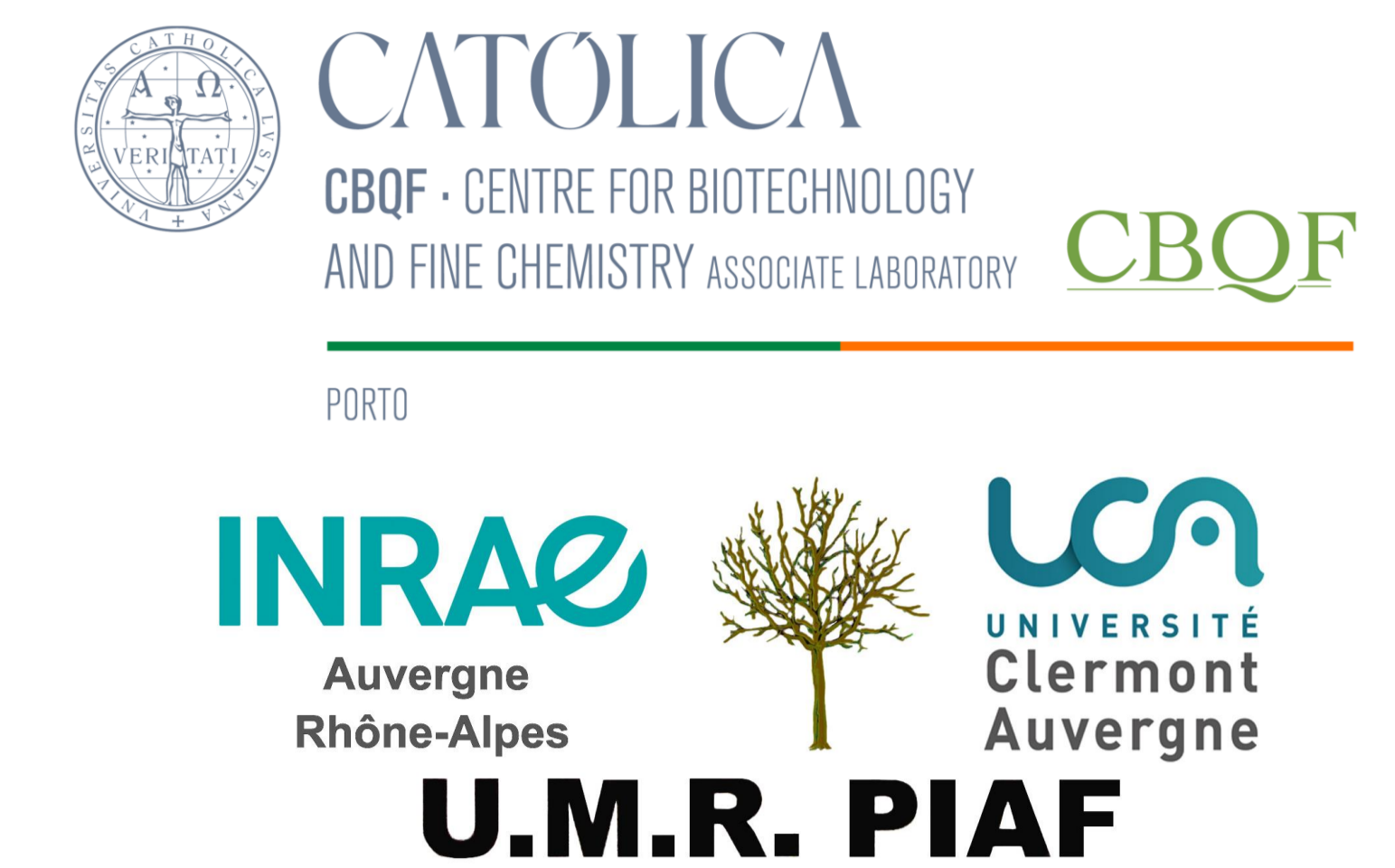


Ectomycorrhizal fungi inocula optimization to aid the health status of trees in the everchanging environment of cities

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Introduction

The **Ectomycorrhizal fungi** improve host trees' vigor and health status and can be used as a **biotechnological tool for the mitigation of stress in urban context**.

Fungi **spores** have the potential to be used as inocula in hash environments, but its **dormancy may affect performance**.

