



**AOR**<sup>TM</sup>  
Advanced Orthomolecular Research

Monograph

# Probiotic-3

A revolutionary formula to support gut health

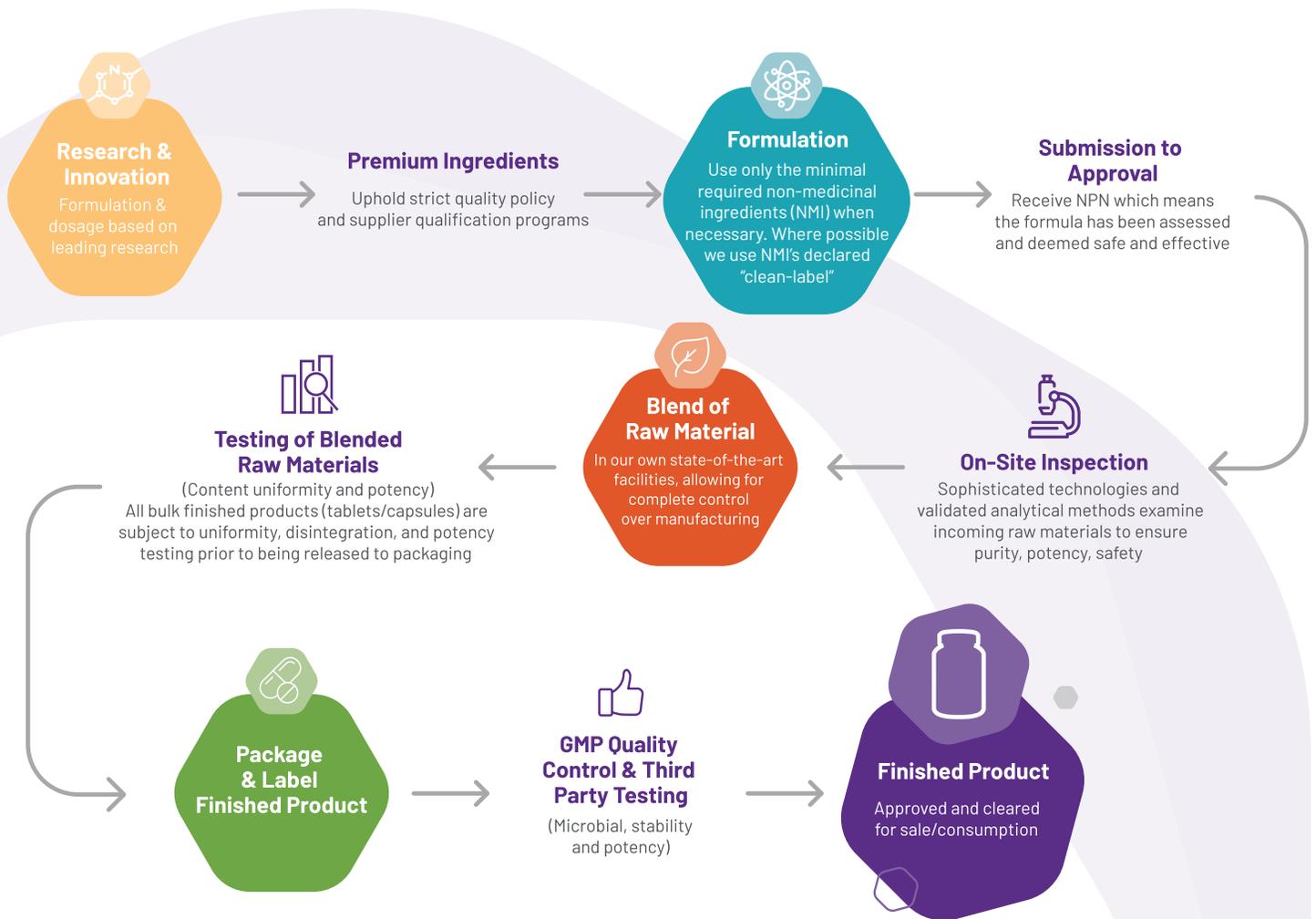


### ABOUT AOR

Advanced Orthomolecular Research (AOR) is a Canadian company headquartered in Calgary, Alberta. AOR has an established reputation as the most advanced supplement formulator in the country and throughout the world. It is through ethical discipline and evidenced-based science that we lead and advance the natural health industry. For more information, please visit [aor.ca](http://aor.ca).

