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Impact of temperature on the growth of *Klebsiella aerogenes*, which causes pearl millet stem rot

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Abstract
Temperature has a significant impact on bacterial growth and is a major factor in influencing the pace of metabolism, reproduction, and overall survival of bacteria. This study looks at how various temperature regimes affect the growth patterns of bacterial isolates of stem rot causing bacteria. Through the use of temperature ranges of 20±2 °C to 40±2 °C, we were able to observe different growth curves for seven different *Klebsiella aerogenes* bacterial isoaltes. It was discovered that 25±2 °C was the ideal growth temperature for each of the seven *Klebsiella aerogenes* isolates. The findings demonstrate the adaptive strategies used by bacteria to endure and multiply in a variety of thermal settings, including the synthesis of particular enzymes and structural proteins that are effective at their respective ideal temperatures. Comprehending these growth dynamics is important for several domains, such as agricultural field microbiology, biotechnology, and their habitat, where bacterial growth management is essential to prevent crop damage.

Keywords: Bacteria, growth, isolates, *Klebsiella aerogenes* and temperature

Introduction

One of the most important and ancient staple millets for millions of poor people living in tropical dry and semi-arid areas is pearl millet [*Pennisetum glaucum* (L.) R. Br.], a plant belonging to the Poaceae family. Pearl millet, the world's fifth-most significant cereal crop after rice, wheat, maize, and sorghum, is essential to maintaining food and energy security, especially in locations that receive rain. The All India Coordinated Research Project on Pearl Millet (2022) reports that this crop is cultivated on more than 30 million hectares globally, with Africa accounting for the majority of its cultivation (>18 million ha) and Asia for the majority (>10 million ha). According to Yadav *et al*. (2012), 90% of the world's pearl millet land is found in Africa and India. Interestingly, since 1980, millet production has increased by 130% in central and west Africa, accounting for 50% of the world's total production. Rajasthan is the state that produces the most, and India is the leader in production. Over 68.40 million hectares, pearl millet was grown in India in 2021–2022, with a total production of 97.80 million tonnes and a productivity rate of 1,430 kg/ha. With a productivity rate of 2,318 kg/ha, the 4.83 lakh hectares of cultivable land in Haryana produced 11.19 lakh tonnes of output. Districts Bhiwani, Mahendragarh, Rewari, Hisar, Charkhi Dadri, Jhajjar, Rohtak and Palwal are the main ones in Haryana that grow pearl millet.

The diseases that can affect pearl millet in India include downy mildew (also known as green ear disease), which is caused by *Sclerospora graminicola*; rust, which is caused by *Puccinia substriata* var. indica; smut, which is caused by *Moesziomyces bullatus*; sugary disease (also known as ergot) caused by *Claviceps fusiformis*; pyricularia leaf spot (blast) caused by *Magnaporthe grisea*; stem rot caused by *Klebsiella aerogenes*; bacterial leaf blight, which is caused by *Pantoea stewartii* subspecies indologenes; and stunting, little leaf and phyllody disease, which is caused by Candidatus. In particular, stem rot, bacterial leaf blight, stunting, small leaf, and phyllody disease were all observed to be prevalent in India in 2021-2022 (Hemalatha *et al*., 2022; Malik *et al*., 2022; Mushineni *et al*., 2021) [6, 8, 10]. The genus *Klebsiella*, which has historically been linked to diseases in humans and animals, has recently become an important plant pathogen. It has been linked to a number of crop diseases, including wilting in many plantations (Ajayasree and Borkar, 2018) [3], stem rot in pearl millet (Malik *et al*., 2022) [8] and top rot in maize (Huang *et al*., 2016) [7].