Aim of the work: assess different storage methods to **preserve** the viability of spores while enhancing their activity by using **activity-inducing** substances.

Methods

DORMANCY/ACTIVITY ASSESSMENT



BIOLOGICAL MATERIAL



Pisolithus tinctorius spores



INDUCTION-SUBSTANCES

Phosphate buffer (control) – **PB**
Glucose
Activated Charcoal
Yeast Extract



CONSERVATION

Sand (4°C) – **BOX**
 Plastic container (4°C) – **FRIDGE**
 Plastic container (-20°C) – **FROZEN**
 Sand (4°C) + 2 weeks Room T° – **ROOM**



MTT METHOD

Measures **cellular metabolic activity**
 Incubation 24h/dark/23°C
 Isopropyl Alcohol – solvent
 Formazan Reading – Spectrophotometer 570nm



ANALYSIS

Activity quantification:
absorbance/mass of spores
 Test for normality and homogeneity
 One-Way ANOVA followed by by Duncan's multiple range test (P <0.05)
 Different letters - statistically significant differences

Results and Discussion

Comparison of Conservation Methods (figure 1)

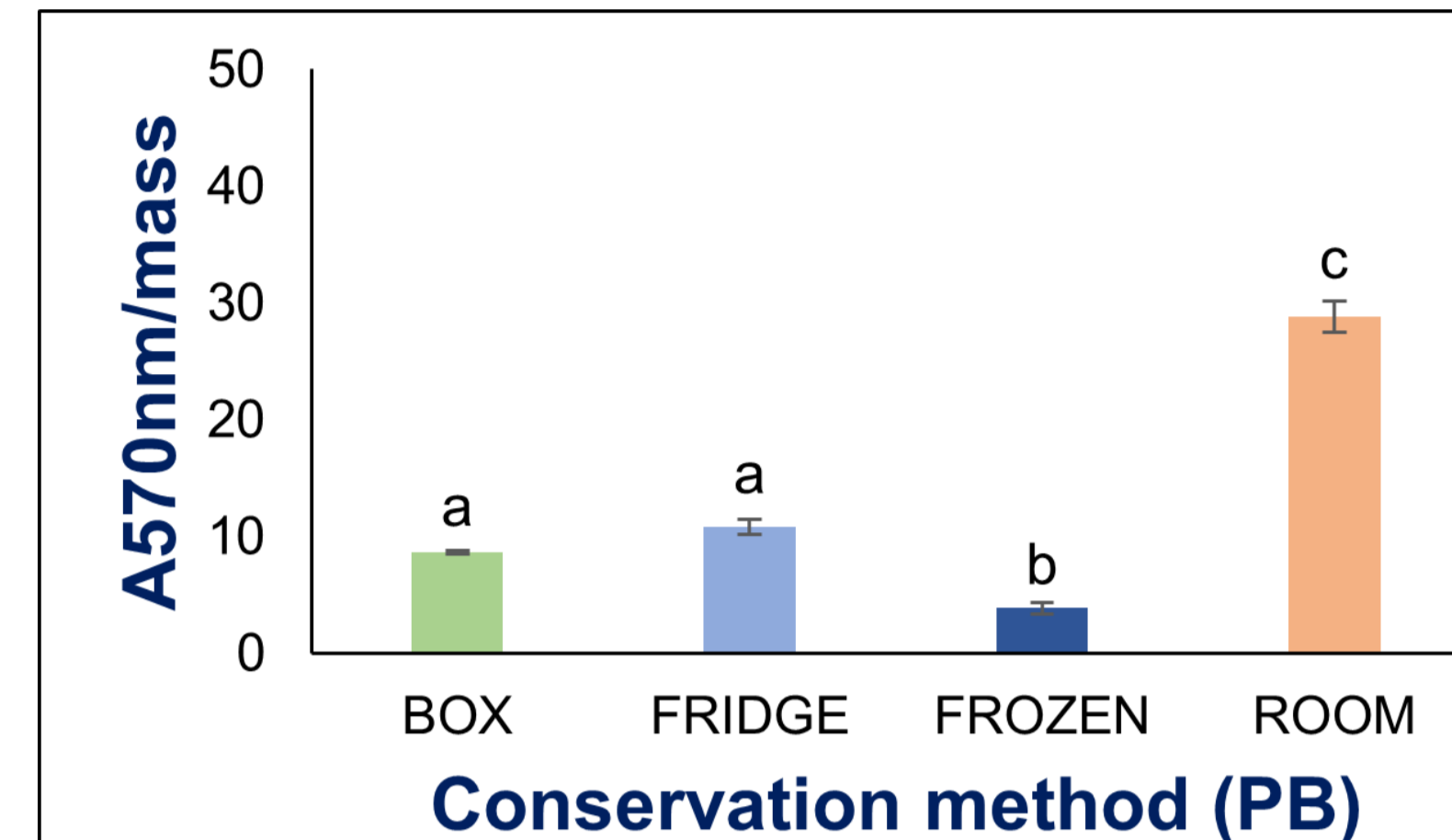


Figure 1: The conservation method where the spores were stored in an initial phase in sand at 4°C and later matured at room temperature (ROOM) exhibited the spores with the highest respiratory activity. In contrast, storage of spores at -20°C (FROZEN) preserved the dormancy of spores, showing the less activity of the four conservation treatments.

Effect of inducing-substances in the activity of spores (figure2 A-D)

