Journal of Microbiological Methods 181 (2021) 106148

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

The influence of agar brands and micronutrients in the growth optimization of *Granulicella* sp. (*Acidobacteriota*)

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ARTICLE INFO

Keywords:
Agar brand
Microbial growth
Acidobacteriota
Micronutrients
Metals

ABSTRACT

Acidobacteriota are highly abundant in soils, however, few cultured representatives are available. The purity of the reagents can influence microbial growth in laboratory conditions and successful isolation. Here we investigated the impact of different agar brands in culture medium and advocate that agar origin should be carefully considered for *Acidobacteriota* strains growth and microbial isolation.

One of the major hurdles of studying bacteria of phylum *Acidobacteriota* is their isolation and propagation in culture media. Despite their high abundance and ubiquity in different environments, especially soils, few representatives have been cultured. They are slow growers, usually taking weeks to months to develop colonies (de Castro et al., 2013; Eichorst et al., 2011). To date, 62 species have been described, compared to only 14 species in 2011 (de Castro, 2011). The number of species of *Acidobacteriota* and isolates has recently increased due to changes in traditional culture methods and the use of unconventional culture media composition. Media modifications include low concentration of nutrients (Janssen et al., 2002; Stevenson et al., 2004), unusual or complex polysaccharides as carbon sources (Eichorst et al., 2011; Pankratov et al., 2008), longer incubation periods (de Castro, 2011), addition of humic acids and quorum-sensing molecules (Stevenson et al., 2004), and the application of soil solutions and inhibitors for unwanted microorganisms (de Castro et al., 2013; Foesel et al., 2013). Although the modifications allowed the isolation of new *Acidobacteriota* genera, another factor that can influence microbial colony development in laboratory conditions is the purity of the reagents, since minor differences in medium composition can impact microbial growth (Atlas, 2010). In this context, few studies addressed impact of the differences in composition between agar brands in microbial growth and no study has addressed those issues in the growth of *Acidobacteriota* genera. Therefore, here we evaluated the impact of three different agar brands (Bacto TM Agar BD 214010 - standard quality, detrimental ions such as iron and copper are reduced - (BD, EUA); Sigma Agar A1296-500 Agar-Agar - purified agar - (Sigma-Aldrich, EUA) and Fluka Agar 05038–500 - highly purified, essentially free of impurities - (Sigma-Aldrich, EUA)) on the colony development of two strains belonging to class *Acidobacteriaceae* (subdivision 1), *Granulicella* genus (5B5 and WH15). Both strains are Extracellular Polymeric Substances (EPS) producers (Kielak et al., 2017) and belong to the culture collection of the Netherlands Institute of Ecology (NIOO-KNAW), Department of Microbial Ecology. *Granulicella* genus 5B5 and WH15 strains were isolated from wood in advanced decay stage, in association with the white-rot fungus *Hypholoma fasciculare*, in the Netherlands (Valášková et al., 2009). The optimized culture medium PSYL5 was used in this study. PSYL5 medium was composed of (g/L): 1.8 KH_2PO_4, 0.2 $MgSO_4.7H_2O$, 30 sucrose and 1.0 yeast extract; pH adjusted to 5.0 (Campanharo et al., 2016), with 20.0 g/L of each respective agar added individually. Assays were performed in 90 mm polystyrene petri dishes. Plates were inoculated with 100 μL of cells at OD_{600nm} = 1 nm/mL, homogenized and incubated at 20 °C and 30 °C for 15 days, when growth and EPS production at both temperatures were visually evaluated. All experiments were executed in duplicates.

Our results showed that *Granulicella* strain 5B5 was not able to grow on Sigma Agar A1296-500, which also poorly supported the growth of *Granulicella* strain WH15. Fluka Agar 05038–500 supported the growth of both strains, but results demonstrated the *Granulicella* strains performed the best in BD agar (Fig. 1), and was not temperature-dependent.

The metal ion composition of the different agar brands has been previously addressed by Bosmans et al. (Bosmans et al., 2016). The

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https://doi.org/10.1016/j.mimet.2021.106148
Received 27 November 2020; Received in revised form 4 January 2021; Accepted 12 January 2021
Available online 20 January 2021

