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# Biofilm formation of *Staphylococcus lugdunensis* strains is associated with clonal lineage and dependent on iron availability

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## Background

*Staphylococcus lugdunensis* is a virulent coagulase-negative staphylococcal species<sup>1</sup>. The ability to form biofilm appears to be one of its main virulence factors<sup>2</sup>, and is promoted by iron-limitation condition<sup>3</sup>. Nevertheless, little is known about biofilm-associated genotypes.

In this context, the objective of this study was to compare the impact of iron restriction on **fitness** and **biofilm formation** of strains belonging to all clonal complexes (CCs) defined by Multilocus sequence typing<sup>4</sup> to date.

## Materials

### Strains

- The study included **49** clinical strains from **different clonal complexes** and **geographical origins** (French and Swedish cities) (Table 1)

Clonal complex	CC1	CC2	CC3	CC4	CC5	CC6	CC7	Singleton*
Number of strains	11	5	11	4	5	5	4	4
Geographical origins	Strasbourg, Rouen, Tours, Kronenberg, Nancy	Strasbourg, Montpellier, Nantes, Nancy, Rouen	Rouen, Strasbourg	Strasbourg, Rouen, Bordeaux	Strasbourg, Bordeaux, Rouen	Kronenberg, Nantes, Strasbourg, Rouen	Strasbourg, Rouen	Rouen, Strasbourg

Table 1: *S. lugdunensis* strains categorized by clonal complexes and geographical origins. \*Singleton Sequence Type (ST)

### Fitness

- Growth kinetics** over 24 hours performed in triplicates for all strains (Tecan spark®):

✓ In **rich media** (Tryptic Soy Broth [TSB])

✓ In **iron-restricted condition**

(RPMI 1640 with 1% casamino acids)

✓ Determination of **generation time** ( $G = \ln 2 / \mu$ ) and **maximal growth rate** ( $\mu$ ) (Figure 1)

