Colourless agar for enhanced colour contrast between microbial colonies and solid medium

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Conflict of Interest

The authors declare no conflict of interest.

Author's contributions

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

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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 microbial diversity. Lack of colour 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 colourless agar - which when placed on coloured paper of suitable hue – would enhance the colour contrast between agar and colonies of any colour. Nevertheless, the concept is confounded by formation of coloured compounds between medium components during autoclave sterilisation, which, in this study, was prevented by dissolving glucose and ammonium chloride in two separate solutions containing other medium components. Upon mixing the two solutions after heat sterilisation, a colourless agar was obtained – which remained colourless even after adding sterile yeast extract (max: 1 g/L) for providing essential vitamins to microbes unable to synthesise 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. Additionally, identical viable cell concentration and colonies of similar morphologies were observed on both the colourless and LB agar; thus, suggesting that no inhibitory compounds were formed during agar preparation. Collectively, using commonly used buffer components, salts and nutrients, a colourless agar was prepared by segregating chromogenic compounds during heat sterilisation; thereby, opening up its potential use in enhancing colour contrast between colonies and agar medium for revealing finer details of pure culture colonies, or more accurate automated colony identification and counting in screening and viable cell count assays.

Keywords: colour contrast; colourless; automated cell counting; viable cell count; agar medium

Subject areas: Microbiology; Bioengineering; Environmental Sciences

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