# Protocol Optimization for Addition of Selected Excipients in a Dry Functional Probiotic Mix Formulation

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**Introduction**

In the current era of health conscious consumers and demand for functional foods, probiotics acts as major functional food ingredients which also confer health benefits incurred due to probiotic strains. Dried formulation of probiotics in adequate numbers retrieved advantages such as ease of handling and application and with increase in shelf life.Food suppliers have ample scope to create market of new functional food ingredients and probiotics are considered one of the best among them. The probiotic strain *L. helveticus* MTCC 5463 used in the study was a human virginal isolate, exclusively studied under *in vitro* and *in vivo* conditions for the its applications as probiotics. The strain has proven bile tolerance and cholesterol reduction properties.

**Objective**

The study was under taken to standardize the protocol and check its suitability for preparation of a dry formulation containing probiotic *Lactobacillus helveticus* MTCC 5463 by evaluating selected excipients which could have best suited as reducing and bulking agents.

**Methodology**

Two reducing agents, L-cysteine (R1) and L-ascorbic acid (R2) and four bulking agents namely skimmed milk powder (B1), chicory powder (B2), maltodextrin (B3) and polydextrose (B4) were selected for blending with probiotic organism. Study was divided in three phases. Phase I was to make freeze dried culture and estimating it for viability and activity, so as to get the idea about the amount of freeze dried culture that would be needed for formulating. Phase II was evaluation of best suitable combination of reducing agent and bulking agent in dried probiotic formulation. The rate of addition of culture (C), reducing agent (R) and bulking agent (B) in formulation was fixed as 10, 10 and 80% (w/w) respectively i.e. (C:R:B = 10:10:80) and total formulation weight was kept as 2 g and subsequently different combinations viz. CR1B1, CR1B2, CR1B3, CR1B4, CR2B1, CR2B2, CR2B3 and CR2B4 were evaluated. Phase III was to optimize concentration of culture, reducing agent and bulking agent in the best formulation combination obtained from Phase II. Four concentration combinations C:R:B viz. 5:5:90, 10:10:80, 15:15:70 and 20:20:60) taken to analyze the viability and activity of the culture in formulation along with control i.e. without any reducing and bulking agents.

**Result and Discussion**

Results obtained on the basis of analyses, Phase I concluded that the inoculation rate for control samples were 0.02% (w/v), where as for formulation samples were 0.2% (w/v) of the freeze dried culture to get viability around10.59±0.03 log cfu/g). Phase II and III resulted as CR2B1 (Culture + L-ascorbic acid + skim milk powder) showed the highest viable count (10.36 log cfu/g) with 97.92 % viability after 3 days of storage at room temperature.

**Conclusion**

Ascorbic acid and skim milk powder in the ratio of C: R: B= 20: 20: 60 (w/w) with *L. helveticus* MTCC 5463 could be used for preparation of probiotic dry mix to get maximum viable counts.Study also showed potential that it could be needed further exploitation as functional ingredient for dairy, food and pharmaceutical industry as probiotic carrier vehicle.

ABSTRACT