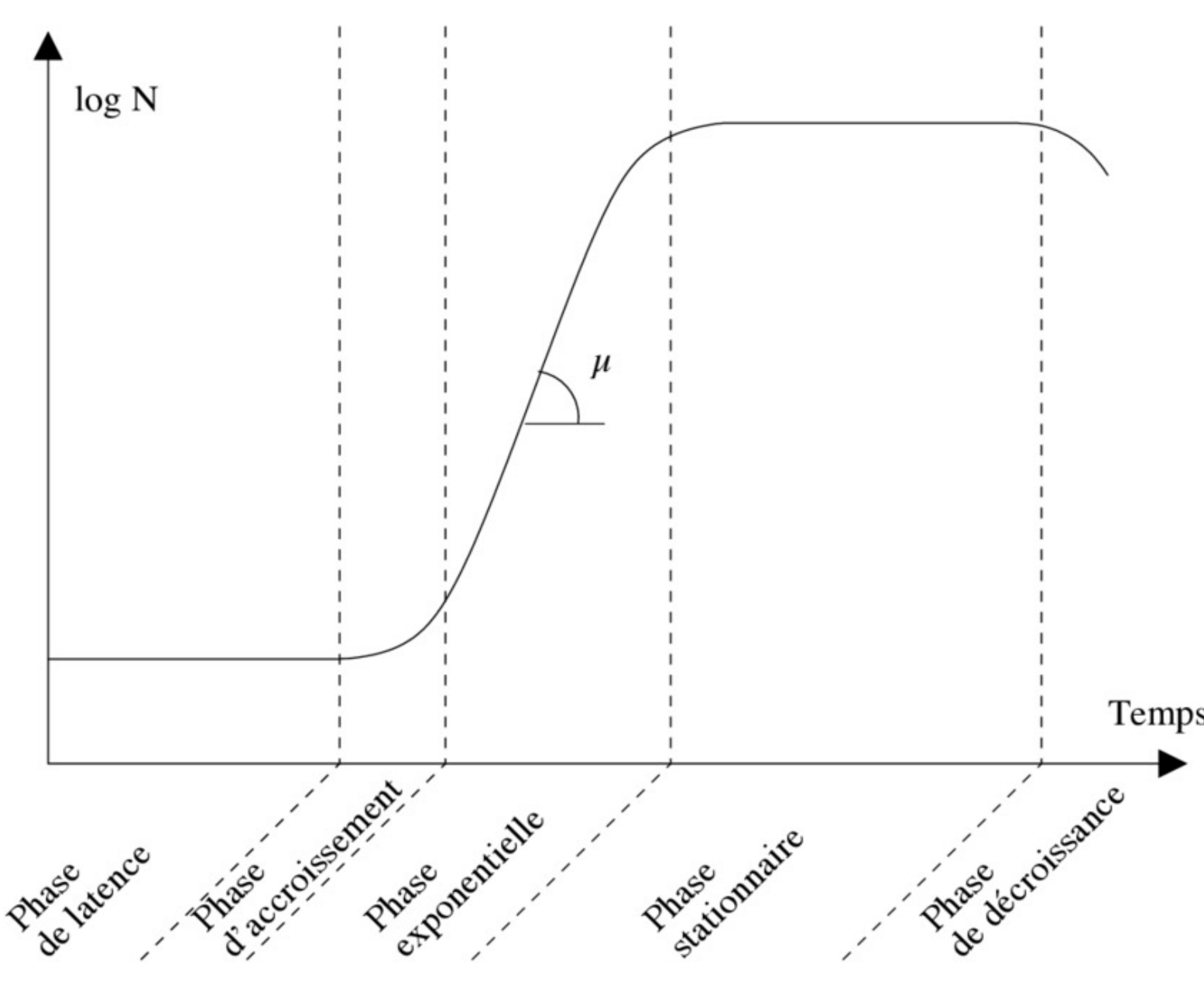


Figure 1 : Diagram of a growth curve

### Biofilm formation

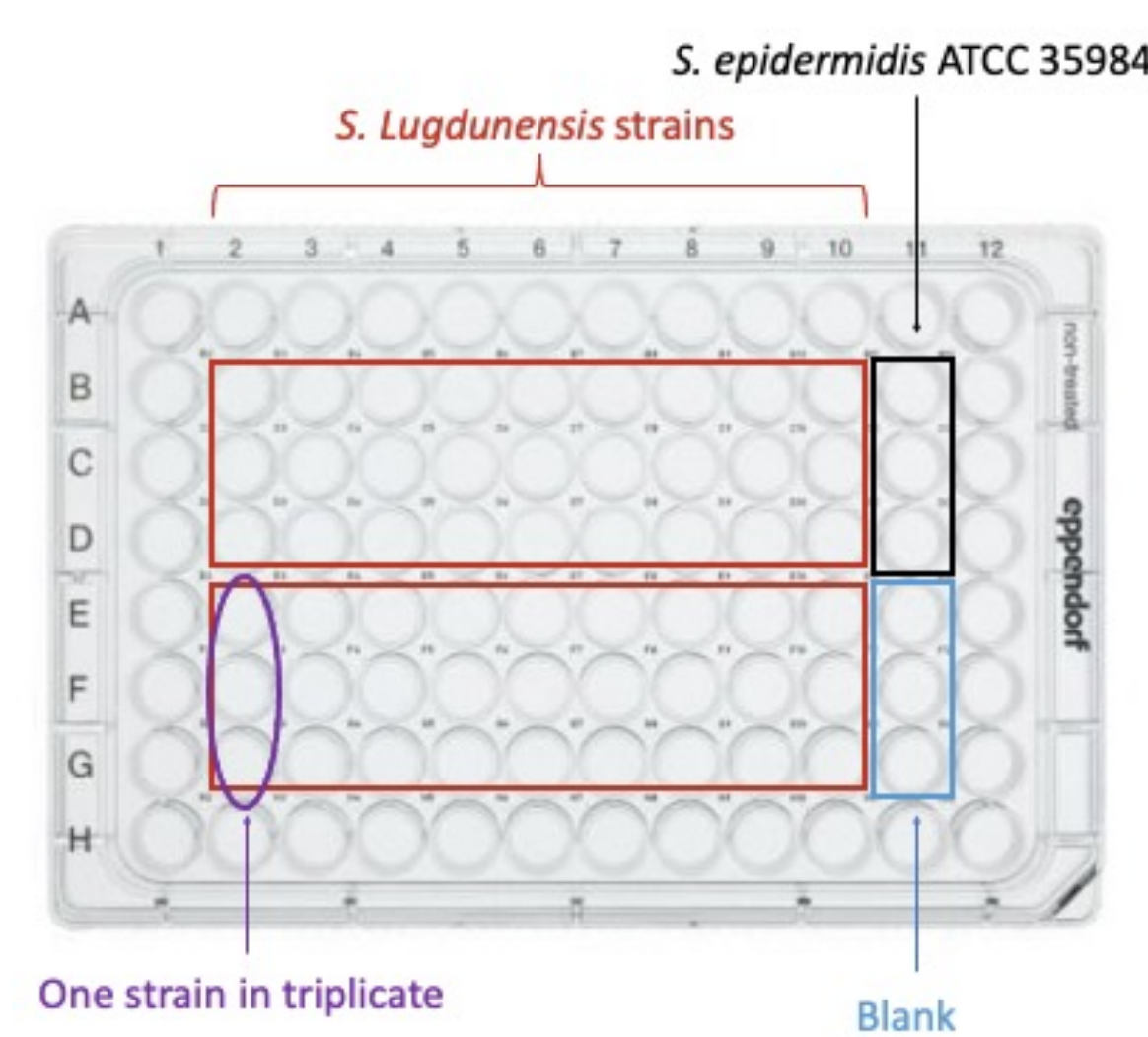


Figure 2: Biofilm formation on polystyrene microtiter plates

- 24h biofilm formation** studied using **crystal violet staining** method on 96-well polystyrene microtiter plates in quadruplicates:

✓ In **rich media** (TSB with 1% glucose) for all strains

✓ In **iron-restricted condition** for 34 strains (Figure 2)

✓ Using *S. epidermidis* ATCC 35984 as biofilm positive control

## Results

### Fitness

	Generation time (G)	Maximal growth rate ( $\mu$ )
<b>Rich medium (TSB)</b>	37.34 minutes $\pm$ 4.13	1.139 $\pm$ 0.132
<b>Iron restricted medium (RPMI + 1% CAS)</b>	80.72 minutes $\pm$ 33.14	0.634 $\pm$ 0.20

Table 2: Generation time and maximal growth rate of 49 *S. lugdunensis* strains in rich and iron restricted media

- No difference** in growth either according to the strains or according to the CCs, whatever the medium (Table 2)

