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Comparable Expressions of *Bacillus* species on Different Growth Media

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***Abstract:** An investigation was carried out using Pikovskaya Broth (PKB), Luria Bertani Broth (LBB), and Peptone Water (PW) to analyse growth expressions of constructed Bacillus subtilis sub sp and compared to a commercial Bacillus subtilis RIK 1285. The aim was to determine the effect of carbon, nitrogen and other elements at different variations on the metabolic activities under different conditions. The results obtained showed growth density of 4.1 g/ml at 70°C and 3.1 g/ml at pH 6.0; 3.3 g/ml at 70°C and 2.8 g/ml at pH 6.0; 3.8 g/ml at 60°C and 2.6 g/ml at pH 7.0 from PKB, LBB and PW respectively. The growth density of the commercial strain recorded 3.8 g/ml at 50°C and 2.8 g/ml at pH 7.0; 3.1 g/ml at 50°C and 2.3 g/ml; 3.0 at 50°C and 2.3 at pH respectively. The investigation showed importance and relevance of gene metabolic upgrade on the utilization of multiple nutrients present from one media to another.*
***Keywords:** media formulation, microbial reaction, growth promoters, growth density*

I. INTRODUCTION

Microbial growth expression within an environment is directly dependent on strain metabolic efficiency, nutrient availability, moisture content and most importantly temperature (°C) and pH. This was confirmed in a report by ([3], [15]), that growth yield factors can be defined not only for nutrients that are built into the biomass, but also for electron donors and acceptors that serve as energy sources and terminal electron acceptors. *Bacillus subtilis* amongst several industrial relevant microorganisms have been modified by several techniques for optimal performance under extreme conditions ([7], [13]). Also, improvement of culture media with variable rations of carbon, nitrogen and other nutritional elements has also been used to justify the performance of the these bacteria and this has led to development of different model for optimal expression. By assessing the relevance of a growth culture medium for an experiment, the main concern is often with the effect of multiple variance growth factors in the cultivation system which helps to determine the maximum concentration of the biomass. Reports have also shown that the relationship between bacterial growth rate and environmental conditions involves different response mechanisms regulated through a two-component system that is activated by an external or internal signal ([4]). To further justify these various claims, optimal growth expression of newly constructed *Bacillus subtilis* sub specie was compared to a commercial *Bacillus subtilis* RIK 1285 using Luria Bertani broth, Peptone broth and pivoskaya broth. The aim was to ascertain interaction of a system to its environment considering differential nutritional properties.

II. MATERIALS AND METHODS

1) *Bacillus* specie: Specimen A was a B*acillus subtilis* RIK 1285 purchased from a culture bank and stored in 4°C for further analysis. Specimen B was newly constructed *Bacillus subtilis* sub specie using *Bacillus* expression Takara Infusion Kit ([6]). To confirm high efficiency of the newly constructed strain, 1% Luria Beratni agar infused with 50 µl kanamycin was used to grow the isolate at 37°C for 12 hr.
2) *Growth media*: 1000 ml of the following culture media: i. Luria Bertani Broth (10 g tryptone 10g, yeast extract 5g, NaCl, 10g), ii. Peptone water (Peptone 10g, Sodium chloride 10g), and iii. Pivoskaya Broth (Dextrose 10g, Calcium phosphate 5g Yeast extract 0.5g, Ammonium sulphate 0.5g, Potassium chloride 0.2g, Magnesium sulphate 0.1g, Manganese sulphate 0.0001g, Ferrous sulphate 0.0001g), were prepared separately and sterilized at 121°C for 10 minutes under 15psi. The sterilized media were allowed to cool and then used for the investigation.

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3) Growth analysis: The culture media were labelled LBB (Luria Bertani Broth), PW (Peptone Water) and PKB (Pivoskaya Broth). 10 ml from each balanced suspension were dispensed into sterile screw cap test tubes in a set of 2 batches. One batch was used to determine optimal growth at 30 – 100^{o}C, the second batch was used to determine optimal growth at pH 4 – 10. Into separate batch set A and B of each 10 ml of suspension, 1 ml of *Bacillus subtilis* RIK 1285 and newly constructed *Bacillus subtilis* sub specie were inoculated separately into the LBB, PW and PKB respectively. The culture suspensions were incubated at various temperatures (^{o}C) and pH ranges for 24 hr. and further used to determine culture growth density for each specimen.

