



Pilot-plant scale biomass production by *Lactobacillus rhamnosus* GG ATCC 53103: a comparison between batch and fed-batch fermentation

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Abstract

Background and Aim : Probiotics such as Lactobacilli are important in improving normal intestinal flora and hindering the growth of harmful bacteria in the digestive system. Given the above reasons, the industrial production of probiotics and the use of high-yield strains is of great importance. The present study compares the biomass production by *Lactobacillus rhamnosus* GG ATCC 53103 in batch and fed-batch cultures conditions at a pilot plant scale. An optimized medium containing the following compounds (g/L): glucose 112.50, sugar beet molasses 56.25, casein 18.75, yeast extract 18.75, K₂HPO₄ 13.13, Tween 80 1.88, MgSO₄·7H₂O 0.3750, MnSO₄·4H₂O 0.0750, CaCl₂·2H₂O 0.1875 and Simethicone 1.25 was used for biomass production. During the fermentation process, culture conditions such as pH, temperature, and oxygen concentration were monitored using process analytical technology (PAT). Based on the obtained results, the maximum biomass production in the batch condition in the first 20 hours of culture in the optimized medium was about 68.14 g/L. After three stages of fed-batch culture, the biomass production by *L. rhamnosus* GG ATCC 53103 reached 93.5 g/L at 37°C with agitation and aeration rates of 100 rev/min and 300 VV-1min⁻¹, respectively. Therefore, biomass production increased about 2.67-fold more than the basal medium.

Methods : Plackett-Berman method for increasing the biomass production by *L. rhamnosus* GG ATCC53103 Plackett–Burman (PB) method using Design-Expert software 12.0.3.3 (Stat-Ease, Minneapolis, MN, USA) was applied for finding the most significant factors for the biomass production by *L. rhamnosus* GG ATCC53103.

Results : Maximum biomass production by *L.rhamnosus* GG ATCC 53103 was 73.4 dry weight /L, in 22 hours of incubation time at pH 7, which was two-fold more than the basal medium as shown in Figure 3. The obtained experimental results were significant at 95% level with the expected results. After optimization, the production medium contains the following compounds (g/L): glucose 112.50, sugar beet molasses 56.25, casein 18.75, yeast extract 18.75, K₂HPO₄ 13.13, Tween 80.88, MgSO₄·7H₂O 3750.0, MnSO₄·4H₂O 0750.0, CaCl₂·2H₂O 1875.0 and Simethicone 1.25 was used for biomass production in further studies.

Conclusion : As we know, *L. rhamnosus* GG is one of the most widely probiotics, used in the food industry. So, improving the biomass production of this probiotic is considered as grate importance. The results of this study showed that, by optimization of medium ingredients and culture conditions, the biomass production by *L.rhamnosus* GG ATCC 53103 improved in batch and fed-batch fermentations at the pilot-plant scale. By optimizing medium components and culture conditions at the pilot-plant scale, we can obtain biomass production of about 95g/L in fed-batch culture conditions about 2.67-fold more than the basal medium. In comparison to other studies, and as far as we know, this amount of

biomass production by *L.rhamnosus* GG ATCC 53103 has not been reported. Schematic flowchart of biomass production by *L. rhamnosus* GG ATCC 53103 shown in Fig 6. Currently, this condition is used for the production of *L.rhamnosus* GG ATCC 53103 biomass in Zist Takhmir pharmaceutical Co. (Tehran, Iran), and can be considered for large industrial scales of probiotic production.

Keywords : Biomass production, *Lactobacillus rhamnosus* GG ATCC 53103, Process Analytical Technology (PAT), Batch culture, Fed-batch culture