Substantiation Matrix

Outlined below are general health categories that are supported by research and articles on EpiCor labeled 1-17.

Immune Health and Inflammation
1, 2, 4, 5, 8, 9, 12, 13, 14

Sinus
4, 8, 9

Cold and Flu
4, 9

Seasonal Allergies
8

Gut Health
12, 13, 15, 16, 17

Microbiota
12, 13, 16, 17

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List of Journals

All research has been published in journals except for 3 and 7. Those highlighted in yellow are human clinical studies.

1. Discovery of edible fermentation product with unusual immune enhancing properties in humans ***[Published: FASEB J, 2006]***
2. An anti-inflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes *in vitro* ***[Published: Nutrition Research, 2007]***
3. Applying C. *elegans* to natural product development ***[Poster: 16th International C. elegans meeting, 2007]***
4. Effects of a modified yeast supplement on cold/flu symptoms ***[Published in Urol Nurs]***
5. A double-blind placebo-controlled, randomized pilot study: consumption of a high-metabolite immunogen from yeast culture has beneficial effects on erythrocyte health and mucosal immune protection in healthy subjects ***[Published: The Open Nutrition Journal, 2008]***
6. Comparison of chemical and cell-based antioxidant methods for evaluation of foods and natural products: generating multifaceted data by parallel testing using erythrocytes and polymorphonuclear cells ***[Published: J Agric Food Chem, 2008]***
7. Screening natural product prototypes for biological activity using C. *elegans*. ***[Poster: Pathogenesis and Heterochrony Conference, 2008]***
8. Immunogenic yeast-based fermentation product reduces allergic rhinitis-induced nasal congestion: a randomized, double-blind, placebo-controlled trial ***[Published: Adv Ther, 2009]***
9. Immunogenic yeast-based fermentate for cold/flu-like symptoms in nonvaccinated individuals ***[Published: J Altern Complement Med, 2010]***
10. Antioxidant bioavailability and rapid immune-modulating effects after consumption of a single acute dose of a high-metabolite yeast immunogen: results of a placebo-controlled double-blinded crossover pilot study ***[Published: J Med Food, 2011]***
11. A dried yeast fermentate prevents and reduces inflammation in two separate experimental immune models ***[Published: Evidence-Based Complementary and Alternative Medicine, 2012]***
12. A dried yeast fermentate selectively modulates both the luminal and mucosal gut microbiota and protects against inflammation, as studied in an integrated *in vitro* approach ***[Published: J Agric Food Chem, 2013]***
13. The HMI module: a new tool to study the Host-Microbiota Interaction in the human gastrointestinal tract *in vitro* ***[Published: BMC Microbiol, 2014]***
14. Anti-inflammatory properties of a dried fermentate *in vitro* and *in vivo* ***[Published: J Med Food, 2014]***
15. Mitigation of heat stress-related complications by a yeast fermentate product ***[Published: Journal of Thermal Biology, 2016]***
16. A yeast fermentate improves gastrointestinal discomfort and constipation by modulation of the gut microbiome: results from a randomized double-blind placebo-controlled pilot trial. ***[Published: BMC Complementary and Alternative Medicine, 2017]***
17. Yeast fermentate prebiotic improves intestinal barrier integrity during heat stress by modulation of the gut microbiome: ***[Published: Journal of Applied Microbiology, 2019]***

Abstracts/Summaries of Journals and Articles on EpiCor

1. Schauss, A. G.; Vodjani, A. Discovery of edible fermentation product with unusual immune enhancing properties in humans. *FASEB J* **2006**, *20* (4), A143. [*Online reference:* https://www.fasebj.org/doi/abs/10.1096/fasebj.20.4.A143-c]

