

## ANTIMICROBIAL POTENTIAL OF BIO-CATALYZER $\alpha\rho$ NO. 11 (BIO-NORMALIZER) AGAINST ENTERIC MICROORGANISMS

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**Abstract.** A total of 141 bacterial isolates were obtained and identified from 84 gastric and rectal exudates of patients suffering from gastric ulcer, enterocolitis, diarrhea, gastroenteritis, internal and external hemorrhoids. The clinical isolates identified were *Enterobacter aerogenes*, *Escherichia coli*, *Salmonella typhi*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Enterobacter sakazakii*, *Edwardsiella tarda*, *Proteus vulgaris* and *Enterobacter agglomerans*.

The minimum inhibitory concentration of Bio-catalyzer  $\alpha\rho$  No.11 (Bio-normalizer), a health food supplement made by yeast fermentation of *Carica papaya* Linn. and traditional Japanese herbs, was found out to be 500 mg/ml. Its antimicrobial activity against these clinical isolates was determined by paper disc diffusion method.

Bio-normalizer showed thinning of growth on 89% of the isolates. The results suggest that Bio-normalizer has the potential to stop the growth of the most commonly encountered enteropathogenic and opportunistic microorganisms which may abound during abnormal conditions in the gastrointestinal tract.

**Keywords:** antimicrobial, Bio-normalizer, enteric microorganisms

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## INTRODUCTION

Over four thousand antimicrobial agents have been isolated from natural sources and more than thirty thousand compounds are acquired from natural antibiotics. A number of these compounds are used efficiently in the field of infectious diseases [1]. But recently, residual toxicity and side effects as well as cross-resistance of infections had been manifested, thus, upholding the quest for clinically applicable natural antimicrobials.

Bio-catalyzer  $\alpha.p$  No. 11 (Bio-normalizer) is a natural health product made by yeast fermentation of *Carica papaya* Linn. and other tropical herbs with glucose as the main carbon source [2]. It is commercially sold in Japan and in the Philippines as a food supplement. Bio-normalizer is a potent and stable hydroxyl radical scavenger [2,3]. It has the ability to increase gamma interferon in the body suggesting that it can enhance the activity of lymphocytes and helper T-cells in coping with the infection and diseases [4]. Moreover, it can regulate superoxide production as well as stimulate white blood cell and macrophage activity.

Its major component, *Carica papaya* Linn. which is a known medicinal herb, is used as anthelmintic, stomachic, diuretic, laxative, remedy for skin problems, among others [5]. It is bacteriostatic to most commonly encountered microorganisms [6] and a scavenger of free radicals [6,7].

Administration of Bio-normalizer was observed to improve a wide range of medical cases from simple colds to chronic diseases such as cardiovascular disorders, brain dysfunctions, gastrointestinal diseases, among others. The present study was undertaken to look into Bio-normalizer's effect on a number of opportunistic and pathogenic organisms isolated from gastrointestinal specimens.

## EXPERIMENTAL

*Test Sample.* Bio-normalizer was supplied by Sun-O International, Inc., Gifu, Japan.

*Test Organisms.* Standard test organisms such as *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus vulgaris* used in the determination of the minimum inhibitory concentration (MIC) were originally obtained from the American Type Culture Collection and preserved by the Microbiology Section of the University of Santo Tomas Research Center for the Natural Sciences (USTRCNS) (Manila, Philippines). The clinical isolates subjected to antimicrobial assay such as *E. aerogenes*, *E. coli*, *Salmonella typhi*, *Citrobacter freundii*, *P. aeruginosa*, *E. sakazakii*, *Edwardsiella tarda*, *P. vulgaris* and *E. agglomerans* were identified from specimens of patients with gastrointestinal disorders.

*Clinical Specimens.* A total of 84 gastric and rectal exudates were aseptically obtained through endoscopy (visualization and inspection of the interior portion of a hollow organ) and proctoscopy (dilation and inspection of the rectum) by the medical staff of the Gastroenterology Section of the Department of Medicine, UST Hospital (Manila, Philippines) from patients clinically diagnosed to be suffering from gastroenteritis, enterocolitis, diarrhea, gastric ulcer, internal and external hemorrhoids.

*Isolation and Characterization.* Gastric and rectal samples were streaked on to blood agar and nutrient agar plates which were incubated for 24 hrs. at 37°C to yield colonies. Incubation was extended to 48 hrs. when no growth was observed. Isolated colonies were characterized culturally, morphologically and biochemically [8]. Analytical Profile Index (API Systems, France) was used to confirm the identity of the isolates.