Klebsiella aerogenes is the causative agent of pearl millet stem rot. The *Enterobacteriaceae* family of bacteria is distinguished by a wide range of biochemical, morphological, and cultural characteristics. According to genome-based comparative bacterial phylogenetics, *Enterobacter aerogenes* was reclassified as *Klebsiella aerogenes* in 2017 (Tindall *et al*., 2017; Wesevich *et al*., 2019) [11, 12]. On nutrient agar, rod-shaped, gram-negative, catalase-positive, citrate-positive, indole-negative, facultative anaerobe *Klebsiella aerogenes* forms whitish-cream colonies. Some strains of these organisms are naturally occurring parts of the flora of the human gastrointestinal tract, and they can be found on plants, in soil, and in water. *Klebsiella* affects people and animals in addition to plants (Huang *et al*., 2016) [7]. Disease incidence, overall severity of the disease, and the source of the inoculum all affect crop output. In order to identify the ideal circumstances for the pathogen's proliferation, experiments were carried out to assess its growth pattern across a range of temperature regimes.

Materials and Methods

7 different isolates namely KA1, KA2, KA3, KA4, KA5, KA6 and KA7 of *Klebsiella aerogenes* causing stem rot of pearl millet were used during study. Their broths were prepared and from them serial dilutions were performed.

Procedure

1. Arrange test tubes and fill them with 9 ml double distilled water and label them.
2. Add 1ml bacterial suspension from the broth to first test tube.
3. Make up further dilution upto 10^{-5}.
4. Perform plating from the last dilution by pour plate method for obtaining single colonies.
5. Add 1ml of diluted sample to appropriately labelled petri plates after dilution.
6. Incubate the plates as per the temperature range for recording observations.
7. Repeat the same process for all 7 isolates

Observations

Pathogen colonies were counted after 48 hours of incubation using following formula:

$$\text{CFU /ml} = \frac{\text{(No. of colonies x Total Dilution factor)}}{\text{Volume of culture plated in ml}}$$

Bacterial isolates were grown on nutrient agar (Table 1 denotes its composition) plates and their growth were recorded at different temperature regimes (20±2 °C, 25±2 °C, 30±2 °C, 35±2 °C and 40±2 °C).

Table 1: Nutrient Agar medium

Component	Quantity (g/L)
Peptone	5.0
Beef extract	3.0
NaCl	5.0
Agar-agar	20.0

Statistical Analysis

Analysis was performed by using opstat software.

Results and Discussion

7 isolates i.e., KA1, KA2, KA3, KA4, KA5, KA6 and KA7 of *Klebsiella aerogenes* causing stem rot of pearl millet were undertaken in the study and the CFU x 10^5/ml were calculated at 5 different temperature ranges varying from 20±2 °C to 40±2 °C. It was observed that 25±2 °C was found to be the most optimum temperature for all isolates of *Klebsiella aerogenes* as maximum number of colonies were observed at this temperature (Table 2). The least number of colonies were observed at 40±2 °C in all seven isolates of the stem rot causing pathogen. After 25±2 °C, 30±2 °C was second best temperature for *Klebsiella aerogenes* isolates growth followed by 20±2 °C. The findings indicate that *Klebsiella aerogenes* thrives best at 25±2 °C (Figure 1). This temperature likely provides the ideal conditions for the metabolic activities of the bacterium, including nutrient uptake, enzyme activity and overall cellular function. Studies on related bacteria of Yang *et al*., 2024 such as those examining the growth of *Klebsiella pneumoniae*, support these findings, showing optimal stability and growth around 25 °C while studies of Jin *et al*., 2015 on *Klebsiella aerogenes* also support the current findings.

At 20 °C, the bacterial growth was significantly lower, suggesting that the cooler temperature slows down metabolic processes, leading to reduced proliferation. At 30 °C and 35 °C, although the bacteria still grew, the conditions were less favourable compared to 25 °C. This may be due to the increased metabolic strain or the denaturation of proteins at higher temperatures. Notably, at 40 °C, the growth was minimal, likely because the higher temperature approaches the upper limit of the bacterium's thermal tolerance, leading to thermal stress and potential denaturation of critical cellular components. Figure 2 shows individual isolate growth at different temperature range.