   Control of immunity to reduce the risk of chronic and infectious diseases is critical to maintaining health. Unusually low sick leave rates in employees working in a fermentation facility in Cedar Rapids, Iowa, led to the discovery of a strain of Saccharomyces with significant immune enhancing properties. Fractionation analysis revealed a significant number of immune enhancing compounds that become ambient during production thereby "exposing" employees through inhalation. Blood studies of "exposed" and "unexposed" employees found that the exposed group had no change in CD4 (helper cells) but a significant decrease in CD8 (suppressor) cells, increased activity of NK cells, glutathione RBC, saliva secretory IgA, and decreased immune complexes (CIC). Unusually high ROS scavenging activity was found using 5 ROS scavenging assays, fresh human neutrophil cells, and phagocytosis activity (macrophage phagocytosis assay). Cell subsets analyzed for adhesion molecules combined with NK activation after exposure show an unusual ability to kill tumor cells and virally infected cells. A significant effect against E. coli and Candida was also found. These findings and others may explain the lack of influenza infections in these workers for periods of up to 30 years, despite periodic and widespread community-wide outbreaks of influenza.

2. Jensen, G. S., et al. An anti-inflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes *in vitro*. *Nutrition Research* **2007**, *27*, 327-335. [*Online reference:* https://www.sciencedirect.com/science/article/pii/S0271531707000978?via%3Dihub]

   The aim of this study was to evaluate the immunomodulating effects of a consumable yeast-based immunogen, EpiCor, on human leukocytes *in vitro*. The selection of antiinflammatory and lymphocyte activation assays was based on initial evidence for immunomodulating effects of EpiCor from an unusually low incidence of influenza among employees in a factory manufacturing EpiCor, along with a high oxygen radical absorbance capacity value. In the present study, EpiCor significantly reduced the production of reactive oxygen species by neutrophils ($p<0.005$). EpiCor treatment of peripheral blood mononuclear cells (PBMCs) caused induction of the activation markers CD80 and CD86 on B lymphocytes, and CD69 and CD25 on CD3-CD56+ natural killer cells. This induction was also seen on enriched populations of natural killer and B lymphocytes, suggesting a direct effect not dependent on bystander cells. Coculturing of PBMC with EpiCor and phytohemagglutinin resulted in inhibition of phytohemagglutinin-induced T-cell proliferation and reduction of interferon gamma production. Fucoidan, a ligand for the homing molecule l-selectin (CD62L), is known to induce rapid up-regulation of several chemokine receptors on lymphocytes. EpiCor caused strong inhibition of Fucoidan-mediated expression of the chemokine receptors CXCR4 and CCR9 on PBMC. This suggested rapid altering of signal transduction pathways, or a direct competition for cell surface receptors, with an end result being an altered sensitivity to chemotactic signals from tissue. We conclude that EpiCor possesses significant anti-inflammatory activity and induces direct activation and increased chemotactic awareness of lymphocyte subsets *in vitro*. This suggests further study of effects of EpiCor consumption on antiviral defense mechanisms and antibody production.

3. Goble, J.; Reeves, S.; Peloquin, J. P., Applying C. *elegans* to natural product development. *Poster at 16th International C. elegans meeting* **2007**.

   Diamond V Mills is a medium sized Agricultural Biotechnology company with more than 63 years of success in

producing dietary supplements and ingredients. Recently the company has moved into human nutrition through its formation of Embria Health Sciences. Our product catalog has enlarged and we have an increasing number of product prototypes to evaluate.

Our products arise naturally as the result of subtle alterations of complex fermentation processes. Furthermore, our products' effects on animal biology are pleiotropic. To demonstrate efficacy presents some serious economic and logistical challenges in that far more product prototypes can be produced than economically tested in vertebrate models. Because C. *elegans* is an excellent whole-animal model for high throughput host/pathogen interactions (Moy et al. 2006) we have begun to develop a medium throughput C. *elegans*-based screen to identify promising product prototypes.