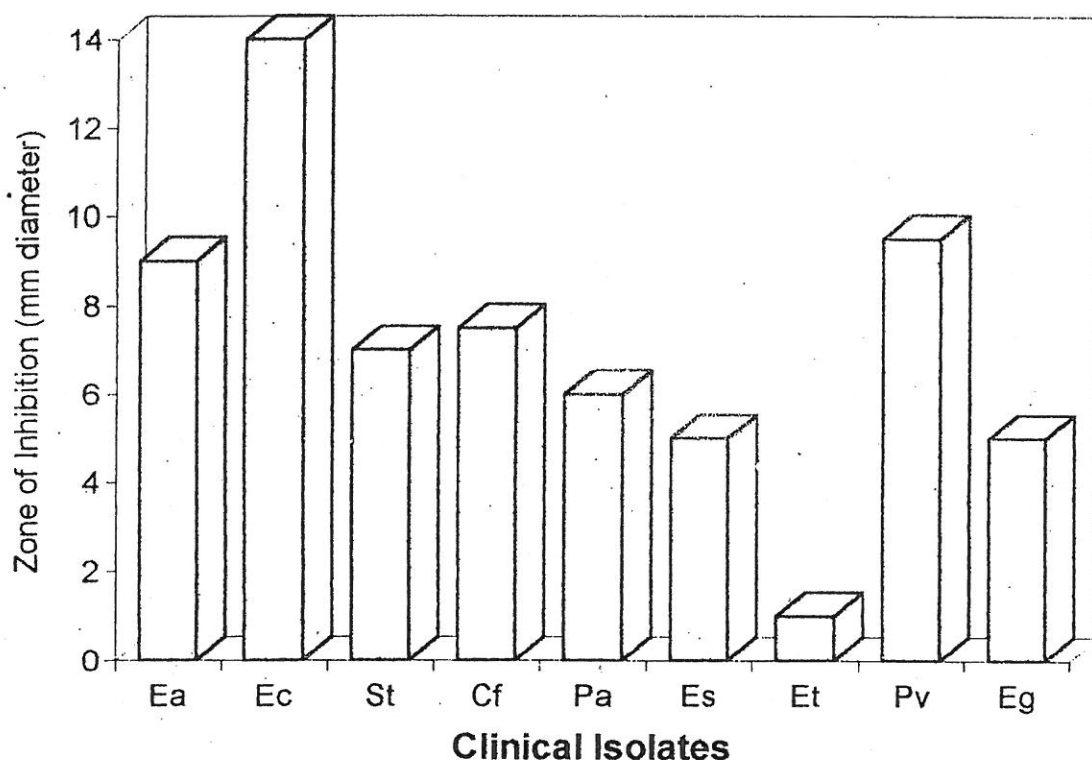
*MIC Determination.* Various concentrations of Bio-normalizer ranging from 100 - 1000 mg/ml were tested against the standard organisms and common clinical isolates using agar cup method and paper disc diffusion method [9,10]. Results showed no significant difference between the two methods used. Bacterial suspensions were normalized to contain  $3 \times 10^8$  organisms/ml by comparing with standards for turbidity measurements (Mc Farland Nephelometer Barium Sulfate Standards) [9].

*Antimicrobial Analysis.* To determine the antimicrobial activity of Bio-normalizer using its MIC, isolates were subjected to paper disc diffusion method [9,10]. Assay for each isolate was done in triplicates. The plates were incubated at 37°C for 24 hrs. and zones of inhibition were measured in mm diameter.

## RESULTS AND DISCUSSION

One hundred and forty-one isolates were characterized and identified from 84 specimens collected from patients with gastrointestinal disorders. The clinical isolates identified, in order of frequency, were *E. aerogenes*, *E. coli*, *S. typhi*, *C. freundii*, *P. aeruginosa*, *E. sakazakii*, *E. tarda*, *P. vulgaris* and *E. agglomerans*. The MIC of Bio-normalizer was found to be 500 mg/ml. A range of 5 - 14 mm thinning of bacterial growth was observed in 89% of the clinical isolates wherein *E. coli* has the largest zone of thinning (Fig. 1). It could be inferred that Bio-normalizer has the potential antimicrobial property since it has the capacity to stop the growth of the microorganisms.

Our study showed that the predominant group of bacteria isolated in the gastrointestinal tract belong to *Enterobacteriaceae*. It includes both the pathogenic bacteria, which infect or parasitize normal individuals, and the opportunistic



**Figure 1.** Antimicrobial activity of Bio-normalizer (500 mg/ml) against 141 isolates from 84 clinical specimens of patients with enterocolitis, diarrhea, gastroenteritis, gastric ulcer, internal and external hemorrhoids. Ea - *E. aerogenes*; Ec - *E. coli*; St - *S. typhi*; Cf - *C. freundii*; Pa - *P. aeruginosa*; Es - *E. sakazakii*; Et - *E. tarda*; Pv - *P. vulgaris*; Eg - *E. agglomerans*.

bacteria, which occur as a result of abnormalities in host defenses. Opportunistic *E. aerogenes*, *P. vulgaris*, *E. tarda* and *C. freundii* are responsible for outbreaks of diarrhea [11,12] while *E. agglomerans*, *E. sakazakii* and *P. aeruginosa* are most frequently implicated with human infections and gastroenteritis [13,14]. *Salmonella* species are some of the most incessantly identified enteric pathogens [15] involved in gastroenteritis [16,17], enterocolitis [16] and diarrhea [18]. Enteropathogenic *E. coli* infections, being a leading cause of diarrhea [11,15,18] most especially among infants in developing countries [20], are also associated with ulcerative colitis [21] due to toxins they produce. The ability of Bio-normalizer to stop the growth of the most common pathogens, may play a role in controlling the

outburst of bacterial colonization, thereby, allowing the immune system to subdue the infection. It may not completely eliminate the infection, but it will prevent the microorganisms from reproducing.

We, therefore, recommend further investigations to elucidate the mechanism of Bio-normalizer's antimicrobial action.

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