Understanding the temperature preferences of *Klebsiella aerogenes* is crucial for various applications, including controlling its growth in field areas, lab conditions and optimizing conditions for industrial processes where this bacterium might cause loss. Additionally, these findings underscore the importance of temperature control in preventing the spread of bacterial infections, as certain temperatures can either inhibit or promote bacterial growth.

Further research into the molecular mechanisms governing temperature-dependent growth could provide deeper insights into the adaptability and resilience of *Klebsiella aerogenes*. This knowledge can inform strategies for managing bacterial populations in both environmental and medical contexts.

Table 2: Growth of seven isolates at different temperature (CFU x 10^5/ml)

Isolate	Temperature (°C)					
	20±2	25±2	30±2	35±2	40±2	Mean (Isolates)
KA1	8.20	14.10	11.30	7.20	2.10	8.58
KA2	15.30	20.50	17.20	8.40	5.10	13.30
KA3	12.10	25.20	20.40	10.10	7.30	15.02
KA4	10.20	15.60	12.10	6.60	2.20	9.34
KA5	5.30	17.50	15.20	3.20	1.40	8.52
KA6	9.40	18.80	16.50	7.30	6.20	11.64
KA7	11.10	22.40	18.30	9.20	4.30	13.06
Mean (Temperature)	10.23	19.15	15.85	7.43	4.09	
CD (p=0.05) Isolate =0.21 Temperature =0.17 Interaction =0.46				SE (m): Isolate =0.07 Temperature = 0.06 Interaction = 0.17		