4. Moyad, M. A., et al. Effects of a modified yeast supplement on cold/flu symptoms. *Urol Nurs* **2008**, *28* (1), 50-5. [*Online reference:* https://www.ncbi.nlm.nih.gov/pubmed/18335698]

   A yeast-based product (EpiCor, a dried *Saccharomyces cerevisiae* fermentate) was compared to placebo to determine effects on the incidence and duration of cold and flu-like symptoms in healthy subjects recently vaccinated for seasonal influenza. In a 12-week, randomized, double-blind, placebo-controlled clinical trial, 116 participants received daily supplementation with 500 mg of EpiCor or placebo for 12 weeks. Data collected included periodic in-clinic examinations and serologic evaluations at baseline, 6- and 12-weeks. Subjects also utilized a standardized self-report symptom diary during the study. Participants receiving the yeast-based product had significantly fewer symptoms and significantly shorter duration of symptoms when compared with subjects taking a placebo.

5. Jensen, G. S., et al. A double-blind placebo-controlled, randomized pilot study: consumption of a high-metabolite immunogen from yeast culture has beneficial effects on erythrocyte health and mucosal immune protection in healthy subjects. *The Open Nutrition Journal* **2008**, *2*, 68-75. [*Online reference: https://benthamopen.com/ABSTRACT/TONUTRJ-2-68*]

   This double-blind, randomized, placebo-controlled pilot study was designed to evaluate effects of consumption of EpiCor. Twenty-five healthy participants consumed 0.5 gram EpiCor or placebo daily for 5 weeks. The hematocrit increased significantly in the EpiCor group ($p<0.04$). A mild increase in saliva sIgA upon EpiCor consumption ($p=0.16$) prompted a subsequent 8-week open-label study involving 22 people showing significant increase in sIgA ($p<0.05$). EpiCor consumption led to a mild increase in serum IL-10 ($p<0.2$); no other differences in Th1/Th2 cytokines were observed. Minor health complaints decreased in the EpiCor group compared to the placebo group ($p<0.02$). Seasonal allergies increased in the placebo group, but were not observed in the EpiCor group; this was reflected by increased serum IgE in the placebo group compared to the EpiCor group ($p<0.13$). We conclude that consumption of EpiCor supported the health of red blood cells and mucosal immune protection.

6. Honzel, D., et al. Comparison of chemical and cell-based antioxidant methods for evaluation of foods and natural products: generating multifaceted data by parallel testing using erythrocytes and polymorphonuclear cells. *J Agric Food Chem* **2008**, *56* (18), 8319-25. [*Oniline reference:* https://www.ncbi.nlm.nih.gov/pubmed/18717566]

   The objective of this study was to compare three tests frequently used for evaluation of antioxidant potential in natural products: (1) oxygen radical absorbance assay (ORAC), (2) cell-based antioxidant protection in an erythrocyte model (CAP-e), and (3) reactive oxygen species formation in polymorphonuclear cells (ROS PMN). The methods were applied to four natural products, all containing antioxidants capable of entering and protecting cells in the CAP-e assay. The magnitude of this effect was not directly correlated to the ORAC value of each product. Furthermore, the products showed different effects in the ROS PMN assay. Acai provided strong inhibition of ROS formation, indicating anti-inflammatory properties. In contrast, Immunel and EpiCor mildly enhanced ROS formation, suggesting activation of the innate immune response. HA Joint Formula showed a complex, nonlinear dose-response in the ROS PMN assay. This illustrates that complex natural products may

have similar antioxidant properties but different effects on human cells. Cell-based antioxidant protection is addressed best in the CAP-e assay, since some natural products contain compounds that may provoke cellular signaling in other cell types. The PMN cell type is a useful model for assessment of overall anti-inflammatory versus immune supportive properties of a product. The sequential use of the three methods serves to bridge analytical and biological testing methods.

