



**Fig. 8.** (A) Measurement of ammonium production from the tested staphylococci on Christensen urea medium (333 mM urea/ 0.1% peptone/0.5% NaCl/0.1% glucose/20 mM Na/K phosphate buffer adjusted to pH 6.5). O/N culture (1/100 vol) was inoculated into Christensen urea medium and incubated at 37°C under static cultivation. The generated ammonium ion was measured by indophenol blue reaction described as below. Ten microliters of the culture supernatant was diluted with 3 ml of PBS (pH7.4) and mixed well with 3  $\mu$ l of 3 mM MnCl<sub>2</sub>, 75  $\mu$ l of 1% sodium hypochlorite solution, and 90  $\mu$ l of phenate reagent (2.5% NaOH and 10% phenol). The mixture was incubated at room temperature for 10 min to complete the reaction. The color of indophenol was measured at OD<sub>630</sub>. Concentration of ammonium ion was calculated with the NH<sub>4</sub>Cl standard solution ranging from 0.04 to 5 mM. *Staphylococcus saprophyticus* ATCC 15305 shows a significant high ammonium production within 2-h incubation. Ammonium production on the *Staphylococcus epidermidis* culture can be detected after 18-h incubation (not shown). (B) The growth curve on Christensen urea medium together with the measurement of ammonium ion (A). The growth of *S. saprophyticus* ATCC 15305 was transiently attenuated after 2-h incubation to alkaline pH 8.8, whereas the culture pH of *Staphylococcus aureus* N315 was not significantly changed from initial pH 6.5 at 2-h incubation despite well growing likewise *S. saprophyticus*. (C) The potent urease inhibitor acetohydroxamic acid inhibits the ammonium production by the mid-log phase cell lysate, suggesting that the specific urease activity contributes to the production in *S. saprophyticus*. (D) A positive reaction producing ammonium ion on Christensen urea agar was detected within 2 h in *S. saprophyticus* ATCC 15305.