OPTIMIZATION OF CULTURAL CONDITIONS FOR MAXIMUM 
FRUCTOOLIGOSACHARIDE PRODUCTION BY **BACILLUS SPECIES**

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**ABSTRACT**

Fructooligosaccharides (FOS) are oligomers of fructose exhibiting the prebiotic properties. The FOS are found naturally in products like honey, onion etc. Plants and microbes have the potential to synthesize FOS by expressing an enzyme Fructosyltransferase (Ftase). The present investigation of production of FOS by indegineously isolated **Bacillus sp** from soil sample. The bacteria produced 10.02% total FOS after 12h of incubation time. The optimum temperature and pH were 60°C and 6.00 respectively. The total FOS production increased to 29.60 % after optimization.

**KEYWORDS:** Fructooligosaccharides (FOS), Fructosyltransferase.

**INTRODUCTION**

Fructooligosaccharides (FOS) are popular prebiotics having its prime usage as artificial sweeteners. The FOS has paved its way to the health market with increasing popularity day by day.\(^{[1-3]}\) The additional health benefits observed after its intake is no rise in blood sugar level although sweet in taste. Apart from taste, it confers excellent prebiotic properties like low calorie and non-carcinogenic nature.\(^{[4]}\) It is popularly labelled as functional food. Artificial sweeteners are witnessing an increased demand in global food market. The objective of present work is to analyse the FOS production potential of an bacterial isolate **Bacillus spp**. The production conditions were optimized for maximum FOS production.

**MATERIALS AND METHODS**

*Identification and Maintenance*

The bacterial isolate was identified to be **Bacillus spp** by different biochemical and morphological observations. It was maintained on nutrient agar medium, pH 7.0 at 4°C. Sub
culturing was done to keep it viable. The pure culture was maintained on nutrient agar with 3% sucrose. The isolate was stored on slants at 4 °C till further use.

**Crude enzyme preparation**

The selected isolate was grown by batch fermentation in the basal medium (g/l): Sucrose-10.0 g; NaNO₃ - 2.5 g; MgSO₄ .7H₂O - 0.5 g; KH₂PO₄ - 1.5 g; pH-7.0. The supernatant was used as a source of crude enzyme.

**Analysis of FOS**

The qualitative and quantitative FOS analysis was done using HPLC. The standards used were of sigma for identification of GF-2, GF-3, GF-4, GF-5 and GF-6.

**Optimization of FOS production in reaction mixture**

**Effect of incubation period on FOS production**

The reaction mixture contained 40 ml of 55% sucrose and 10 ml of crude enzyme and the 1ml of sample was withdrawn after every 2h for FOS analysis.

**Effect of temperature on FOS production**

The reaction mixture was incubated for 12 h at 40°C, 50, 60, 70 and 80. The FOS production was recorded at all temperatures.

**Effect of initial pH on FOS production**

The reaction mixture was incubated for 12 h at 60 at different pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. The FOS production was recorded for all reaction mixtures incubated at different pH.

**RESULTS AND DISCUSSION**

**Identification of the isolate**

The bacterial isolate was identified to be *Bacillus sp* by different biochemical and morphological observations. The crude enzyme was derived from the supernatant of batch fermentation.
Optimization of FOS production in reaction mixture

*Effect of incubation period on FOS production*

The reaction mixture contained 40 ml of 55% sucrose and 10 ml of crude enzyme and 1 ml of sample was withdrawn after every 2 h for FOS analysis. The FOS produced are given in Fig-1. The optimum incubation period was 12 h expressing total FOS % to be 10.02%.

![Fig-1: Effect of incubation period on total FOS production.](image)

*Effect of temperature on FOS production*

The reaction mixture was incubated for 12 h at 40°C, 50°C, 60°C, 70°C and 80°C. The FOS production was recorded at all temperatures. The % FOS produced at all temperatures are given in Fig-2. The optimum temperature was found to be 60°C.

![Fig-2: Effect of temperature on total FOS production.](image)

*Effect of initial pH on FOS production*

The reaction mixture was incubated for 12 h at 60°C at different pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. The FOS production was recorded for all reaction mixtures incubated at different pH. The maximum FOS % produced was at pH-6.
Fig-3: Effect of pH on total FOS production.

The FOS production has been consistently reported by microbes of classes like molds, yeasts and bacteria.[5] (Belorkar et al., 2013). The pH favouring the FOS production is reported to be between 5.0 to 6.0.[6] (Sanchez et al., 2010). The incubation time for FOS production also varies with the source of enzyme and time period.[7-8]

The present work has highlighted its ability in FOS production also. The optimum conditions for maximum FOS production were 12h incubation period, 6.0 pH and 60°C temperature. The yield of total FOS % reached 29.6% comparable to observations of Park et al.[9]

REFERENCES