7. Peloquin, J. P. Screening natural product prototypes for biological activity using C. *elegans*. *Poster At Pathogenesis And Heterochrony Conference, Madison, WI* **2008.**

Diamond V Mills has produced innovative and effective natural fermentation products for more than 64 years with our complex proprietary process. Diamond V's Innovation Program continually generates new product prototypes we must evaluate for biological activity and efficacy, thus presenting many economic and logistical challenges. Importantly, common vertebrate models are less than optimal when screening multiple prototypes in the early stages of product development. Because of these challenges, Diamond V developed a screen using C*aenorhabditis elegans* to identify promising new product prototypes. C. *elegans* is an excellent whole-animal model for host-pathogen interactions (Moy et al. 2006) and to show the influence of natural products on aging (Wilson et al. 2005). Presently, we rely on lifespan alteration as an indicator of biological activity when comparing our prototypes to appropriate controls. We further investigate the most promising of these prototypes in targeted vertebrate models (*in vitro* and *in vivo*) to better understand the biological and health effects of our prototypes. Our C. *elegans* model has helped Diamond V reduce our use of vertebrate animals, ensure efficacy of our products, and significantly increase the efficiency and cost-effectiveness of our new product development process.

8. Moyad, M. A., et al. Immunogenic yeast-based fermentation product reduces allergic rhinitis-induced nasal congestion: a randomized, double-blind, placebo-controlled trial. *Adv Ther* **2009**, *26* (8), 795-804. [*Online reference:* https://www.ncbi.nlm.nih.gov/pubmed/19672568]

Allergic rhinitis (AR) impacts around 25% of the worldwide population. However, cost, safety, and a high dissatisfaction rate with numerous conventional medications continues to be an issue in the largest patient surveys, due primarily to a lack of efficacy on nasal congestion. Our previously published randomized trial demonstrated a significant reduction in cold and flu-like symptoms, and a secondary potential observation of a decrease in nasal congestion with an oral yeast-derived compound; therefore, the objective of this study was to test the effects of this same product on nasal congestion and other notable AR symptoms.

A 12-week, randomized, double-blind, placebo-controlled clinical trial of 96 healthy subjects with a recent clinically documented history of seasonal allergies and AR was conducted. Participants received once-daily supplementation with 500 mg of a dried, modified *Saccharomyces cerevisiae* oral fermentation product (EpiCor, Embria Health Sciences, Ankeny, Iowa, USA) or placebo during the 12-week period of the highest recorded concentrations of total pollen counts for this Midwest geographic area. Clinical outcome measurements included in-clinic examinations, validated questionnaire and standard diary, and serologic analysis at baseline, 6 and 12 weeks. During the highest pollen count period (weeks 1-6), EpiCor significantly reduced the mean severity of specific AR symptoms, including a significant reduction in nasal congestion ($p=0.04$), rhinorrhea ($p=0.005$), and a nonsignificant reduction in ocular discharge symptoms. A significantly ($p=0.04$) reduced total number of days with nasal congestion (12.5 fewer days) favored EpiCor compared with placebo, as did the nasal congestion section of the quality of life questionnaire ($p=0.04$). Subjects receiving the intervention also experienced significantly ($p=0.03$) higher salivary IgA levels. Adverse events were similar to placebo.

This yeast-derived product appeared to be safe and efficacious, and should receive more clinical research with and without standard medications to reduce the impact of seasonal allergies, especially AR-induced nasal congestion.

9. Moyad, M. A., et al. Immunogenic yeast-based fermentate for cold/flu-like symptoms in nonvaccinated individuals. J *Altern Complement Med* **2010**, *16* (2), 213-8. [*Online reference:*

https://www.ncbi.nlm.nih.gov/pubmed/20180695]

The common cold has a profound impact on employee attendance and productivity. Seasonal influenza is responsible for approximately 200,000 hospitalizations and 36,000 deaths per year in the United States alone. Over-the-counter medication efficacy has been questioned, and seasonal vaccination compliance issues abound. Our previously reported randomized trial of an oral fermentation product found an adjuvant benefit for vaccinated individuals in terms of a significantly reduced incidence and duration of cold and flu-like symptoms.