### Biofilm formation

#### In rich medium

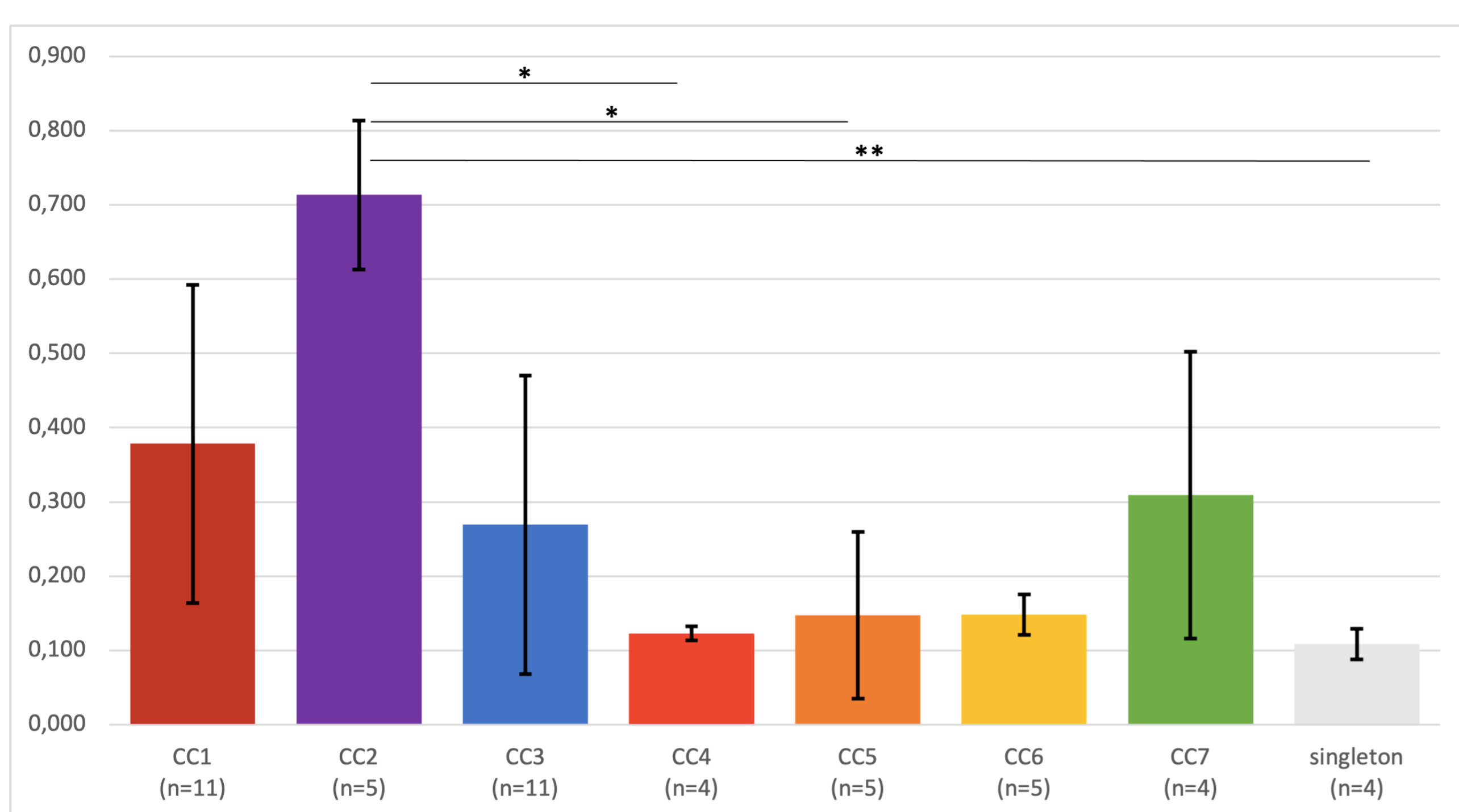


Figure 3: Biofilm formation of 49 *S. lugdunensis* strains after a 24h incubation at 37°C in rich medium (TSB + 1% glucose) according to the CC (OD mean values). Error bars represent the standard deviation within each CC.

- All strains were biofilm producers ( $DO_{590nm} > 0,1$ ) in rich medium after 24h
- CC2** strains produced significantly **more biofilm** than **CC4** ( $p = 0.0203$ ), **CC5** ( $p = 0.0215$ ) and **singleton STs** ( $p = 0.0045$ ) strains (Figure 3)