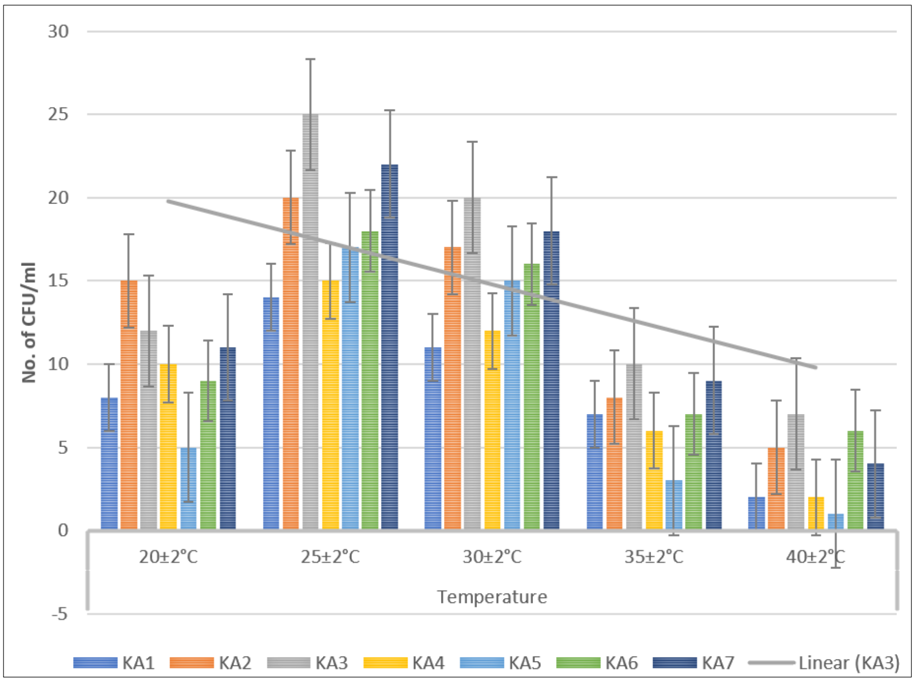
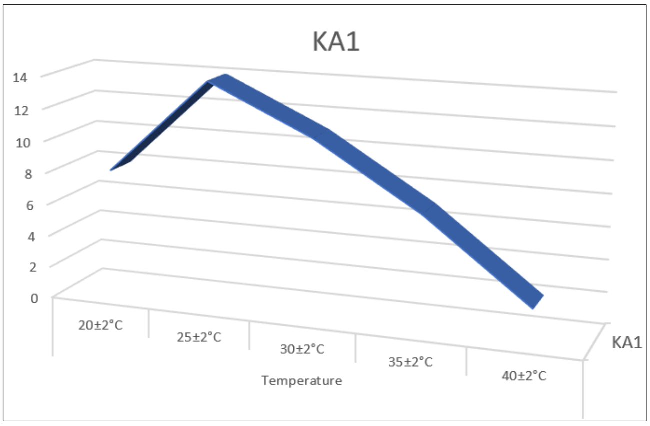
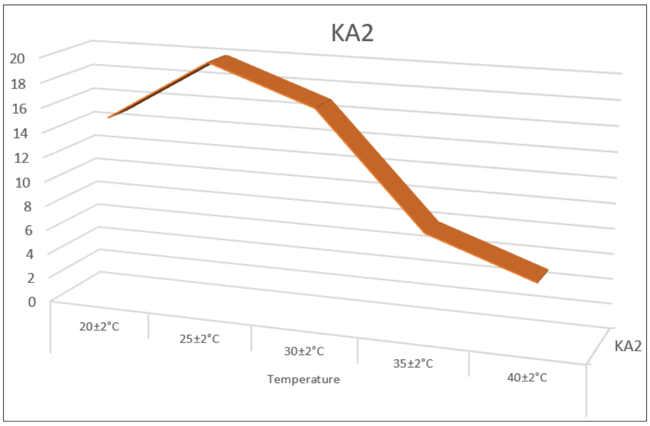
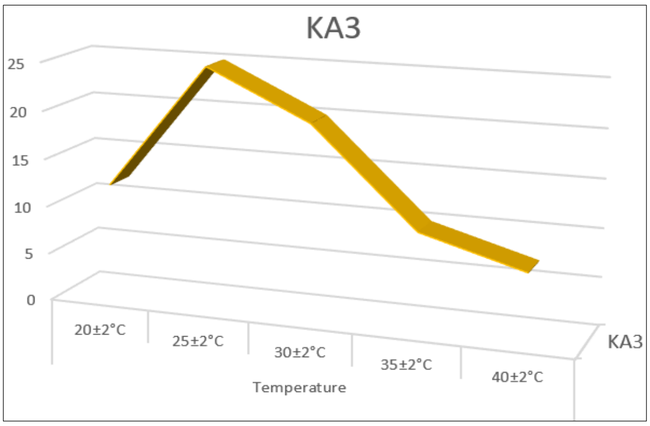
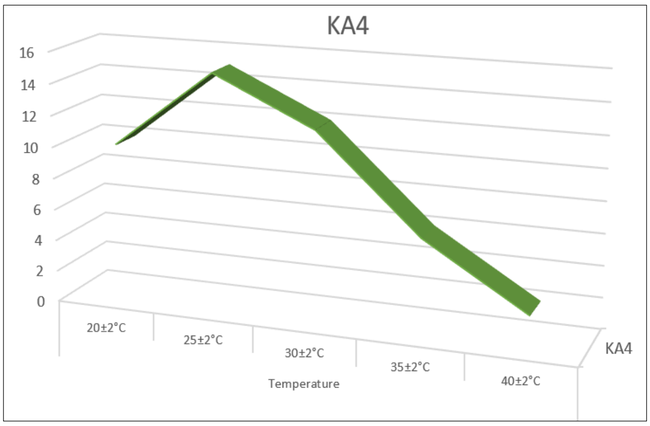


