

Production of bioactive compound(s) with the marine fungus *Calcarisporium* sp.

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Introduction

Increasingly, unique bioactive compounds are being obtained from fungi isolated from marine environments. A *Calcarisporium* sp. (KF525) isolated from the German Wadden Sea has recently been described as producing interesting cyclic and linear polyesters (calcarides) with antibacterial activity (Silber et al. 2013a) and unrelated, unique calcaripeptides (Silber et al. 2013b).

Calcarisporium sp. KF525 is a slow-growing fungus ($\mu_{max} \sim 0.06 \text{ h}^{-1}$) and the calcarides are produced during the later stages of growth (or during stationary phase). Large amounts may accumulate in cultures allowed to stand for months, but this is not practical as a sustainable production method. Therefore, production of calcaride A was assessed in flask and bioreactor cultures (Fig. 1). These studies provide a basis for developing an improved production process, which could provide sufficient material for further bioactivity testing.

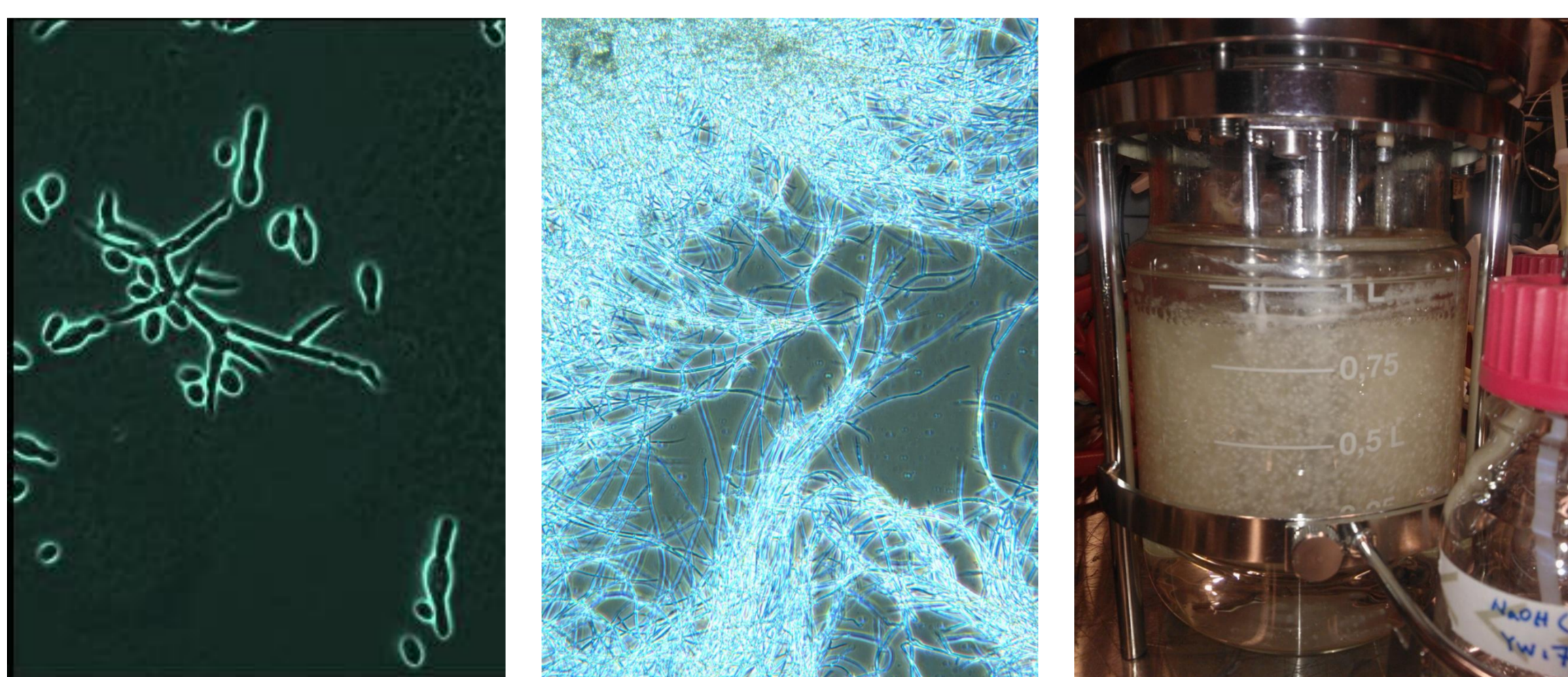


Fig. 1. *Calcarisporium* sp. KF525 (spores and mycelia, left) was grown in 1 L Biostat Q bioreactor (right), inoculated with 10% (v/v) pre-cultures as small pellets.