#### In iron-restricted conditions

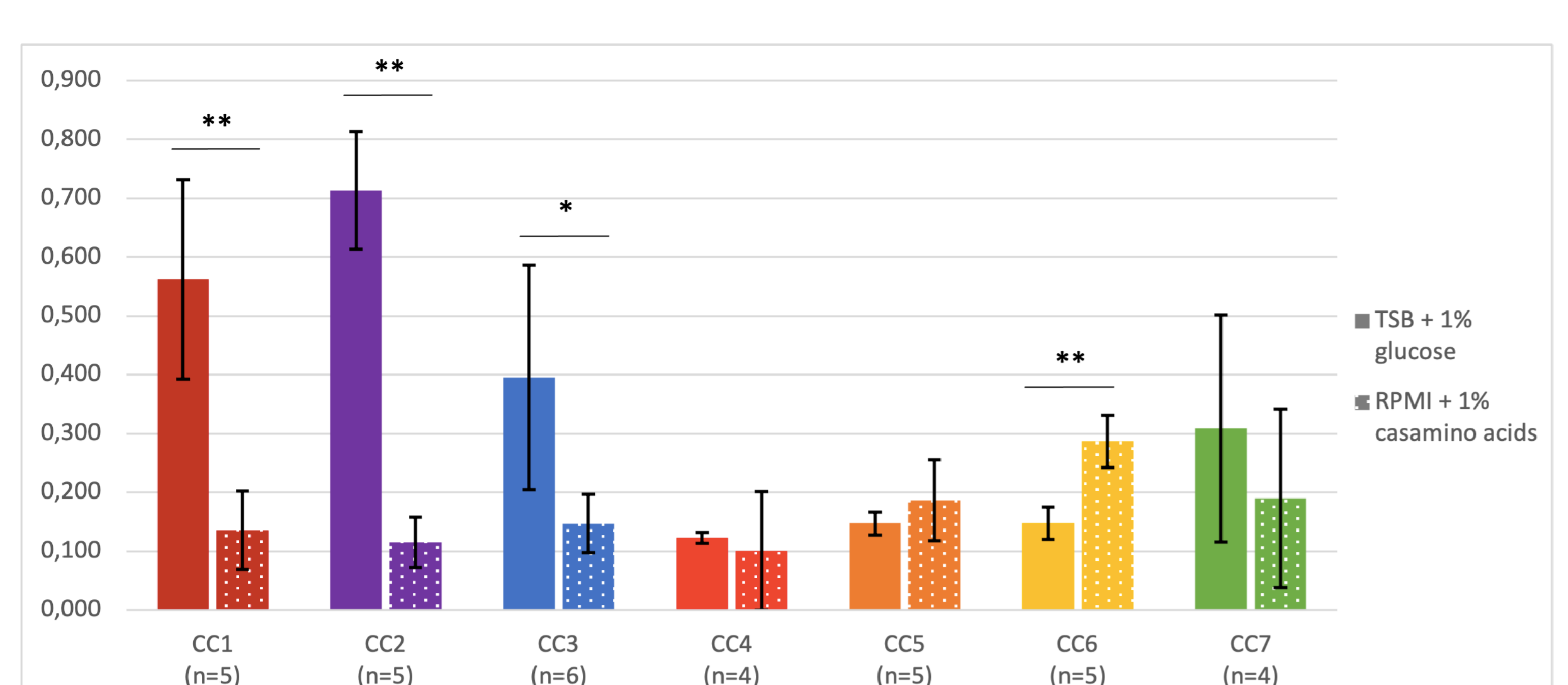


Figure 4: Biofilm formation of 34 *S. lugdunensis* strains after a 24h incubation at 37°C in rich medium (TSB + 1% glucose [full bars]) or iron-restricted medium (RPMI + 1% casamino acids [hatched bars]) according to the CC (OD mean values). Error bars represent the standard deviation within each CC.

- In **iron-restricted conditions**, **CC1** ( $p = 0.0079$ ), **CC2** ( $p = 0.0079$ ) and **CC3** ( $p = 0.026$ ) strains produced significantly **less biofilm** than in rich medium
- On the contrary, **CC6** ( $p = 0.0079$ ) strains were significantly **more productive** (Figure 4)

## Conclusions

- This study shows for the first time that the **biofilm-forming ability** of *S. lugdunensis* strains is strongly **correlated with CC affiliation**.
- In addition, two different **CC-dependent behaviors** are observed in an **iron-restricted environment**. These results are to be confirmed with a larger number of strains.