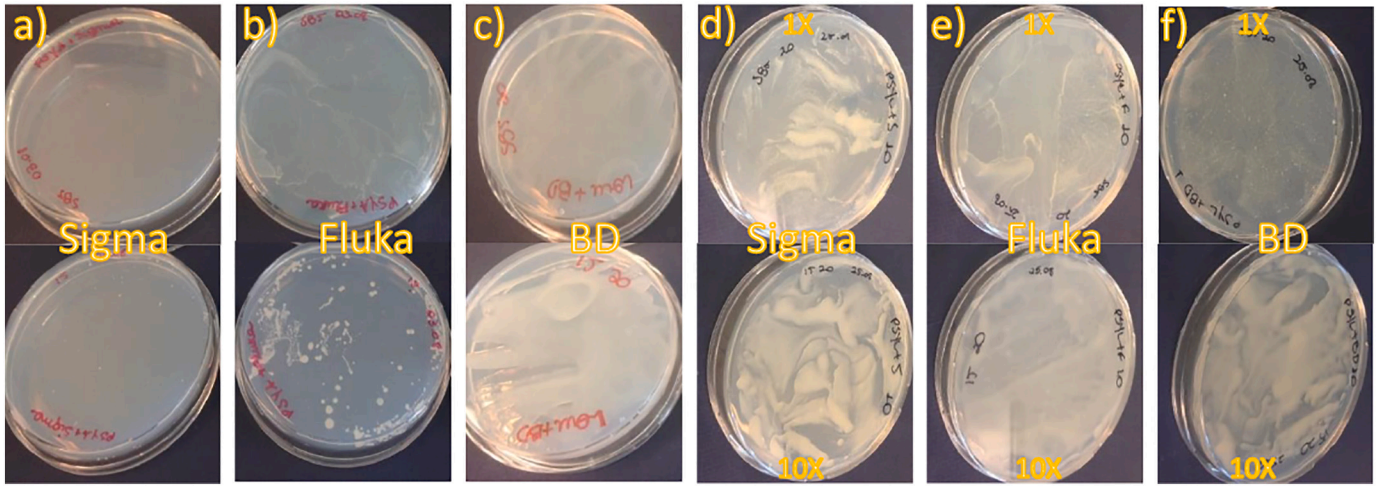

Fig. 1. Growth of *Granulicella* strains 5B5 (top) and WH15 (bottom) in PSYL5 culture medium containing different agar brands after incubation for 15 days at 20 °C. a) Sigma agar, b) Fluka agar c) BD agar. Growth of *Granulicella* strains 5B5 (top) and WH15 (bottom) after the addition of trace element solution and incubation for 15 days at 20 °C. d) Sigma agar, e) Fluka agar, f) BD agar; 1×: trace element solution SL10 1 mL/L; 10×: trace element solution SL10 10 mL/L.

Table 1
Summary of *Granulicella* strains growth and EPS production in culture medium containing different agar sources at different concentrations of SL10 solution in two temperatures.

	20 °C		30 °C	
	WH15	5B5	WH15	5B5
Agar brand	**PSYL 5 (growth/ EPS)**	**PSYL5 (growth/ EPS)**	**PSYL 5 (growth/ EPS)**	**PSYL5 (growth/ EPS)**
Sigma	+/+	−/−	–	–
Sigma+SL10 1×	+++/+	+/+	+++/+	+/+
Sigma+SL10 10×	+++/+++	++/+	+++/+++	+++/++
Fluka	++/++	+/+	++/++	+/+
Fluka+SL10 1×	+++/++	++/+	+++/+	+++/+
Fluka+SL10 10×	+++/++	++/+	+++/++	+++/+
BD	+++/++	+++/+	+++/+++	+++/+
BD + SL10 1×	+++/++	+++/+	+++/+++	+++/+
BD + SL10 10×	+++/++	+++/++	+++/+++	+++/++

SL10 1×: trace element solution SL10 1 mL/L; SL10 10×: trace element solution SL10 10 mL/L. +:<100 bacterial colonies/low EPS production, ++: <500 bacterial colonies/moderate EPS production; +++: uncountable number of bacterial colonies/abundant EPS production. The visual comparison was performed using the Bacto TM Agar BD.

products used in the current study had varied metal ion concentration, which could be affecting the performance of the strains in solid media. Hence, we further tested if the addition of two concentrations of the trace element solution SL10 (Atlas, 2010) to the solid culture medium could enhance the growth of the *Granulicella* strains on media with the different agar brands. To further determine the effect of trace elements solution SL10 (μM composition in 1 mL solution: $FeCl_2.4H_2O$ 7.54; $ZnCl_2$, 0.51; $MnCl_2.4H_2O$, 0.51; H_3BO_3, 0.10; $CoCl_2.6H_2O$, 0.80; $CuCl_2.2H_2O$, 0.01; $NiCl_2.6H_2O$ 0.10; $Na_2MoO_4.2H_2O$ 0.15) on the bacterial growth on each agar brand separately, we added different concentrations (1 mL and 10 mL per L of PSYL5 culture medium) of SL10 to the autoclaved culture media after cooling to 40 °C. The agars were autoclaved separately and mixed to the media right before solidification in order to avoid hydrolysis and production of inhibitors (Tanaka et al., 2014). Plates were inoculated and incubated as in the previous experiment. Culture media without the addition of SL10 solution were used as controls.

The addition of SL10 (1 mL and 10 mL concentrations) visually enhanced bacterial growth and EPS production, especially for Sigma (Fig. 1d) and Fluka (Fig. 1e) brands, supporting that the metal ion content of these agar brands was not fulfilling the requirements for the growth of our strains. Nevertheless, the best was BD agar (Fig. 1c and f), since no extra amendments were required for bacterial development as shown in Table 1.

Overall, our experiments demonstrated and reinforced the importance of not only purity but also the composition of reagents used for experiments involving microbial growth, particularly *Acidobacteriota* members. For instance, Bosmans et al. (2016) showed that calcium content of the agar strongly affected antimicrobial activity of *Firmicutes* strain ST15.15/036 against *Agrobacterium*. Being able to track brands and reagent batches is important especially when dealing with fastidious microbes that rely on the presence of micronutrients for a good performance in laboratory conditions. Furthermore, those observations may lead to other interesting questions concerning to the role of micronutrients in the metabolism of such microbes (Costa et al., 2020), as the importance of trace elements in microbial metabolism is widely recognized (Chandrangsu et al., 2017).

Funding

Ohana Y.A. Costa was supported by SWB grant from CNPq [202496/2015–5] (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil). This study was supported by the Netherlands Organization for Scientific Research (NWO) grant number 729.004.016.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

Publication number 7136 of the Netherlands Institute of Ecology (NIOO-KNAW).

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