III. RESULTS

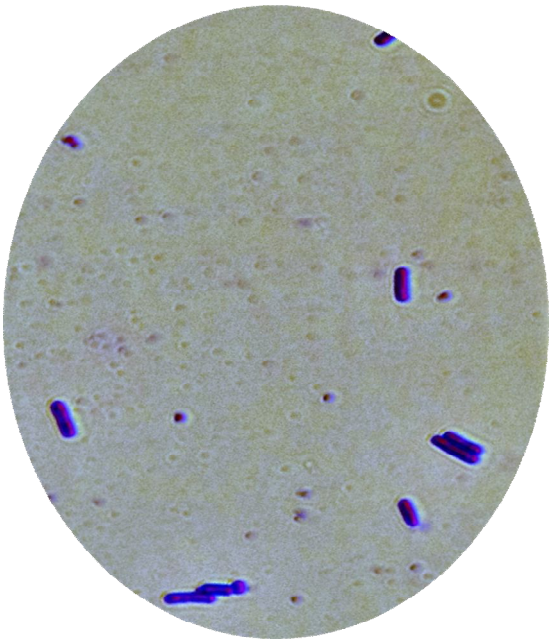


Figure 1: Microscopic characteristics of new constructed *Bacillus subtilis* sup sp. The image was obtained by gram's reaction technique and observed using a fluorescence microscope of X100 magnification. The Figure above clearly shows the positive purple coloured rod shaped cells indication the presence of peptidoglycan with techoic acid allowing pigment retention.

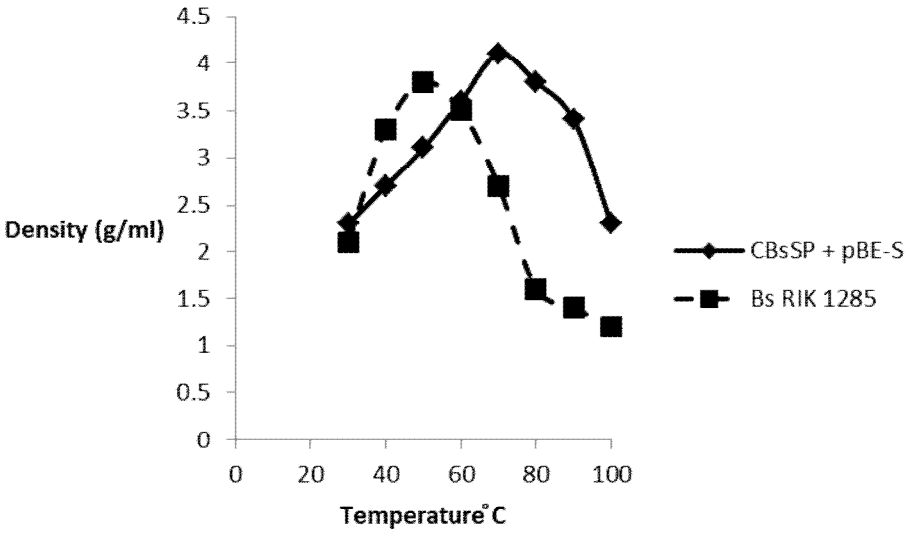


Figure 2: Optimal temprature ^{o}C of growth density for CBsSP + pBE-S (Constructed *Bacillus subtilis* sub sp) and Bs RIK 1285 (Commercial *Bacillus subtilis* RIK 1285) using Pikovskaya Broth.

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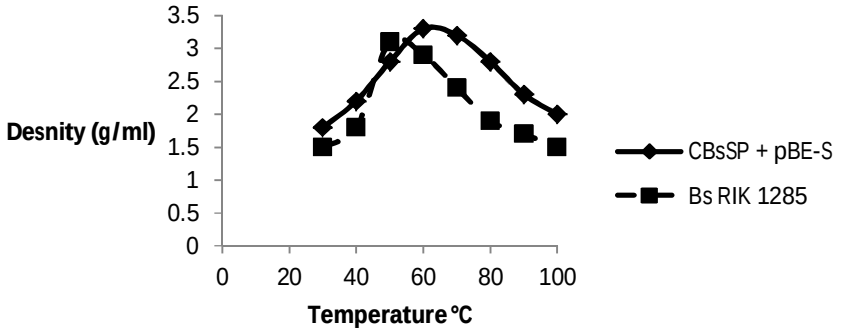


Figure 3: Optimal temprature °C of growth density for CBsSP + pBE-S (Constructed *Bacillus subtilis* sub sp) and Bs RIK 1285 (Commercial *Bacillus subtilis* RIK 1285) using Luria Bertani Broth.