A concurrent 12-week, randomized, double-blind, placebo-controlled clinical trial of 116 subjects with no recent history of seasonal influenza vaccination was conducted. Participants received once-daily supplementation with 500 mg of a dried modified *Saccharomyces cerevisiae* oral fermentate (EpiCor) or placebo. Clinical outcome measurements included periodic interval-based in-clinic examinations and serologic analysis at baseline, 6 weeks, and 12 weeks. Participants utilized a standardized self-report symptom diary. Subjects receiving the intervention experienced a statistically significant reduction in the incidence ($p=0.01$), a nonsignificant reduction in duration ($p=0.10$), and no impact on the severity ($p=0.90$) of colds or flu-like symptoms, but a more favorable safety profile compared with subjects receiving placebo.

This nutritional-based fermentate appeared to be safe and efficacious in a unique at-risk population and should receive more clinical research as a potential method to reduce the incidence of cold and flu-like symptoms, in individuals with and without a history of influenza vaccination.

10. Jensen, G. S., et al. Antioxidant bioavailability and rapid immune-modulating effects after consumption of a single acute dose of a high-metabolite yeast immunogen: results of a placebo-controlled double-blinded crossover pilot study. *J Med Food* **2010**, *14,* 1002–1010. [*Online reference:* https://www.ncbi.nlm.nih.gov/pubmed/21501093]

The objective of this pilot study was to investigate the acute effects on circulating lymphocyte subsets, antioxidant status, and cytokine profile after consumption of EpiCor (Embria Health Sciences, Ankeny, IA, USA), a dried fermentate produced from *Saccharomyces cerevisiae*, using a placebo-controlled randomized crossover study design with 12 healthy adult human subjects. EpiCor contains high levels of bioavailable antioxidants and strongly activates natural killer (NK) cells *in vitro*. EpiCor consumption has been shown to increase erythrocyte hematocrit levels, boost mucosal immune protection, reduce cold/flu symptoms, reduce seasonal allergy symptoms and the need for rescue medication, and increase salivary secretory immunoglobulin A levels. This warranted further study on immune effects in humans. A within-subject analysis of data collected before and at 1 and 2 hours after consumption of a single dose of 500 mg of EpiCor versus placebo was performed. A transient reduction in circulating T and NK cell numbers was observed 2 hours post-consumption, suggesting that homing and recirculation of these cells, as part of healthy immune surveillance, were supported by EpiCor. The increased expression of activation markers on the CD3(-) CD56(+) NK cell population was significant for CD69 at 1 hour post-consumption (CD25, $p<0.07$; CD69, $p<0.05$), whereas for CD25 it was significant at 2 hours after consumption (CD25, $p<0.03$; CD69, $p<0.15$). A rapid increase in serum interferon-ɣ was observed at 1 hour post-consumption ($p<0.07$; after removal of two outlying data sets, $p<0.05$) and may have contributed to the effects seen on NK and T cell subsets. Significant increase in serum antioxidant protection was seen 2 hours after consumption ($p<0.04$). Thus consumption of a single 500 mg dose of EpiCor provides a rapid and transient effect on the trafficking and activation status of specific lymphocyte subsets, as well as increased antioxidant protection.

11. Evans, M., et al. A dried yeast fermentate prevents and reduces inflammation in two separate experimental immune models. *Evidence-Based Complementary and Alternative Medicine* **2012**, *2012, 7*. [*Online reference:* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3328167/]

Diverse and significant benefits against cold/flu symptoms and seasonal allergies have been observed with EpiCor in multiple published randomized trials. To determine if EpiCor may influence other immune conditions, two separate animal studies were conducted. Study 1 examined the ability of EpiCor to prevent or reduce

inflammation when given orally for 14 days to rats prior to receiving 1% carrageenan (localized inflammation model). EpiCor significantly ($p<0.05$) reduced swelling at all time points (1, 2, 3, 6, 12, and 24 hours) versus the control. Edema severity and PGE2 levels were reduced by approximately 50% and 25% ($p<0.05$), respectively. Study 2 examined the ability of EpiCor to treat established inflammation induced by type-2 collagen in mice over 4 weeks (autoimmune arthritis model). Significantly reduced arthritis scores, antibody response to type-2 collagen, and interferon-gamma levels were observed compared to controls (all parameters $p<0.05$). EpiCor favorably impacts multiple acute and potentially chronic immunologic inflammatory control mechanisms and should be further tested in clinical trials.