### AOR PRODUCT PATH

From raw material selection to manufacturing and shipping of final packaged goods, AOR adheres to the strictest of quality control standards. Our products represent innovative ideas and thoughtfully researched concepts with advanced techniques to develop products of superior quality and effectiveness - bringing you "Innovation you can trust".



## SUPPLEMENT FACTS

**Probiotic-3**

NPN: 80070555

AOR 04219 90 capsules

**Serving Size**

1 capsule

**Medicinal Ingredients:***Enterococcus faecium* T-110*Clostridium butyricum* TO-A*Bacillus subtilis* TO-A

‡ Colony Forming Units

18 million CFU ‡

0.6 million CFU ‡

0.6 million CFU ‡

**Non-medicinal Ingredients**Lactose, potato starch, polyvinyl alcohol and providone. **Capsule:** hypromellose.**Adult dosage:** Take two to three capsules daily with/without food, or as directed by a qualified health care practitioner. Take at least two to three hours before or after taking antibiotics.**Cautions:** Consult a health care practitioner prior to use if you are pregnant or breastfeeding, if you have fever, vomiting, bloody diarrhea, or severe abdominal pain. Do not use if you have an immune compromised condition (e.g. AIDS, lymphoma, patients undergoing long-term corticosteroid treatment). If symptoms of digestive upset (e.g. diarrhea) occur, worsen or persist beyond three days, discontinue use and consult a health care practitioner. This product contains lactose, dextrin, and potato starch, do not use if you have such allergies.

## PRODUCT SPOTLIGHT

**Probiotic-3**

A revolutionary formula to support gut health

 Gluten Free
  Non-GMO
  MyBlueprint™



## 1. SUMMARY

Probiotic-3 is a unique probiotic formula that contains three bacterial species with specific health-promoting attributes: *Enterococcus faecium* T-110, *Clostridium butyricum* TO-A, and *Bacillus subtilis* TO-A. While these three strains are new to the North American market, they have been widely used in hospitals and pharmacies throughout Asia for over 50 years.

*E. faecium* T-110 is a natural resident of the human gastrointestinal tract that produces lactic acid, which reduces the gastrointestinal pH, thus preventing the growth of harmful bacteria. *C. butyricum* TO-A is also a natural resident of the human gastrointestinal tract. It breaks down dietary fibre into several beneficial nutrients, one of which is butyric acid, which feeds cells of the GI tract. *B. subtilis* TO-A supports the growth of *E. faecium* and *C. butyricum*, as well as the growth of several strains of the beneficial Bifidobacterium species.

Clinical studies have shown wide-ranging health benefits including allergy reduction, improved immunity, reduced bloating, constipation and diarrhea, and even enhanced detoxification. Additionally, Probiotic-3 is the only probiotic product in Canada that contains a strain of bacteria from the Clostridium family, which has been shown to have antagonistic effects against several pathogenic bacteria such as *C. difficile*, *H. pylori*, and *E. coli*.

## 2. BACKGROUND

The  $1 \times 10^{14}$  microbes present in the human gut are vitally important to normal health.<sup>1</sup> About 1,000 species of bacteria from four taxonomic groups comprise the majority of these microbes. Although the population is highly dynamic, it is critical in developing and shaping the gut immune system; consequently, disruption of the microbiota, a condition known as dysbiosis, is associated with both acute and chronic disorders of the gut. These include inflammatory disorders such as Crohn's disease and ulcerative colitis, as well as infections by *Clostridium difficile*, *Helicobacter pylori*, and various other enteropathogenic bacteria. The local and global burdens of these conditions is considerable.<sup>2,3</sup> Increasing evidence suggests that it is possible to alleviate these disorders by resolving the dysbiosis. However, since conventional treatments for these disorders actually cause or aggravate dysbiosis, there is a need for approaches to support gut health by restoring microbial homeostasis.