Results

- *Calcarisporium* sp. KF525 grew well on a variety of carbon sources.
- Calcaride A was produced well on sucrose, malt extract and starch, substrates which provide slow release of glucose (Fig. 2).
- Production was reduced or delayed on glucose and fructose (Fig. 2).
- Less calcaride A was produced from fructose than from sucrose in bioreactor cultures, and sucrose was used as carbon source in further experiments.
- *Calcarisporium* sp. KF525 produced $0.8 \pm 0.1 \text{ mg calcaride A [g biomass]}^{-1}$ in sucrose containing medium with C/N ~23.

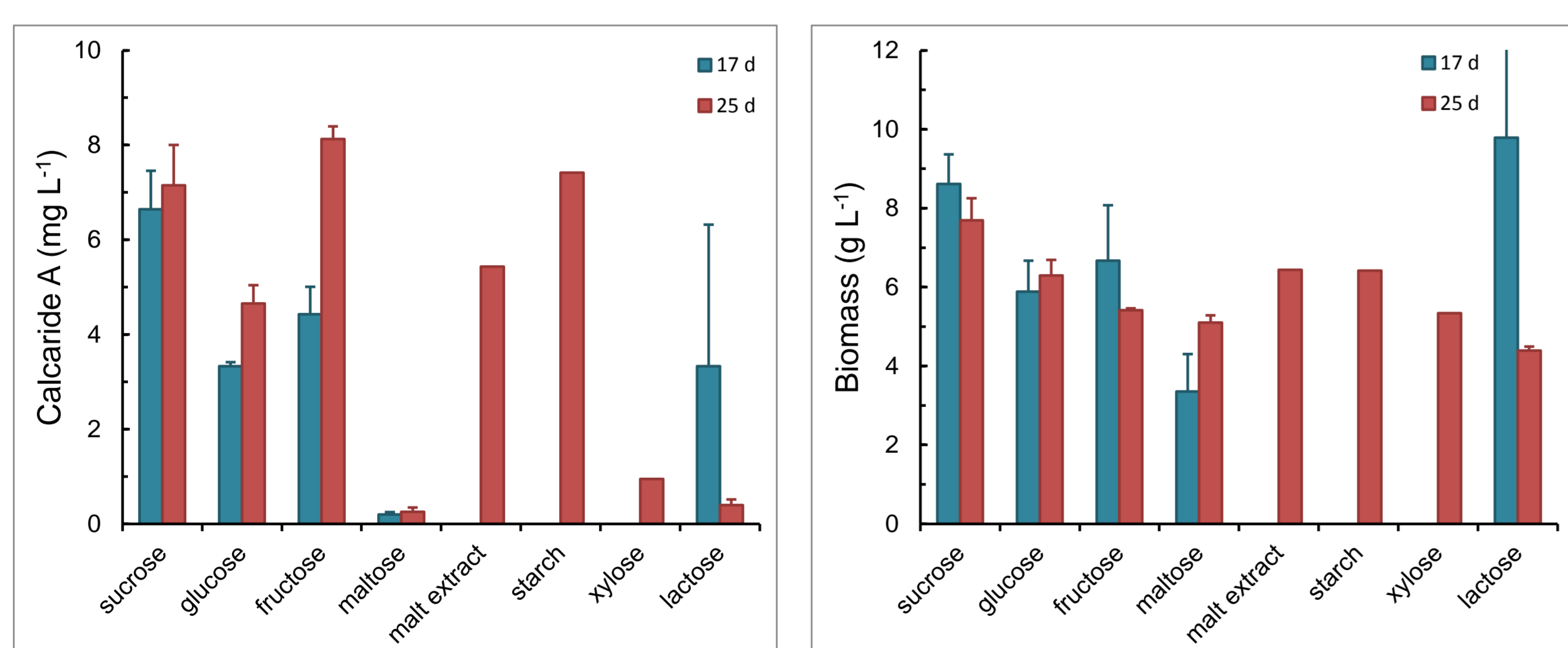


Fig. 2. Calcaride A and biomass production by *Calcarisporium* sp. KF525 in defined medium, flask cultures, pH ~5.3, C/N ~23, with 3% marine salt, 22 C.