12. Possemiers, S., et al. A dried yeast fermentate selectively modulates both the luminal and mucosal gut microbiota and protects against inflammation, as studied in an integrated *in vitro* approach. *J Agric Food Chem* **2013**, *61* (39), 9380-9392. [*Online reference:* https://www.ncbi.nlm.nih.gov/pubmed/24006902]

    EpiCor, derived from *Saccharomyces cerevisiae*, has been shown to have immunomodulating properties in human clinical trials and *in vitro*. However, the underlying mechanisms behind its immune protection via the gut remain largely unknown. Therefore, the aim of this study was to use an integrated *in vitro* approach to evaluate the metabolism of EpiCor by the intestinal microflora, its modulating effect on the gut microbiota, and its anti-inflammatory activity on human-derived cell lines. Using the SHIME model, in combination with a mucus adhesion assay, has shown that low doses of EpiCor have a prebiotic-like modulatory effect on the luminal- and mucosa-associated microbiota. These include gradual changes in general community structure, reduction of potential pathogens, quantitative increase in lactobacilli, and qualitative modulation of bifidobacteria. Moreover, by combination of the SHIME with Caco-2 cells and Caco-2/THP1 cocultures, a significant decrease in proinflammatory cytokines was observed at the end of the treatment period.

13. Marzorati, M., et al. The HMI module: a new tool to study the Host-Microbiota Interaction in the human gastrointestinal tract *in vitro*. *BMC Microbiol* **2014**, *14* (1), 133. [*Online reference:* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4039060/]

    Recent scientific developments have shed more light on the importance of the host-microbe interaction, particularly in the gut. However, the mechanistic study of the host-microbe interplay is complicated by the intrinsic limitations in reaching the different areas of the gastrointestinal tract (GIT) *in vivo*. In this paper, we present the technical validation of a new device - the Host-Microbiota Interaction (HMI) module - and the evidence that it can be used in combination with a gut dynamic simulator to evaluate the effect of a specific treatment at the level of the luminal microbial community and of the host surface colonization and signaling.

    The HMI module recreates conditions that are physiologically relevant for the GIT: i) a mucosal area to which bacteria can adhere under relevant shear stress (3 dynes cm^{-2}); ii) the bilateral transport of low molecular weight metabolites (4 to 150 kDa) with permeation coefficients ranging from 2.4×10^{-6} to 7.1×10^{-9} cm sec^{-1}; and iii) microaerophilic conditions at the bottom of the growing biofilm ($PmO2 = 2.5 \times 10^{-4}$ cm sec^{-1}). In a long-term study, the host's cells in the HMI module were still viable after a 48-hour exposure to a complex microbial community. The dominant mucus-associated microbiota differed from the luminal one and its composition was influenced by the treatment with a dried product derived from yeast fermentation. The latter - with known anti-inflammatory properties - induced a decrease of pro-inflammatory IL-8 production between 24 and 48 h. The study of the *in vivo* functionality of adhering bacterial communities in the human GIT and of the localized effect on the host is frequently hindered by the complexity of reaching particular areas of the GIT. The HMI module offers the possibility of co-culturing a gut representative microbial community with enterocyte-like cells up to 48 h and may therefore contribute to the mechanistic understanding of host-microbiome interactions.