Probiotics, as defined by the World Health Organization, are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host".<sup>4</sup> The most common commercial probiotics are lactic acid bacteria – Lactobacilli – found in beverages, yoghurts and similar "functional foods". Some probiotic bacteria, such as *Clostridium butyricum*, merit attention for their specific uses in reducing disease incidence or risk of recurrence. This non-pathogenic species of Clostridium reduces inflammation and infections in the colon through several approaches. First, it is a robust producer of short-chain fatty acids (SCFAs), including butyrate for which it is named. These small hormone-like molecules exert specific protective effects on immune cells within the colonic epithelium, increasing immune tolerance to commensal bacteria and reducing inflammation. Second, SCFA production by *C. butyricum* lowers the pH of the intestinal environment, and thus has a bacteriocidal effect against pathogenic species that are acid-intolerant. Third, it directly kills pathogenic bacteria that colonize the intestinal mucosa, by both competing with these species for resources and by production of an assortment of anti-bacterial peptides. Experimental studies to determine which bacteria promote intestinal homeostasis identified several bacterial strains, all of which were Clostridia, and which included *C. butyricum*.<sup>5,6</sup> These strains were markedly deficient in the colons of individuals with inflammatory bowel disease.<sup>6</sup> These findings support a strong role for *C. butyricum* in regulation of intestinal health.

Probiotic-3 is a probiotic supplement containing three bacterial strains. The first, *C. butyricum*, delivers the protective effects outlined above. The second, *Enterococcus faecium*, works in synergy with *C. butyricum* to eliminate enteropathogenic bacteria. Finally, *Bacillus subtilis* acts as a probiotic by supporting the proliferation of the other two strains, as well as of beneficial commensals of the Bifidobacteria species. The synergistic actions of the constituent strains make Probiotic-3 a synbiotic agent,<sup>7</sup> a characteristic that distinguishes it from most probiotics similarly available as supplements.

### 3. CLINICAL STUDIES

#### ULCERATIVE COLITIS

A single-arm pilot study was designed to evaluate the effect of Probiotic-3 against symptoms associated with treatment-refractory ulcerative colitis.<sup>8</sup> Twenty patients with mild to moderate colitis took three Probiotic-3 capsules, three times a day, for four weeks. The primary outcome measure was resolution of symptoms. In total, 45% of patients exhibited complete remission, with 10% showing partial remission. Analysis of their colonic microbiota showed that populations of Bifidobacteria increased significantly in most patients. Furthermore, this positive response was associated with a normalization of intestinal populations of other commensal bacteria.

A subsequent randomized, placebo-controlled trial evaluated the use of Probiotic-3 to maintain remission, in ulcerative colitis patients previously treated with the standard of care.<sup>9</sup> Here, 46 patients were randomized to receive three Probiotic-3 tablets twice a day, or placebo, for 12 months. The primary outcome measure was incidence of relapse. There were 50% fewer relapses in the Probiotic-3 group than in the control group.

#### GASTROINTESTINAL INFECTIONS

A randomized, double-blind, placebo-controlled trial was designed to test the ability of Probiotic-3 to reduce symptoms associated with acute infectious diarrhea.<sup>10</sup> The study focused on 304 children between three months and six years of age admitted to a primary care centre with viral or bacterial infections. The primary outcome measure was the duration of diarrhea. The children either received a daily dose of Probiotic-3 for seven days, or a placebo. Duration of diarrhea was 30% shorter in children receiving Probiotic-3, and was associated with a 30% shorter hospital stay. Probiotic-3-supplemented children had elevated stool counts of Bifidobacteria, increased production of the tolerogenic IL-10 cytokine, and decreased production of the inflammatory TNF $\alpha$  cytokine.

The standard of care to resolve *Helicobacter pylori* infections involves administration of a proton pump inhibitor along with an antibiotic cocktail. However, this failed to clear the infection in about a third of patients.<sup>11</sup> In a clinical study designed to assess the effect of Probiotic-3 supplementation on *H. pylori* clearance, 182 patients were randomized to standard of care supplemented with daily Probiotic-3, or to standard of care alone, for 14 days.<sup>12</sup> The clearance rate with Probiotic-3 supplementation was 84%, as compared to 63% for standard therapy.

Taken together, the results from these studies suggest that supplementation with Probiotic-3 contributes significantly to reduction of symptoms associated with gastrointestinal inflammation and infections.

