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Enhanced enzyme production and probiotic viability in oilseed cakes fermented with *Bacillus subtilis* for animal nutrition

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Abstract: *The present study explores the potential of 3 OSCs [flax (FSC), pumpkin (PSC)], and hemp (HSC)] as substrates for Bacillus subtilis ATCC 6051a (BS) during solid-state fermentation (SSF) to enhance enzyme production and probiotic viability for animal feed. Results indicate that bacterial growth and sporulation varied significantly among substrates ($p < 0.05$), with FSC exhibiting the highest spore resistance (86.48%), followed by PSC (82.87%), and HSC (81.23%). Enzyme activity confirmed the production of extracellular enzymes (protease, cellulase, xylanase, and phytase) pH variation indicated a shift toward alkalinity, particularly in PSC and HSC. The in vitro digestion model assessed BS survivability under simulated GI conditions. FSC displayed the most stable bacterial population throughout digestion, suggesting its potential as an optimal carrier for probiotic applications. These findings highlight the viability of OSC-based SSF in producing bioactive, probiotic-enriched feed ingredients, enhancing the digestibility and nutritional value of agro-industrial by-products for sustainable animal nutrition.*

• Material and method

Microorganism and vegetable material
Vegetative cells, spores resistance, and pH

Solid-State Fermentation (SSF) conditions
Moisture content and dry weight

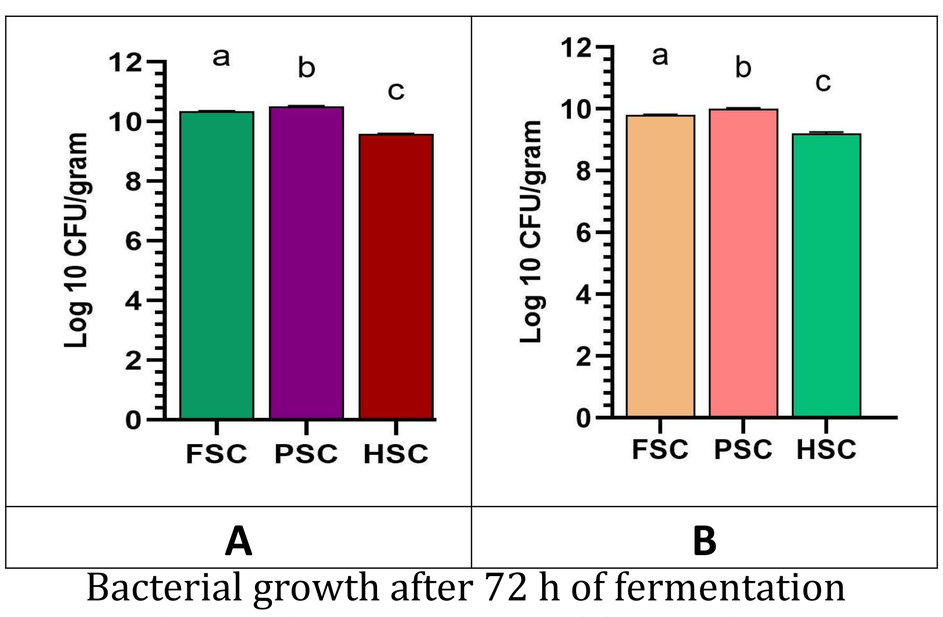
Dose:
8.32×10^{10} CFU/ g^{-1} feed
(*Bacillus subtilis* ATCC 6051a-BS)

