of *B. coagulans* obtained from the soil. The identity of *B. coagulans* BCP92 has been fully investigated and confirmed by phenotypic, genotypic and complete genome sequencing analysis. The available evidence using conventional phenotypic analysis, in combination with genotypic analysis and whole genome sequencing, confirms the identity of BCP92 as a strain of *B. coagulans*. *B. coagulans* BCP92 is manufactured according to current good manufacturing practices (GMP), by a fermentation process using food grade ingredients. The proposed use of *B. coagulans* BCP92 at maximum use levels of $2x10^9$ cfu/serving will result in a conservative high-end estimated daily intake of 36.4×10^9 cfu/day of *B. coagulans* BCP92.

B. coagulans was first isolated and described in 1932. As *B. coagulans* forms spores, it possesses high heat and acid resistance providing advantages for its use in food. The available information suggests that *B. coagulans* has been in use for over 50 years. *B. coagulans* is used in the production of a protein-rich food known as *ugba* in African countries. This microorganism is also used to improve and maintain ecological balance of the intestinal microflora. *B. coagulans* is marketed as dietary supplement for human consumption to improve and maintain ecological balance of the intestinal microflora. *B. coagulans* has been used for the prevention and treatment of acute diarrhea and intestinal infections, as well as for gastrointestinal side effects due to antibiotic therapy. *B. coagulans* is approved for use in the preparation of enzymes used for food production. As per 21 CFR § 184.1372, insoluble glucose isomerase enzyme produced from *B. coagulans* is recognized as GRAS. The available information suggests that *B. coagulans* is well-tolerated, non-pathogenic and non-toxicogenic and there is a common knowledge of safe use of *B. coagulans* for several decades. *B. coagulans* is classified as BSL-1 organism, thus indicating the organism is not known to cause disease in healthy human adults.

[6]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

The safety of *B. coagulans* BCP92 is supported by whole genome sequence analysis. For this analysis, all coding sequences were examined, including those of potential safety concern. The available information from genome sequencing analysis suggests that *B. coagulans* BCP92 is does not harbour any resistance genes that might be transferred. The genes known to confer resistance to antibiotics relevant to human or veterinary importance were not found in the genomic sequences of *B. coagulans* BCP92. Neither were toxin genes or hazardous virulence factors found in the genome. Phenotypic analysis shows an absence of antibiotic resistance and no production of biogenic amines. No evidence of pathogenicity has been reported, and the species is generally regarded as non-pathogenic as well as non-toxigenic. No indications of toxicity were found in acute and repeated-dose studies of oral toxicity or in genotoxicity assays with other strains of *B. coagulans*, and no adverse effects were reported when the spores are administered to humans. All of these findings support the conclusion that the intended use of *B. coagulans* BCP92 spore preparation is safe.

The safety of multiple strains of *B. coagulans* has been extensively examined in a variety of animal toxicity studies. Findings from acute toxicity, subchronic toxicity, chronic toxicity (one year) and reproductive toxicity studies suggest that *B. coagulans* is not toxic. The longest duration oral toxicity study of *B. coagulans* was a one-year repeat dose toxicity study of *B. coagulans* GBI-30, 6086.

The safety conclusion of *B. coagulans* BCP92 is based on the totality of the available evidence, including phenotypic and genotypic characterization, whole genome sequence and bioinformatics analysis, traditional and current uses, and animal and human studies, including those for other similar strains. The data reviewed in this GRAS satisfy the common knowledge element of the GRAS standard and provide evidence that there is reasonable certainty that consumption of *B. coagulans* BCP92 for its intended use in conventional foods up to a maximum addition level of $2x10^9$ cfu/serving is safe.

The safety of *B. coagulans* BCP92 has also been established using the decision tree (Pariza et al., 2015) for determining safety of microbial culture to be consumed by Humans or Animals and are summarized below.

Decision Tree:

1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? **YES**

2. Has the strain genome been sequenced? **YES**

3. Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? **YES**

4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? **YES**

5. Does the strain produce antimicrobial substances? **NO**

6. Has the strain been genetically modified using rDNA techniques? **NO**

7. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? **NO.** (The strain was isolated from soil.)

8. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? **NO**

Therefore, taken together*, B. coagulans* BCP92 (subject of this GRAS) can be deemed to be safe for use in conventional foods for human consumption (Pariza et al., 2015).

6.4.1 Conclusion of the Expert Panel

The intended use of *B. coagulans* BCP92 spore preparation has been determined to be safe through scientific procedures and the safety was shown by genomic analysis of the strain, a record of safe ingestion of numerous strains of *B. coagulans*, phenotypic characterization of *B. coagulans* BCP92, toxicity of other strains, and research in humans, concluding that the expected exposure to *B. coagulans* BCP92 spore preparation is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of *B. coagulans* BCP92 has been made through the deliberations of an Expert Panel consisting of Madhusudan G. Soni, Ph.D., FATS and David Ribet, Ph.D., who reviewed a monograph prepared by Brincor Associates, LLC for Pellucid Lifesciences, as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They independently critically reviewed and evaluated the publicly available information and the potential human exposure to *B. coagulans* BCP92 spore preparation anticipated to result from its intended use, and individually and collectively determined that no evidence exists in the available information on *B. coagulans* BCP92 that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of *B. coagulans* BCP92 spore preparation. It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion regarding the safety of *B. coagulans* BCP92 under its intended conditions of use.

In summary, on the basis of scientific procedures[7], including reports of exposure to other *B. coagulans* strains, resulting in a maximum estimated daily intake of 36.4×10^9 cfu/day or 2×10^9 cfu/serving of *B. coagulans* BCP92 spores is considered safe. The intended uses are compatible with current regulations and is produced according to current good manufacturing practices (cGMP).

6.4.2 Affirmative Statement Concerning Data and Information

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with Pellucid Lifesciences's conclusion of GRAS status under the conditions of intended use.

Brinda Mahadevan, Ph.D., ERT., FATS
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[7] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.