Investigation into the Mechanism of Capsular-type Switching and its Influence on Antibiotic Susceptibility in *Acinetobacter baumannii* OCU_Ac16b

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異分野研究交流会

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Introduction

Acinetobacter baumannii OCU_Ac16b is a strain isolated from a patient at our university hospital[1]. When grown on solid media, this strain exhibits two distinct phenotypes: the L-type, forming relatively large colonies, and the S-type, forming smaller colonies. We previously found that the L-type has an extremely thick capsule, which we hereafter call a hypercapsule, whereas the S-type has either no capsule or a much thinner one (Fig. 1). Interestingly, L-type cells easily convert to the S-type during cultivation in liquid media, while switching from the S-type to the L-type has not been observed (Fig. 2). This study aims to characterize these two phenotypes more deeply and to unravel the mechanism behind this capsular-type switching.

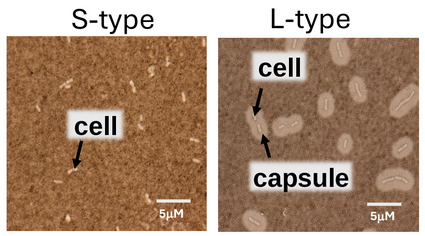

Fig. 1: The difference in capsule thickness between the S- and L-types. Cells were observed under a microscope after India ink-negative staining.

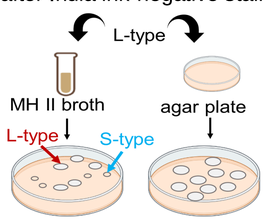

Fig. 2: Observation of the switching from L-type to S-type during cultivation in liquid media.

Methods

1. Seven separately isolated S-type variants were subjected to genome sequencing (one using PacBio Sequel IIe and Illumina HiSeq, and the others using only Illumina HiSeq).
2. We examined the growth rates and fitness of L-type and S-type cells at shaking speeds of 60 rpm and 180 rpm at 37°C.
3. The effect of low oxygen conditions was tested using an AnaeroPack (microaero).
4. Antimicrobial susceptibility tests were performed using the Etest.

Results

1. Genome Sequencing

All genome-sequenced S-type strains were found to have a mutation in the capsular polysaccharide synthesis gene cluster (Fig. 3), suggesting that these mutations play a direct role in capsule-type switching.

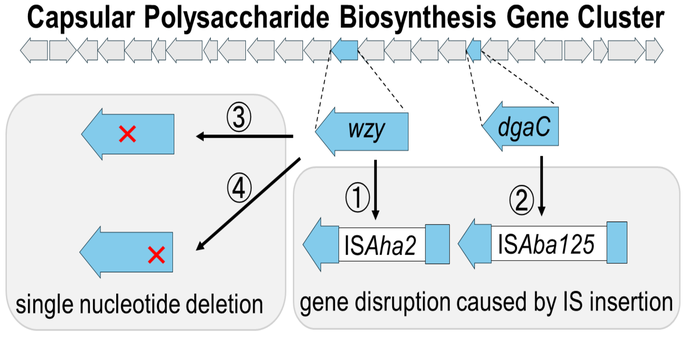

Fig. 3: Mutations identified in the capsular polysaccharide synthesis gene cluster of the S-type variants.

2. Time Course of Capsule-type Switching and the Influence of Shaking Speed

When we tested the effect of shaking the culture at high, medium and low rates, capsule-type switching was observed when medium and low shaking speed was used. A competition assay between S- and L-types under different shaking speeds revealed that the S-type has a growth advantage when shaken slowly (Fig. 4).

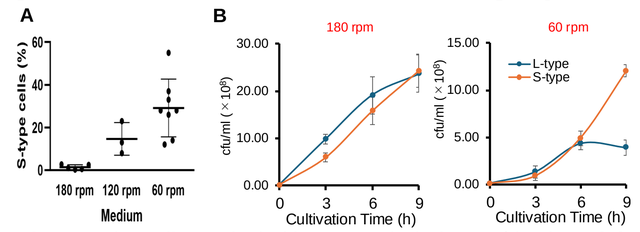

Fig. 4: The effect of shaking speed during cultivation. **(A)** Investigation of the conversion to S-type at different shaking speeds. **(B)** The fitness of each type evaluated by a competition assay.

3. The Effect of Oxygen Concentration

When oxygen concentration was lowered with an AnaeroPack, switching from L-type to S-type was observed even with shaking at 180 rpm. Higher fitness of the S-type compared to the L-type was confirmed in a competition assay under low oxygen concentration (Fig. 5).

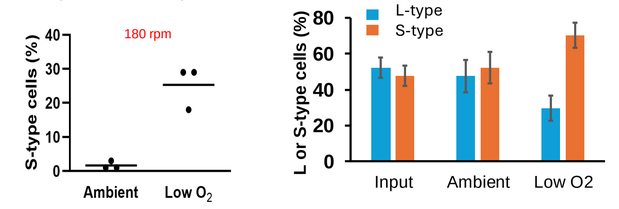

Fig. 5: Effect of limiting oxygen with Anaero-Pack. **Left**: The ratio of L- and S-types after cultivating the L-type for 24 h at 180 rpm. **Right**: Competition assay results.

4. Hypercapsule of L-type Contributes to Antimicrobial Resistance

The L-type exhibited higher resistance to the β-lactam antibiotics, imipenem and cefepime, than the S-type (Table 1), suggesting that the hypercapsule contributes to increased antimicrobial resistance.

	β-lactam antibiotics						Tetracycline		New quinolone	Amino glycosides		Other
	ABPC	PIP	CAZ	FEP	IPM	MEM	MIN	TGC	LVX	KM	AMK	CST
ATCC 19606	>256	16	4	8	0.25	0.25	0.064	1	0.25	8	16	0.094
L-type	>256	>256	>256	>256	16	>32	0.064	0.25	0.5	4	4	0.125
S-type	>256	>256	>256	64	8	>32	0.047	0.25	0.5	4	4	0.094

Table 1: Results of antimicrobial susceptibility testing using the Etest.
ABPC: ampicillin. PIP: piperacillin. CAZ: ceftazidime. FEP: cefepime. IPM: imipenem. MEM: meropenem MIN: minocycline. LVX: levofloxacin. KM: kanamycin. AMK: amikacin. CST: colistin. TGC: tigecycline

Conclusions

This study revealed that capsule-type switching is likely driven by different kinds of mutations. We additionally found that the shaking speed significantly affects the switching, presumably because a slower shaking speed results in lower dissolved oxygen concentration. Furthermore, antimicrobial susceptibility tests revealed the possible role of the hypercapsule in antimicrobial resistance. Future research should aim to uncover the precise mechanisms underlying these observations.

Reference

1. Oinuma K, Suzuki M, Sakiyama A, Tsubouchi T, Saeki, K, Sato K, Niki M, Yamada K, Shibayama K, Kakeya H, Kaneko Y. Genomic characterization of triple-carbapenemase-producing *Acinetobacter baumannii*. JAC-Antimicrobial Resistance, 2021;3:dlab191.