14. Jensen, G. S., et al. Anti-inflammatory properties of a dried fermentate *in vitro* and *in vivo*. *J Med Food* **2014**, *18*(3), 378-84. [*Online reference:* https://www.ncbi.nlm.nih.gov/pubmed/25105458]

    The aim of this study was to document anti-inflammatory properties of a dried fermentate derived from

Saccharomyces cerevisiae (EpiCor), hereafter referred to as dried fermentate *in vitro* using cell-based bioassays, and *in vivo* using a skin irritation model in healthy humans. *In vitro* testing involved parallel assessment of primary human polymorphonuclear (PMN) cell formation of reactive oxygen species (ROS) and migration toward the inflammatory mediator Leukotriene B4. *In vivo* evaluation used a single-blind placebo-controlled design, where dermal histamine-induced inflammation was used as a model for the complex intercellular signals involved in the initiation, escalation, and resolution of the inflammatory response. Microvascular blood perfusion was evaluated using noninvasive laser Doppler probes applied to the inner forearms of 12 healthy human subjects, where parallel sites were treated with either dried fermentate or saline (placebo). Subjective scores of dermal irritation were also collected. Treatment of PMN cells *in vitro* resulted in reduced ROS formation and migratory activity toward Leukotriene B4. Clinical results demonstrated significantly reduced microvascular inflammatory responses to histamine-induced skin inflammation, and significantly reduced subjective scores of irritation at the inflamed sites treated with dried fermentate compared with the sites treated with placebo ($p < 0.05$). Treatment of inflammatory cells *in vitro* with dried fermentate resulted in reduced inflammatory responses. This was confirmed *in vivo*, suggesting that the dried fermentate facilitates the resolution of inflammatory responses. The effects using a topical skin model suggest that similar events may happen when the dried fermentate is introduced across mucosal membranes after consumption.

15. Ducray, H.A.G, et al. Mitigation of heat stress-related complications by a yeast fermentate product. *Journal of Thermal Biology* **2016**, *60*, 26–32. [*Online reference:* https://www.ncbi.nlm.nih.gov/pubmed/27503713]

    Heat stress results in a multitude of biological and physiological responses which can become lethal if not properly managed. It has been shown that heat stress causes significant adverse effects in both human and animals. Different approaches have been proposed to mitigate the adverse effects caused by heat stress, among which are special diet and probiotics. We characterized the effect of the yeast fermentate EpiCor (EH) on the prevention of heat stress-related complications in rats. We found that increasing the body temperature of animals from 37.1±0.2 to 40.6±0.2 °C by exposure to heat (45 °C for 25 min) resulted in significant morphological changes in the intestine. Villi height and total mucosal thickness decreased in heat-stressed rats pre-treated with PBS in comparison with control animals not exposed to the heat. Oral treatment of rats with EH before heat stress prevented the traumatic effects of heat on the intestine. Changes in intestinal morphology of heat-stressed rats, pre-treated with PBS resulted in significant elevation of lipopolysaccharides (LPS) level in the serum of these animals. Pre-treatment with EH was effective in the prevention of LPS release into the bloodstream of heat-stressed rats. Our study revealed that elevation of body temperature also resulted in a significant increase of the concentration of vesicles released by erythrocytes in rats, pre-treated with PBS. This is an indication of a pathological impact of heat on the erythrocyte structure. Treatment of rats with EH completely protected their erythrocytes from this heat-induced pathology. Finally, exposure to heat stress conditions resulted in a significant increase of white blood cells in rats. In the group of animals pre-treated with EH before heat stress, the white blood cell count remained the same as in non-heated controls. These results showed the protective effect of the EH product in the prevention of complications, caused by heat stress.

16. Pinheiro, I., et al. A yeast fermentate improves gastrointestinal discomfort and constipation by modulation of the gut microbiome: results from a randomized double-blind placebo-controlled pilot trial. *BMC Complement Altern Med* **2017**, *17* (1), 441. [*Online reference:* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5584023/]

    Constipation and symptoms of gastrointestinal discomfort such as bloating are common among otherwise healthy individuals, but with significant impact on quality of life. Despite the recognized contribution of the gut microbiome to this pathology, little is known about which group(s) of microorganism(s) are playing a role. A previous study performed *in vitro* suggests that EpiCor fermentate has prebiotic-like properties, being able to favorably modulate the composition of the gut microbiome. Therefore, the aim of this study was to investigate the effects of EpiCor fermentate in a population with symptoms of gastrointestinal discomfort and reduced bowel movements and to evaluate its effect at the level of the gut microbiome.