References

1. Silber J, Ohlendorf B, Labes A, Erhard A, Imhoff JF. 2013a. Calcarides A-E, antibacterial macrocyclic and linear polyesters from a *Calcarisporium* strain. *Mar Drugs* 11, 3309-3323.
2. Silber J, Ohlendorf B, Labes A, Näther C, Imhoff JF. 2013b. Calcaripeptides A-C, cyclodepsipeptides from a *Calcarisporium* strain. *J. Nat. Prod.* 76, 1461-1467.
3. Vogel HJ. 1956. Convenient growth medium for *Neurospora* (Medium N). *Microb Genet Bull* 243, 112-119.

Results

- Calcaride A production was dependent on culture pH (Fig. 3).
- Production in cultures initially at pH 6.5 increased after the pH was reduced during growth (Fig. 3), but remained low in bioreactor cultures.

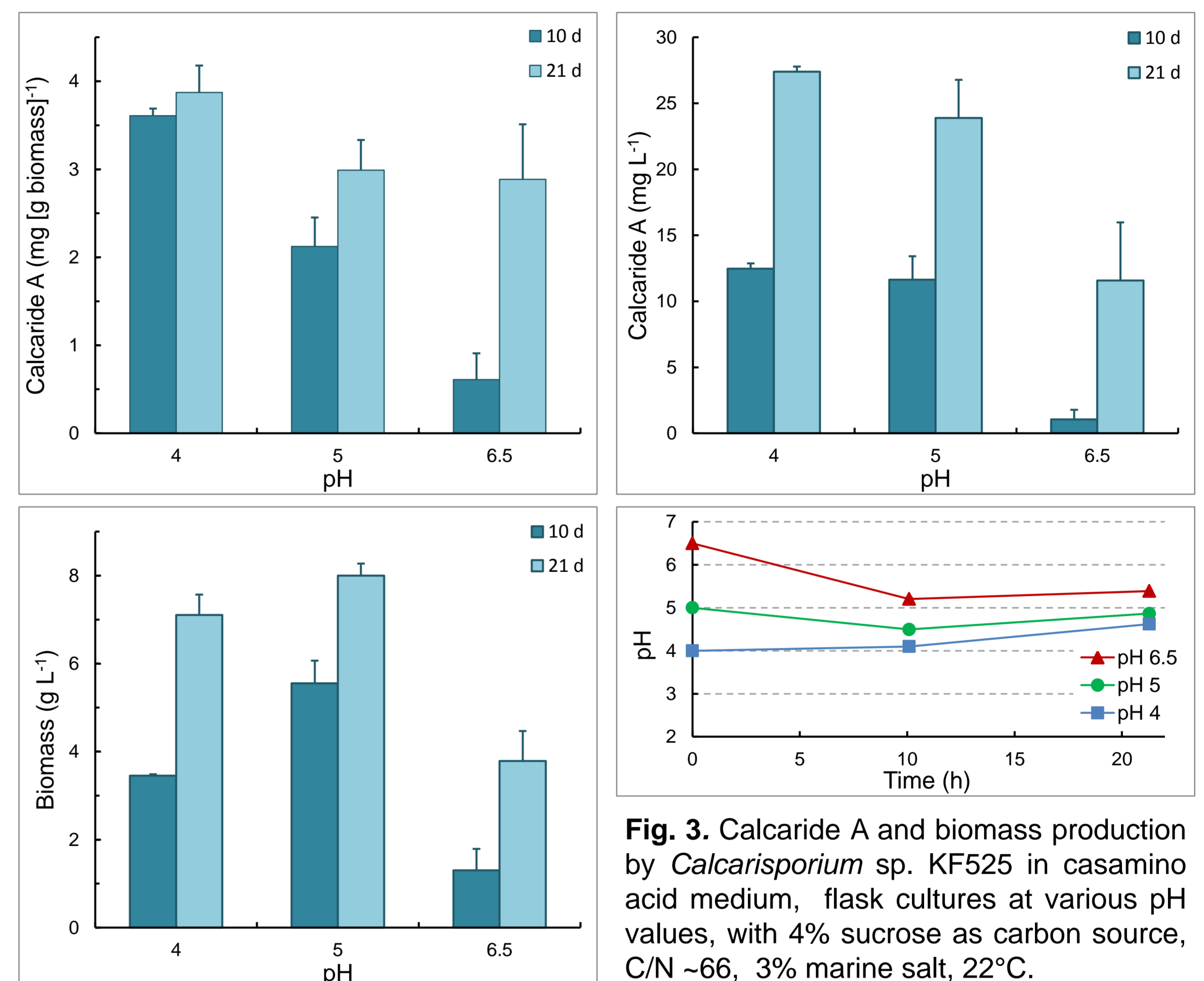


Fig. 3. Calcaride A and biomass production by *Calcarisporium* sp. KF525 in casamino acid medium, flask cultures at various pH values, with 4% sucrose as carbon source, C/N ~66, 3% marine salt, 22°C.