Enzymatic activity → **Protease** → **Cellulase** → **Xylanase** → **Phytase**

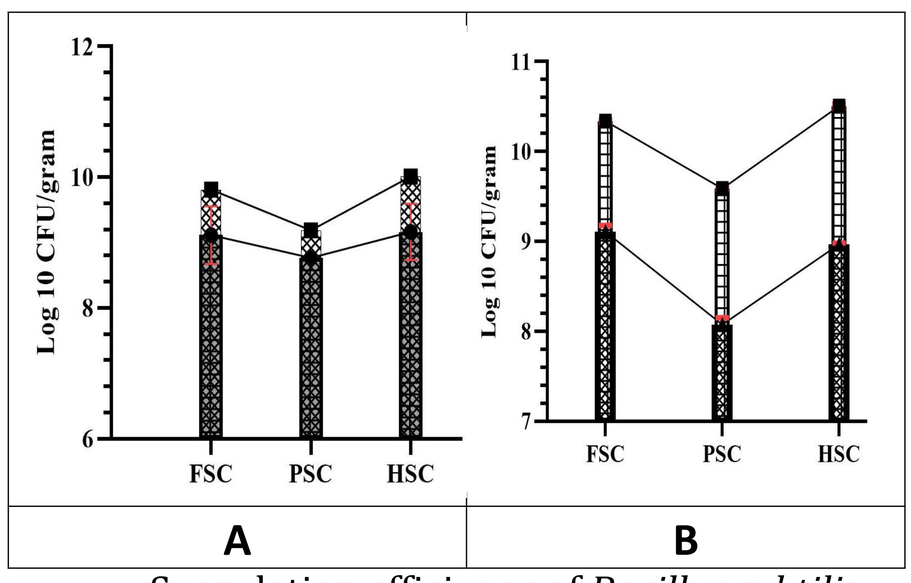
In vitro digestion under simulated conditions
- based on INFOGEST 2.0 protocol

Statistical analysis

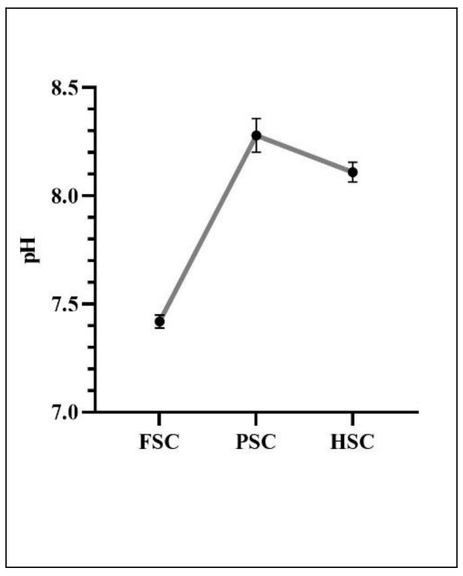
• Results and discussions



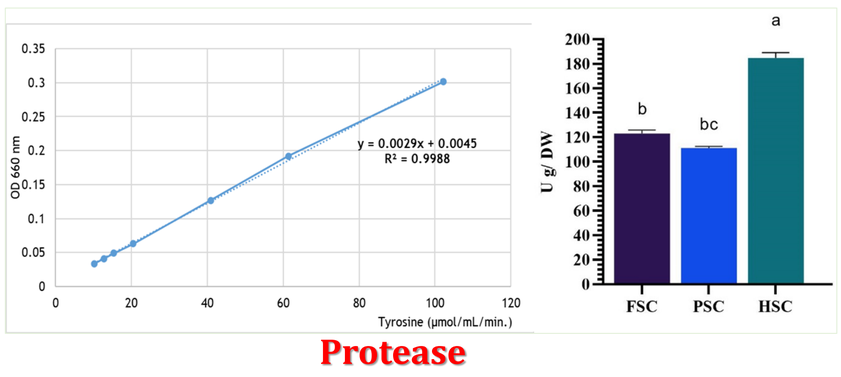
Bacterial growth after 72 h of fermentation
A = Wet-fermented substrates; B = Dried-fermented substrates.