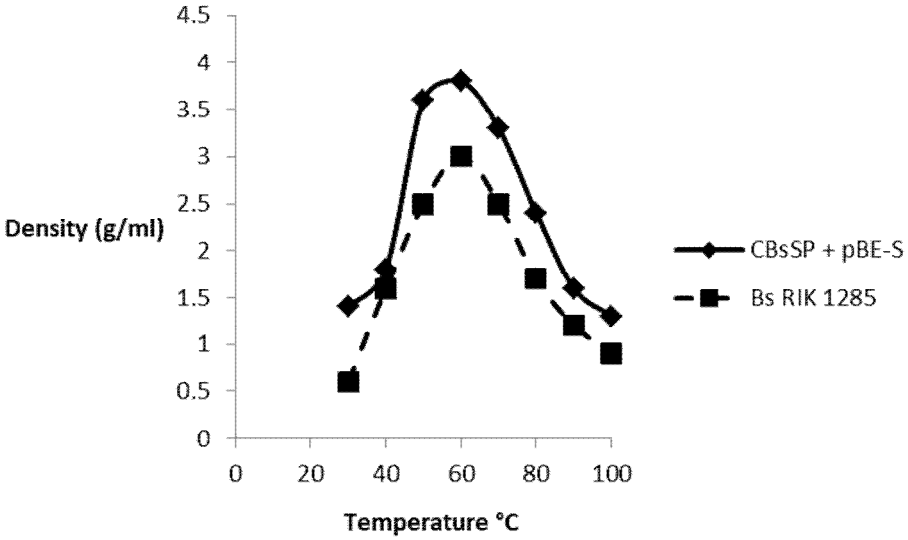


Figure 4: Optimal temprature °C of growth density for CBsSP + pBE-S (Constructed *Bacillus subtilis* sub sp) and Bs RIK 1285 (Commercial *Bacillus subtilis* RIK 1285) using Peptone Water.

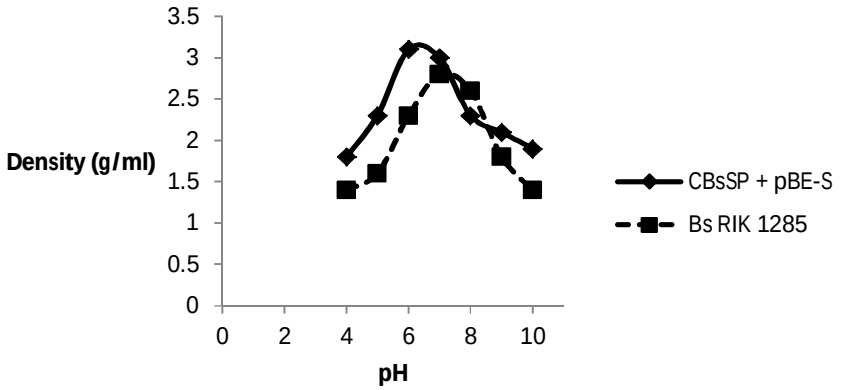


Figure 5: Optimal pH of growth density for CBsSP + pBE-S (Constructed *Bacillus subtilis* sub sp) and Bs RIK 1285 (Commercial *Bacillus subtilis* RIK 1285) using Pikovskaya Broth.

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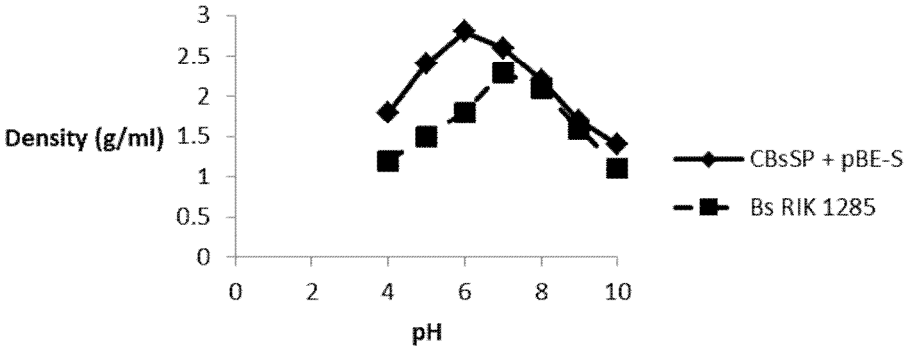


Figure 6: Optimal pH of growth density for CBsSP + pBE-S (Constructed *Bacillus subtilis* sub sp) and Bs RIK 1285 (Commercial Bacillus subtilis RIK 1285) using Luria Bertani Broth.

