Colorless agar for enhanced color contrast between microbe colonies and solid medium

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New in this version

Language and logic flow was improved in this version.

Conflicts of interest

The authors declare no conflicts of interest.

Authors’ contributions

Wenfa Ng conceived the idea, designed and performed the experiments, analyzed the data, and wrote the abstract. Yen-Peng Ting mentored Wenfa Ng, as well as discussed and analyzed the data.

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Abstract

Solid medium enables spatially resolved simultaneous cultivation of different microbes in a simple and relatively low cost format useful for preliminary screening of microorganism diversity. Lack of color contrast between colonies and agar, however, hampers colony identification by automated image analysis – thus, presenting a challenge for phenotype screening experiments and viable cell counting. Since microbes secrete pigments of myriad hues, this research sought to develop a colorless agar - which when placed on colored paper of suitable hue – would enhance the color contrast between agar and colonies of any color. However, realization of the concept is hampered by heat-induced formation of colored compounds between medium components during autoclave sterilization, which in this study, was prevented by dissolving glucose and ammonium chloride in two separate solutions (each containing other medium components). Mixing the two sterilized solutions at 48 °C yielded a colorless agar – which remained colorless even after adding sterile yeast extract (max: 1 g/L) for providing essential vitamins to microbes unable to synthesize them. Culture experiments revealed good growth of *Escherichia coli* DH5α (ATCC 53868), *Pseudomonas protegens* Pf-5 (ATCC BAA-477), *Pseudomonas aeruginosa* (ATCC 15442), and *Bacillus subtilis* (ATCC 8473) with cell yield positively correlated with yeast extract concentration. In addition, identical viable cell concentration and colonies of similar morphologies were observed on both the colorless and LB agar; thus, suggesting that no inhibitory compounds were formed during agar preparation. Collectively, using commonly used buffer components as well as salts and nutrients, a colorless agar was prepared by segregating chromogenic compounds during heat sterilization; thus, opening up its potential use for enhancing color contrast between colonies and agar in revealing finer details of pure culture colonies, or more accurate automated colony identification and counting in screening and viable cell count assays.

**Keywords:** color contrast; colorless; automated cell counting; viable cell count; agar medium;

**Subject areas:** Microbiology; Bioengineering; Environmental Sciences;