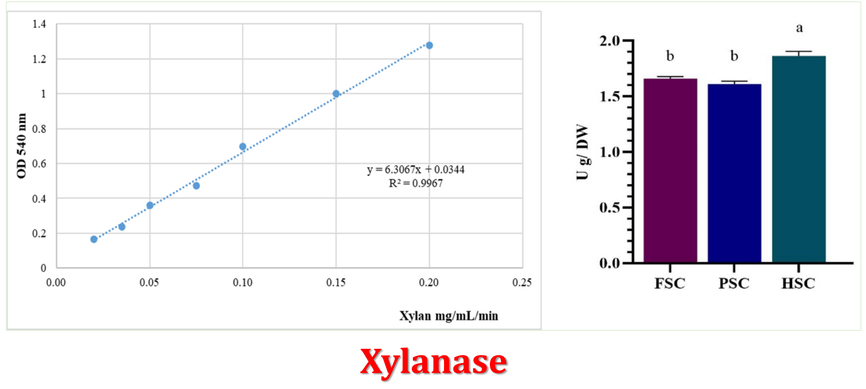
Sporulation efficiency of *Bacillus subtilis*
A = Wet-fermented; B = Dried-fermented



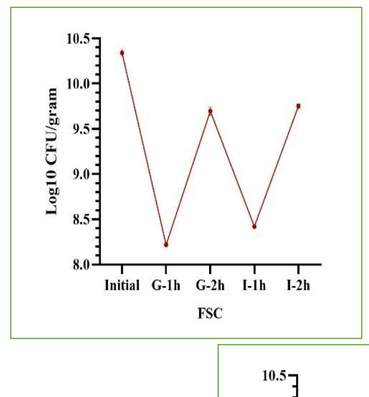
pH variations
in different OSC

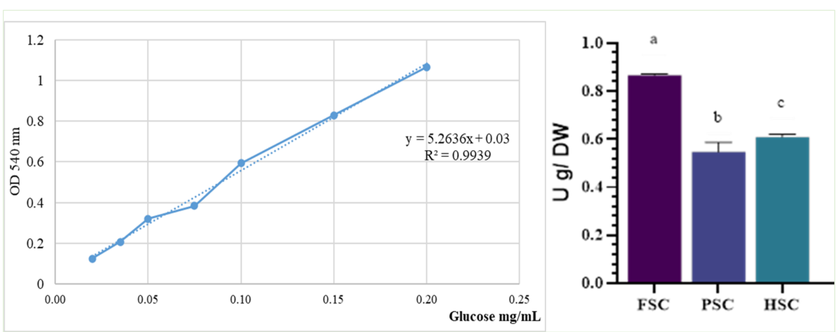


Protease

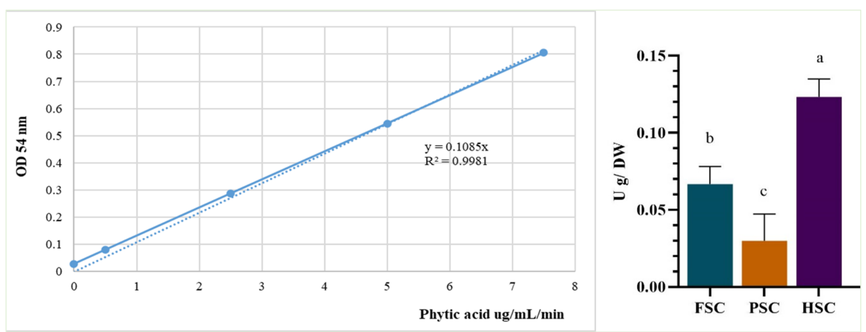


Xylanase

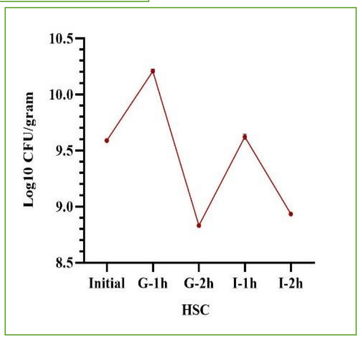




Cellulase



Phytase



***In vitro* digestibility**

• Conclusions

The findings confirm that digestibility and microbial viability in OSCs depend on substrate composition, enzymatic activity, and nutrient bioavailability. FSC emerges as the most digestible substrate, maintaining microbial stability throughout digestion.

HSC supports microbial adaptation but exhibits fluctuations, while PSC allows initial bacterial growth but lacks sustained nutrient release.

These results highlight the importance of substrate selection in optimizing microbial performance and nutrient utilization in biotechnological and nutritional applications.

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