Fig 1: Growth of various isolates at different temperature









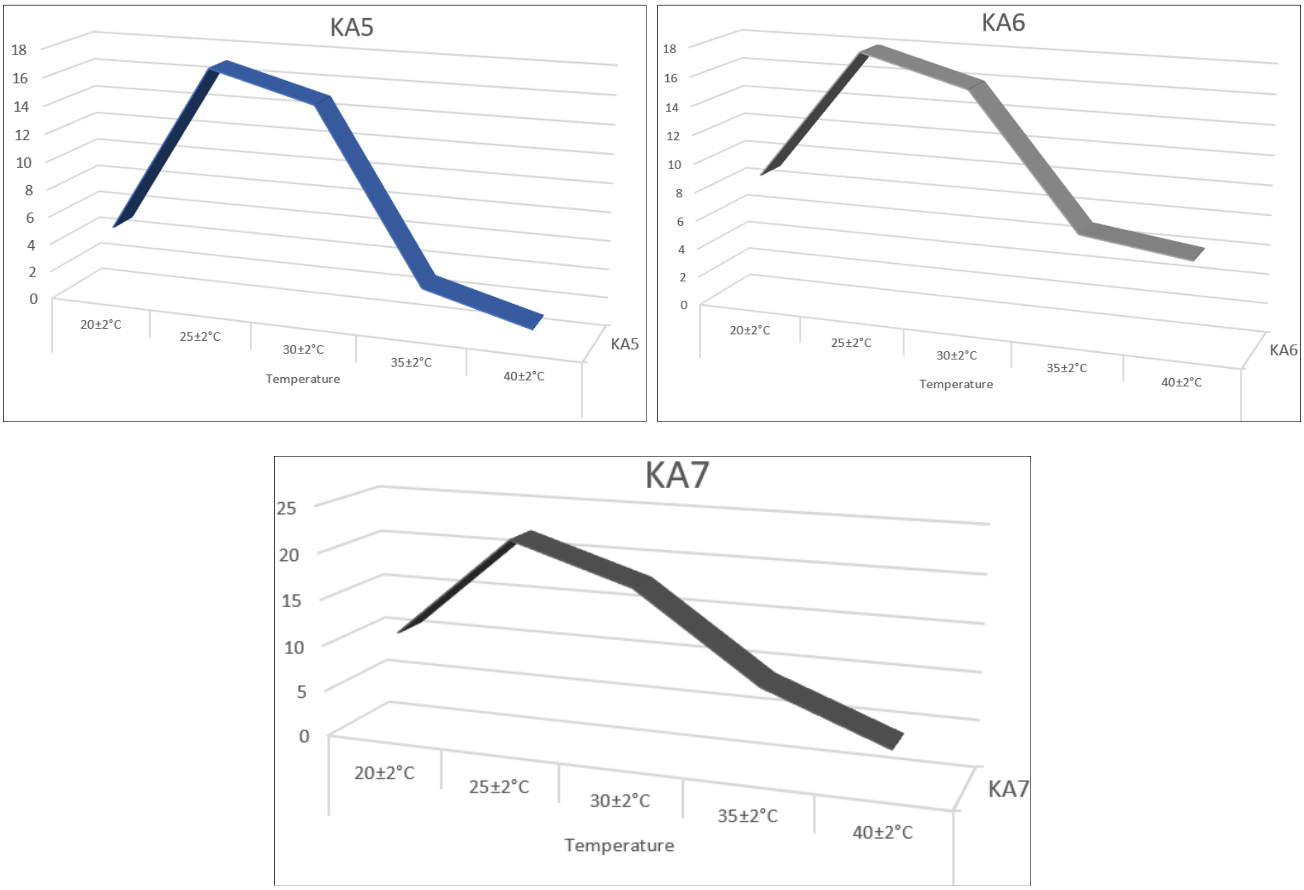


Fig 2: Individual growth graph of separate isolates at different temperature range

Conclusion

In conclusion, this study investigated the temperature preferences of seven isolates of Klebsiella aerogenes responsible for stem rot in pearl millet. Our findings indicate that 25±2 °C is optimal for the growth of these isolates, with maximum colony counts observed at this temperature. Lower temperatures, such as 20±2 °C, slowed bacterial growth significantly, while higher temperatures up to 40±2 °C showed progressively less favorable conditions, likely due to metabolic strain and thermal stress. These results align with previous studies on related bacteria, highlighting the importance of temperature control in managing Klebsiella aerogenes populations. Further research into the underlying molecular mechanisms could enhance our understanding and aid in developing effective strategies for bacterial control in diverse environments and industries.

Conflict of Interest

Author confirms no conflict of Interest.

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