    This pilot study was performed according to a randomized, double-blind, placebo-controlled parallel design.

Eighty subjects with symptoms of gastrointestinal discomfort and constipation were allocated to one of two trial arms (placebo or EpiCor fermentate). Randomization was done in a stratified manner according to symptom severity, resulting in two subgroups of patients: severe and moderate. Daily records of gastrointestinal symptoms were assessed on a 5-point scale, and also stool frequency and consistency were documented during a 2-week run-in and a 6-week intervention phases. Averages over two-week intervals were calculated. Constipation-associated quality of life and general perceived stress were assessed at baseline and after 3 and 6 weeks of intervention. Fecal samples were also collected at these same time points.

EpiCor fermentate led to a significant improvement of symptoms such as bloating/distension ($p=0.033$ and $p=0.024$ after 2 and 4 weeks of intervention, respectively), feeling of fullness ($p=0.004$ and $p=0.023$ after 2 and 4 weeks of intervention, respectively) and general daily scores ($p=0.046$ after 2 weeks of intervention) in the moderate subgroup. A significant improvement in stool consistency was observed for the total population ($p=0.023$ after 2 weeks of intervention) as well as for the severe subgroup ($p=0.046$ after 2 weeks of intervention), and a nearly significant increase in stool frequency was detected for the total cohort ($p=0.083$ and $p=0.090$ after 2 and 4 weeks of intervention, respectively). These effects were accompanied by an improvement in constipation-associated quality of life and general perceived stress, particularly in the moderate subgroup. Members of the families Bacteroidaceae and Prevotellaceae, two groups of bacteria that have been previously reported to be deficient in constipated patients, were found to increase with EpiCor fermentate in the severe subgroup. In the moderate subgroup, a significant increase in *Akkermansia muciniphila* was observed.

Despite the relatively low dose administered (500 mg/day), particularly when comparing to the high recommended doses for prebiotic fibers, EpiCor fermentate was able to modulate the composition of the gut microbiome, resulting in improvement of constipation-associated symptoms. Conversely, the reported increase in bowel movements may have altered the gut microbial community by increasing those groups of bacteria that are better adapted to a faster gastrointestinal transit time.

17. Ducray, H.A.G., et al. Yeast fermentate prebiotic improves intestinal barrier integrity during heat stress by modulation of the gut microbiota in rats. *J Appl Microbiol* **2019**, *127*, 1192-1206. [*Online Reference:* https://www.ncbi.nlm.nih.gov/pubmed/31230390]

To evaluate efficacy of *Saccharomyces cerevisiae* fermentate prebiotic, EpiCor, in protection of intestinal barrier integrity in rats during heat stress, to analyze the impact of heat stress and preventive treatment with EH on the structure of the gut microbiota.

Two groups of rats were treated orally with EpiCor or phosphate-buffered saline for 14 days. On day 15, half of the rats in each group were exposed to heat stress conditions, while control animals were kept at room temperature. Histological and Western blot analyses of the intestine, culture-based microbiological analysis and high-throughput 16S rRNA sequencing for the gut microbiota were performed for each rat. Exposure of animals to heat stress conditions resulted in inhibition of tight junction (TJ) proteins expression, decrease of Paneth and goblet cells, decrease of beneficial and increase of pathogenic bacteria. Oral treatment of rats with EpiCor before stress significantly prevents these adverse effects by elevation of the gut beneficial bacteria, particularly butyrate-producing bacteria.

Essential effect of EpiCor in protection of intestinal barrier integrity during heat stress is connected with beneficial modulation of the gut microbiota. Our results will contribute to the development of new approaches to prevention of heat stress-related complications.

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