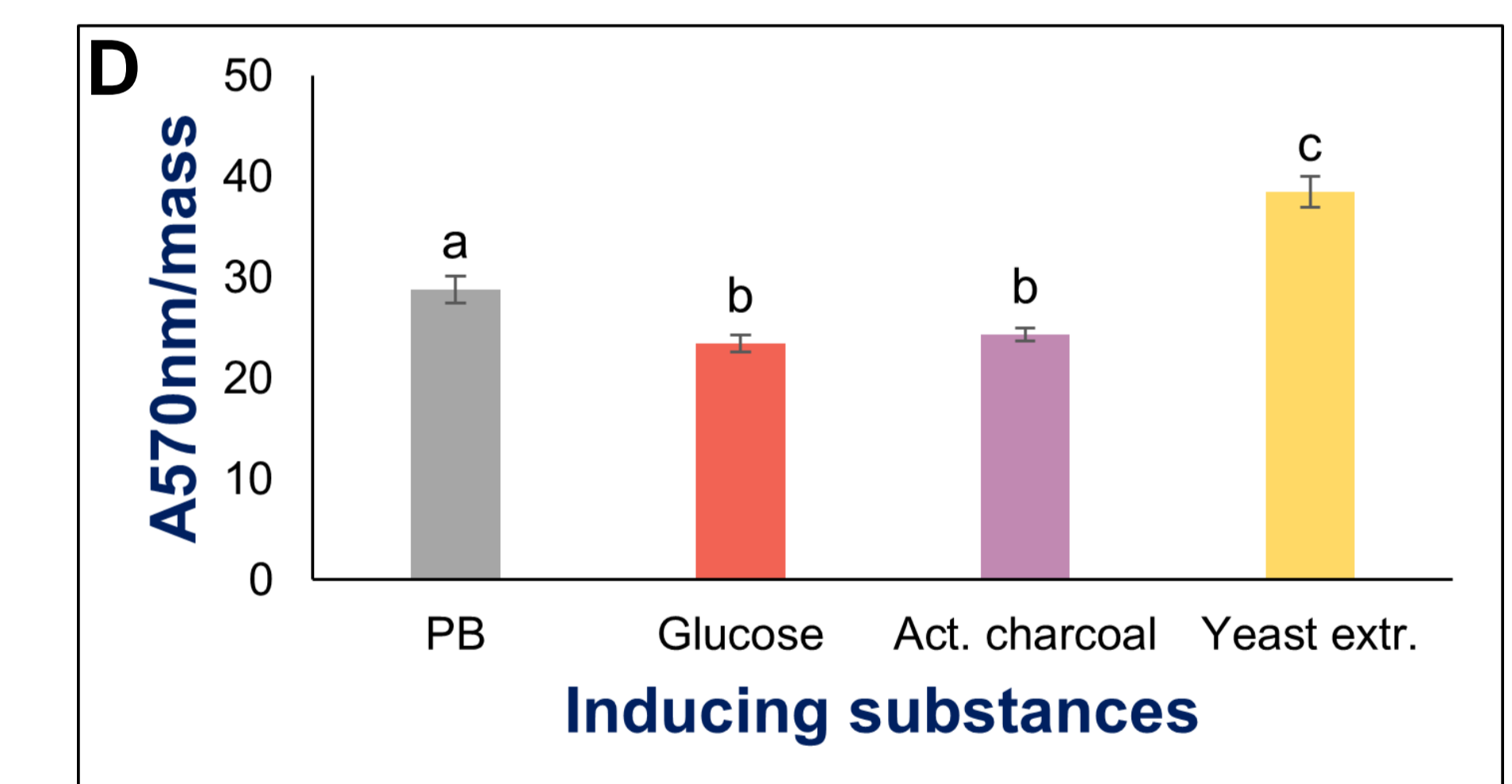
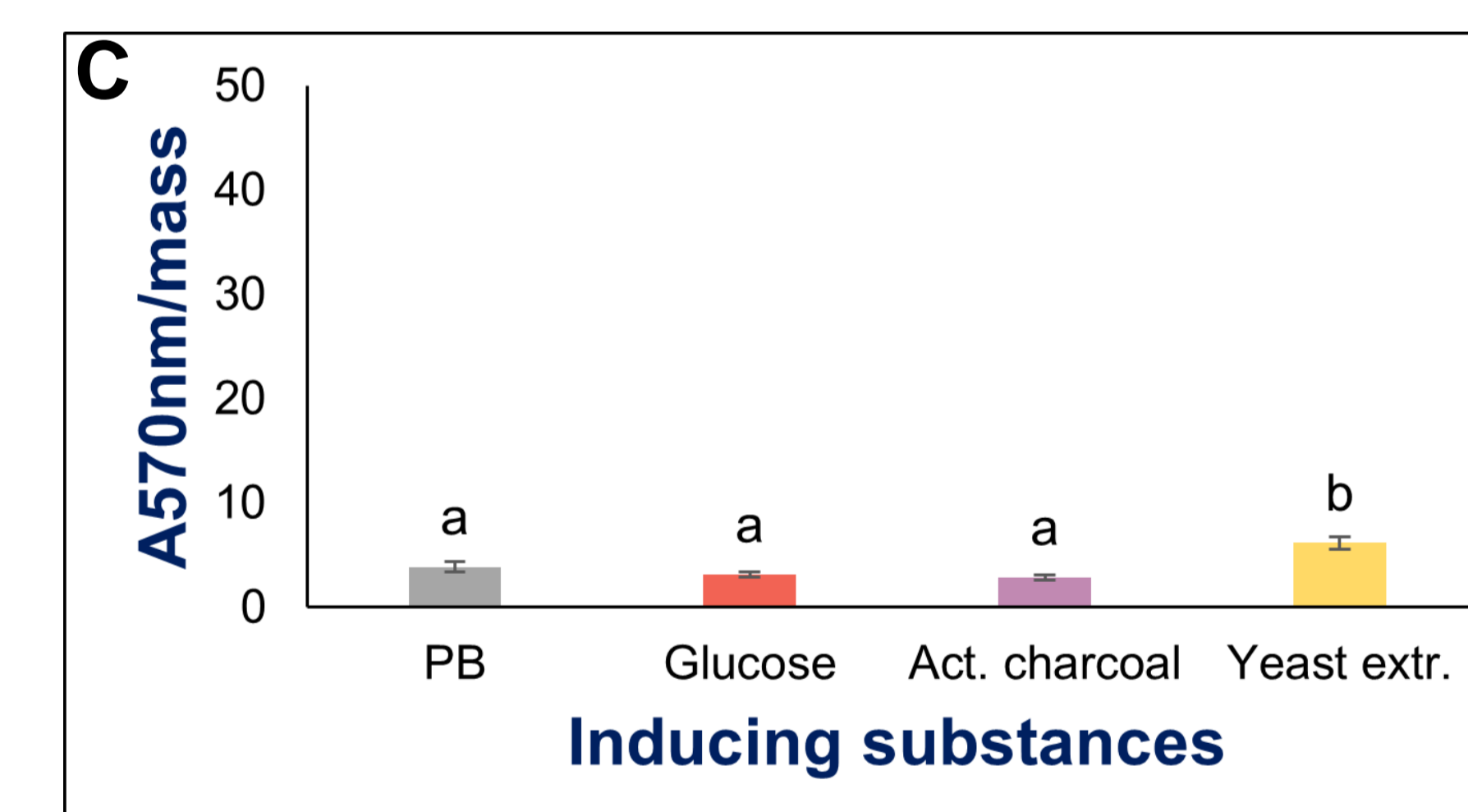
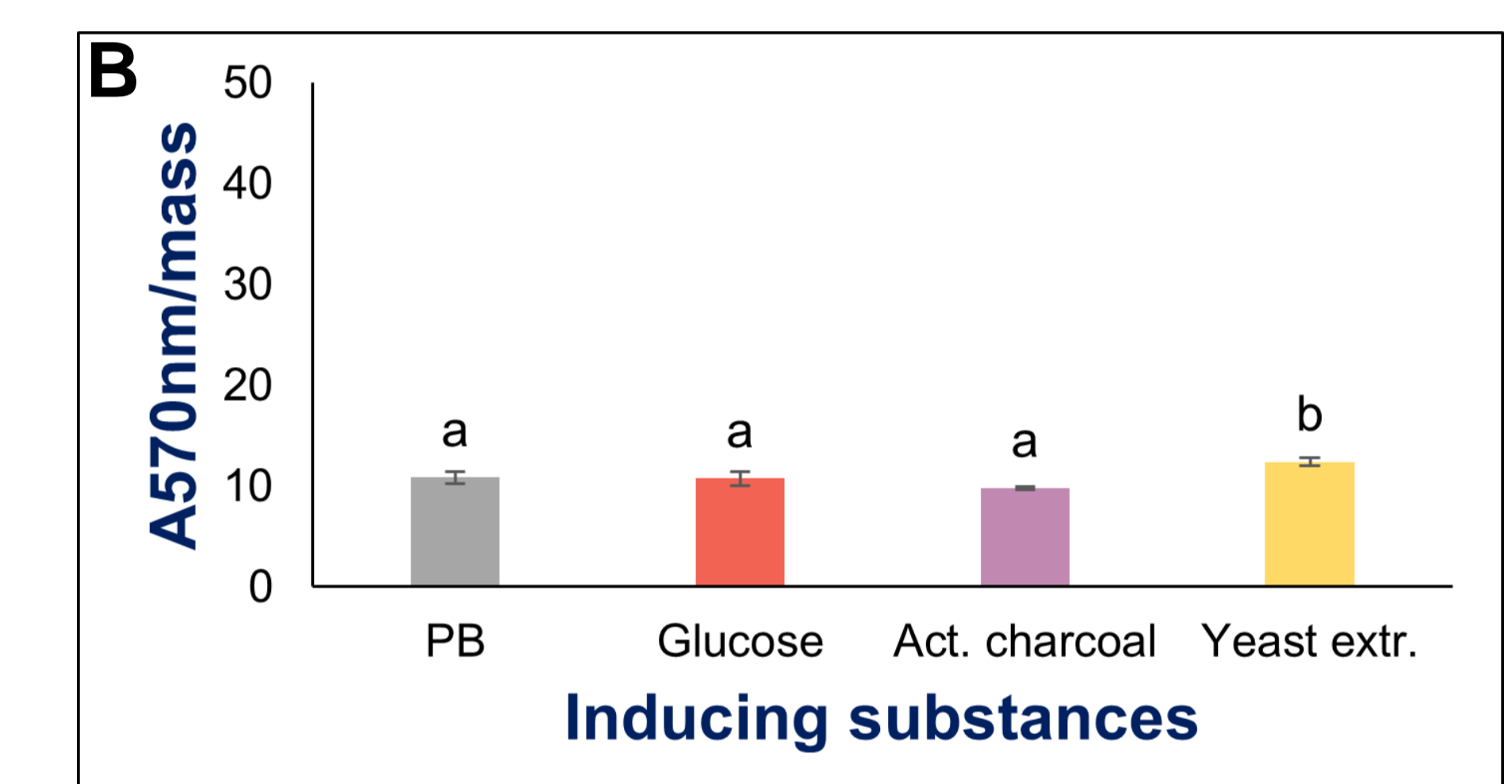
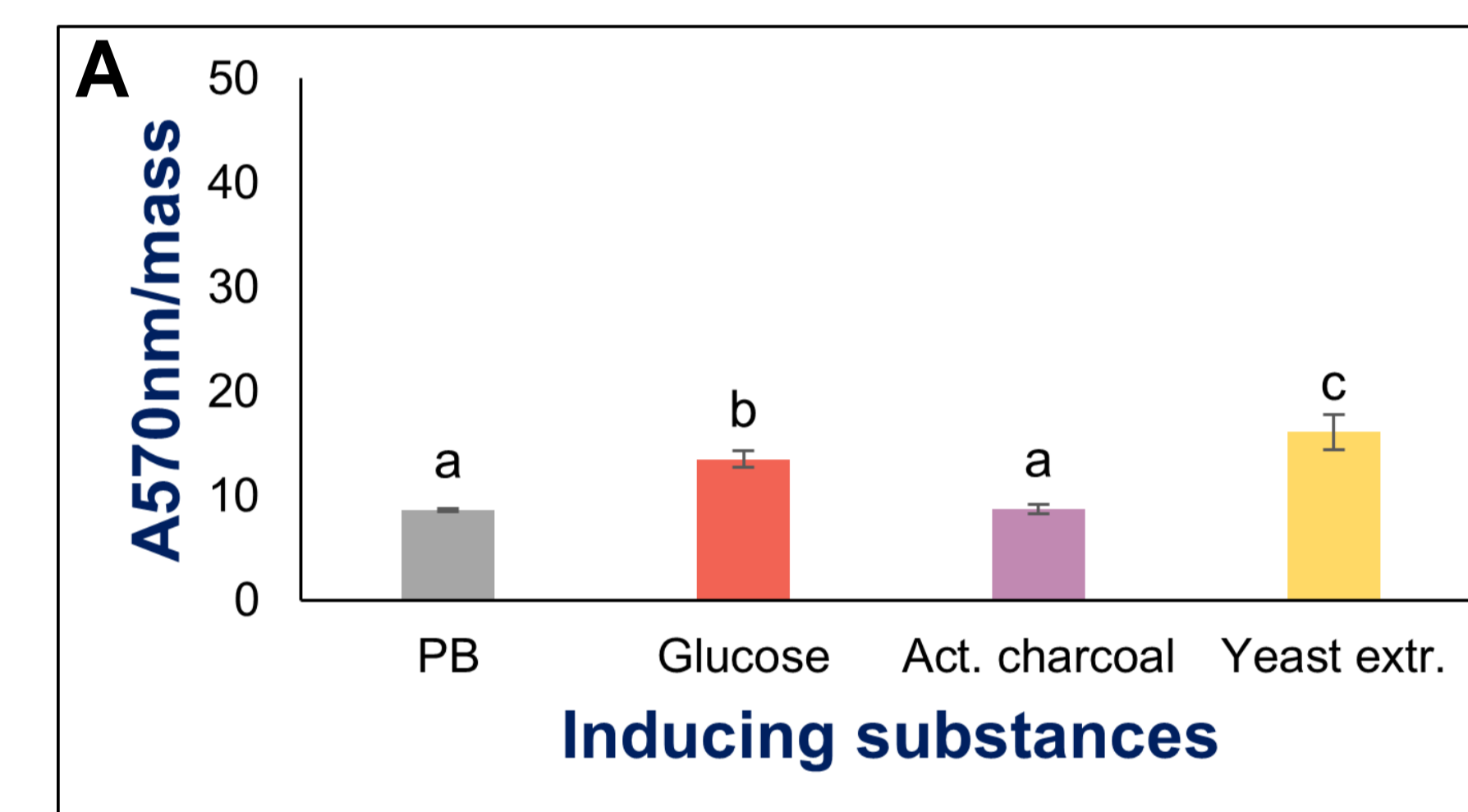


Figure 2: A – Box treatment; B – Fridge treatment; C – Frozen treatment; D – Room treatment. The yeast extract exhibited an activity-inducing effect in all conservation treatments, even in the frozen treatment that maintained the activity of spores at low levels. In the box treatment, glucose also induced the activity of spores. In contrast, glucose and activated charcoal had na inhibitory effect in the activity of spores in the room treatment.

Conclusions

Storage methods and inducing substances greatly impact the activity of EcM spores and represent a step towards the **optimization of the EcM inocula** as a biotechnological tool to help the commonly stressed urban trees in the everchanging environment of cities.

The use of effective EcM inocula can become an **important management practice for maintaining green infrastructures in cities**.

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