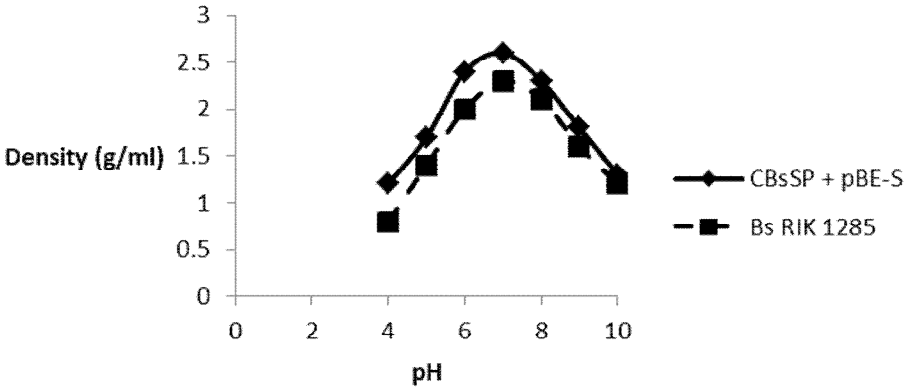


Figure 7: Optimal pH of growth density for CBsSP + pBE-S (Constructed *Bacillus subtilis* sub sp) and Bs RIK 1285 (Commercial *Bacillus subtilis* RIK 1285) using Peptone Water.

IV. DISCUSSION

It is perhaps not surprising that density of similar strains should have similar properties, especially when grown under similar conditions, as only the sporulation is affected by temperature, pH, and available nutrients ([12]). On the hand, several techniques have been used to increase the performance of species such as the constructed *Bacillus subtilis* sub sp in this investigation. The relevance of the upgrade is to improve on stability relating to metabolism and adaptation under extreme conditions. In this investigation, the growth of both Bacillus spp followed the general pattern of a gram positive, rod-shaped bacterium, arranged as single cells or in chains, and can form endospores as described in Figure 1. Hyper activity due to infusion of signal peptide bond to improve growth stability and possess antibiotic resistance ability was also recorded. This was also reported by ([5]). In this investigation, Figure 2, Figure 3 and Figure 4 reported the comparative growth density curves of *Bacillus subtilis* sub specie and *Bacillus subtilis* RIK 1285 at various temperatures from PKB, LBB and PW. It was observed that *Bacillus subtilis* sub sp recorded growth density of 4.1 g/ml at 70^{o}C, 3.3 g/ml at 70^{o}C and 3.8 g/ml at 60^{o}C from PKB, LBB and PW respectively while *Bacillus subtilis* RIK 1285 recorded density of 3.8 g/ml, 3.1 g/ml and 3.0 g/ml at 50^{o}C 3.1 g/ml from the same media. This result was similar to a report on *Bacillus* spp gene infusion technology to enhance growth stability and improve metabolism by ([9], [14]). Another report by ([10]) on media interaction with *Bacillus* sp growth at increasing temperature had been recorded. Similar observations were recorded for ionic concentration as illustrated in Figure 5, Figure 6 and Figure 7. From the figures shown, It was observed that *Bacillus subtilis* sub sp recorded growth density of 3.1 g/ml at pH 6.0, 2.8 g/ml at pH 6.0 and 2.6 g/ml at pH 7.0 from PKB, LBB and PW respectively while *Bacillus subtilis* RIK 1285 recorded density of 2.8 g/ml, 2.8 g/ml and 2.3 g/ml at pH 7.0 from the same media. This is similar to the investigation on effect of culture media on growth density of *Bacillus* development at a range of pH 6.0 – 7.0 as reported by ([11], [8]). Also, a similar significance of nutritional properties on growth development was reported in another investigation by ([1], [2]).

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The presence of microorganisms in all natural environments and their ability to adapt to available conditions has provided positive responses to various challenges in industrial application. Recently, improved expression by *B. subtilis* has become an efficient tool for the production of over 60% of commercially available metabolites and other items such as fermented products, flavor enhancers, sweeteners, and animal feed additive, household detergents, antibiotics, and vitamins and in the development of vaccines. The relevance of *Bacillus subtilis* continues to serve as a model organism in biotechnology and it has helped drive the research of molecular genetics, cell biology, and differential media formulations as one of its greatest component.