## PHARMACOLOGY

### CLOSTRIDIUM BUTYRICUM

*C. butyricum* is an anaerobic bacterium, and preferentially colonizes the distal colon. There are several mechanisms by which it protects the mucosal layer from damage. Firstly, the production of large quantities of SCFAs by *C. butyricum* lowers the pH in the intestine enough to render the environment inhospitable to many pathogenic bacteria.<sup>13</sup> SCFAs also stimulate the proliferation of intestinal epithelial cells, which promotes wound healing and maintains the physical barrier against pathogenic microbes. As there is a large population of commensal microbes in the intestine, a healthy gut immune system must be broadly tolerant to diverse antigens presented by such non-pathogenic entities. *C. butyricum* directs such tolerance by instructing immune cells to produce IL-10 and TGFβ;<sup>14</sup> these cytokines consequently suppress the chronic inflammation associated with disorders such as ulcerative colitis. Notably, early-onset colitis is associated with deficiencies in IL-10 signaling.<sup>15</sup> Through the same mechanism, *C. butyricum* supplementation reduces the development of allergies in animals and humans.<sup>16-18</sup> In sum, the effects of *C. butyricum* colonization of the intestinal surface are to improve physical integrity, to reduce colonization by pathogenic bacteria, to increase immune tolerance, and to reduce inflammatory responses.

### ENTEROCOCCUS FAECIUM

*E. faecium* is a lactic acid bacterium, similar to Bifidobacteria and Lactobacilli. It is particularly effective at killing pathogenic bacteria, both on the mucosal surface and in suspension. *E. faecium* produces three antimicrobial peptides – known as bacteriocins – which are effective against multiple bacterial species.<sup>19</sup> *E. faecium* and *C. butyricum* in synergy are several orders of magnitude more effective in controlling pathogenic bacteria than either strain alone.<sup>20</sup>

### BACILLUS SUBTILIS

*B. subtilis* plays multiple distinct roles in regulation of intestinal homeostasis. It produces a signaling molecule, known as the competence- and sporulation-stimulating factor (CSF), which activates pro-survival pathways in enterocytes. This protects the cells from oxidative damage and maintains the integrity of the intestinal barrier.<sup>21</sup> Remarkably, secretion of CSF and nitric oxide into the gut by *B. subtilis* is associated with longevity in animal models.<sup>22</sup> *B. subtilis* is also effective in controlling pathogenic bacteria, both alone and in combination with *E. faecium*.<sup>23,24</sup> Finally, *B. subtilis* acts as a prebiotic by increasing the proliferation of Bifidobacteria and *E. faecium*. The mechanism involves production and secretion of 3,3-dihydroxyazetidine by *B. subtilis*, which acts as a growth factor for itself and other bacterial strains.<sup>25,26</sup>

## PHARMACOKINETICS

No studies have directly assessed the extent of intestinal colonization by the bacteria in Probiotic-3; however, it is possible to use various biomarkers to obtain similar information. In a clinical study in which participants received daily Probiotic-three capsules for seven days, stool samples were collected on days two, four, and seven for bacteriological analyses.<sup>10</sup> Bifidobacteria and Lactobacilli counts were significantly elevated by day seven. Production of TNFα by patient peripheral blood mononuclear cells was reduced by day two, and IL-10 production was elevated by day six. This data suggests a two to seven-day timeline to action for Probiotic-3 supplementation.

## ADVERSE EFFECTS

The bacterial strains present in Probiotic-3 have been extensively characterized for safety by genetic and toxicological analyses.<sup>27</sup> In acute and repeated-dose studies in rats, there were no abnormal findings on any physiological or metabolic indices, setting the no-observable-effect level at 3 g/kg. The genome of *E. faecium* has been completely sequenced; it lacks most of the key genes for antibiotic-resistance, pathogenesis, and virulence.<sup>28,29</sup> Probiotic-3 supplements are well tolerated. In several clinical trials in adult and child cohorts, there were no reports of adverse effects.<sup>8-10,30,31</sup>

## INTERACTIONS

There are no known drug interactions with Probiotic-3. Since it contains live microbes, its use is not recommended in immunocompromised individuals.

## CLINICAL APPLICATIONS

- Acute infectious diarrhea
- Antibiotic-associated diarrhea
- Ulcerative colitis
- Irritable bowel syndrome
- Inflammatory bowel disease

## CONCLUSION

Probiotic-3 is a safe and effective supplement for use in support of gastrointestinal health, and to help relieve symptoms associated with gastrointestinal inflammation or acute gastrointestinal infections.

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