Results

- *Calcarisporium* sp. KF525 produced calcarides in stirred tank reactors, but specific production was generally lower than in flask cultures.
- Calcaride A production was reduced in the presence of excess nitrogen (Fig. 4).
- Maximum calcaride A production was obtained in < 14 days, depending on conditions.

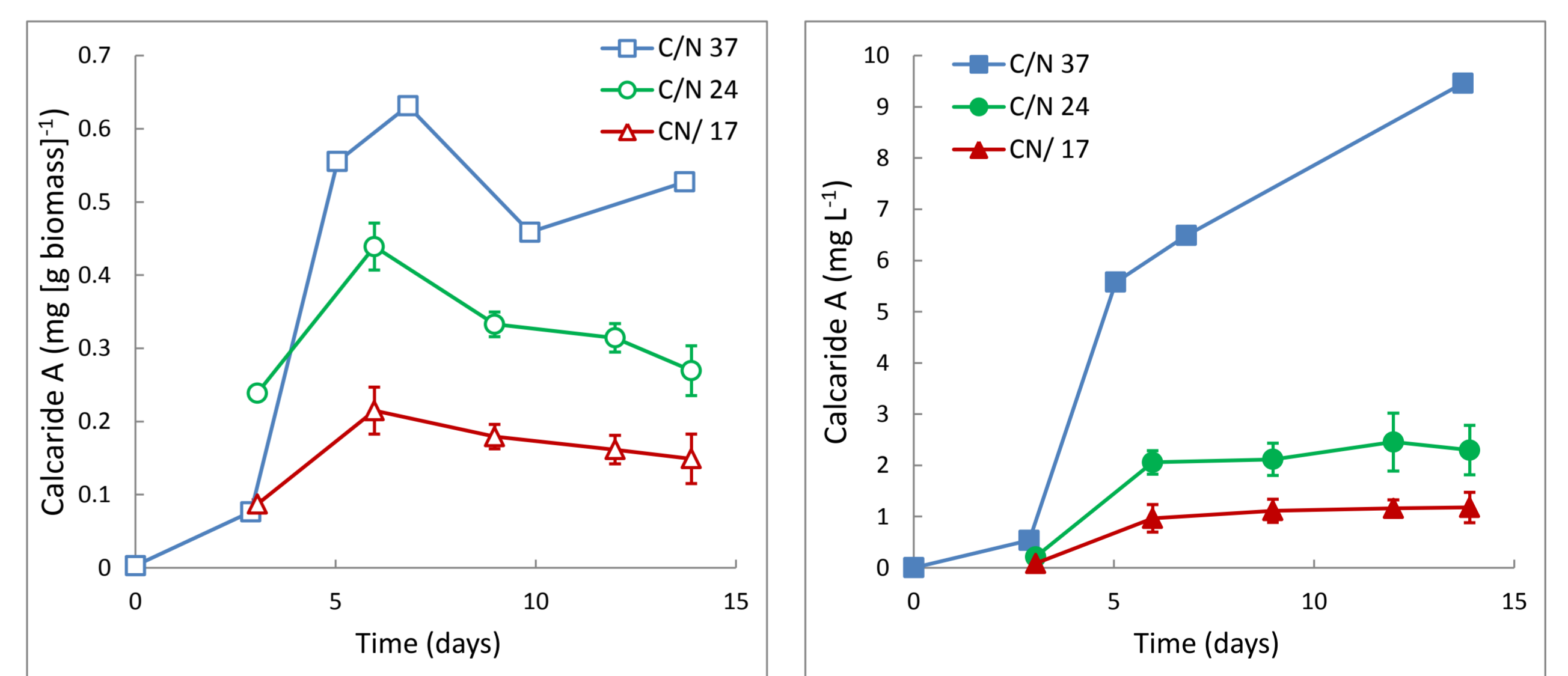


Fig. 4. Calcaride A production by *Calcarisporium* sp. KF525 in bioreactors, in defined medium at pH 5.3, with 4% sucrose as carbon source, 3% marine salt, 22°C. The C/N 37 culture was inoculated with more biomass than the other cultures.

Conclusion

- Calcaride A is produced by *Calcarisporium* sp. KF525 at acidic pH values in N-limited cultures.
- Production was partially inhibited in the presence of glucose.
- The time of maximum production was reduced from >3 weeks to < 2 weeks by ensuring optimal conditions for production.
- Production of calcaride A has been transferred from flasks to bioreactors.