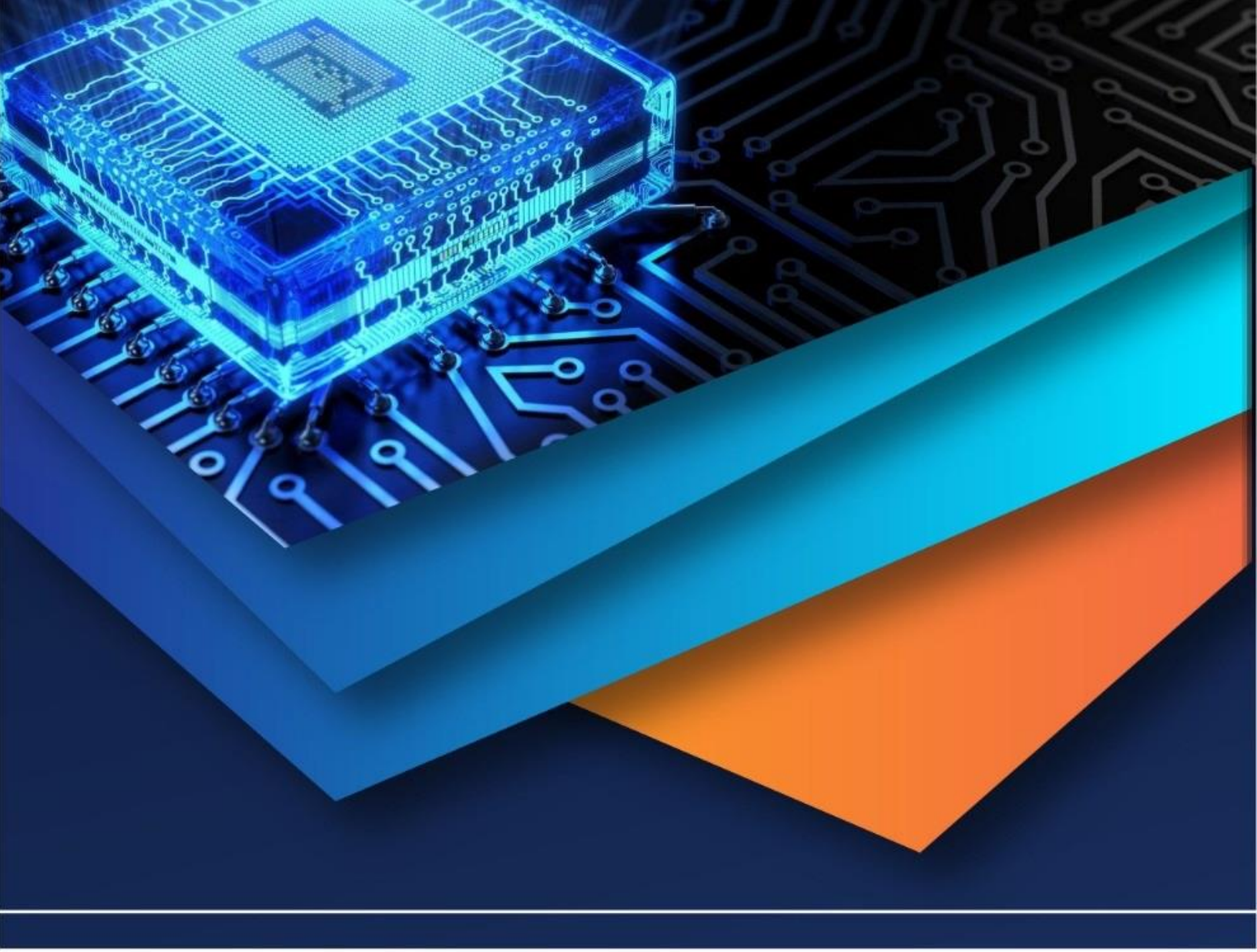
V. CONCLUSION

In this investigation, a new construct of *Bacillus subtilis* sub specie was developed using the Takara *Bacillus* Expression Kit. The strain expressed high metabolic efficiency by growing on antibiotic infused media. Furthermore, tts increased growth stability was also confirmed using different media with comparison to a commercial strain (*Bacillus subtilis* RIK 1285). This investigation confirmed the hyper activity of a transformed strain being able to utilize multiple ingredients in a medium because it had been documented that multiple ingredients often create metabolic stress. The industrial relevance of this investigation illustrated the importance of gene metabolic upgrade which enhances strain ability against contamination, and possibility of improving industrial bioprocessing under extreme conditions.

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