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Bacillus subtilis is a non-pathogenic model Gram-positive bacterium that has been extensively studied for over a century. Microbiologists have used B. subtilis to investigate a broad variety of biological questions, ranging from the intricacies of cell metabolism to community behaviour and evolution (Earl et al., 2008; Shank & Kolter, 2011). As a consequence of the extended use of B. subtilis, multiple strains exist in laboratories and strain collections all over the globe. Some of these strains have been isolated from distinct environments and are used as wild-type reference strains; e.g. NCIB 3610 (from here onwards, 3610) and PS216. Other commonly used strains have been described as ‘domesticated’ due to their prolonged use under laboratory conditions, which have conferred them with characteristics that make them ideal research models, i.e. ease of genetic manipulation and efficient growth on commercially available media. One of the key features of B. subtilis is its ability to form biofilms. Biofilms are complex multicellular communities that can develop in diverse environments and potentially have a major impact on multiple human activities, among others including an ominous progression of common infections or hampering of biotechnological and industrial applications (Bjarnsholt et al., 2013; Valderrama & Cutter, 2013). Formation of biofilms can be desirable under certain circumstances; B. subtilis biofilms, for example, have been implicated in crop protection by prevention of colonization of plant roots by pathogenic organisms (Bais et al., 2004). B. subtilis has become one of the model organisms used for biofilm research. Studies performed over the years have provided many insights regarding the processes involved in the development of these bacterial populations (Mhatre et al., 2014; Mielich-Süss & Lopez, 2015). Strain 168 is the most well-known and widely used laboratory strain; it is an easily transformable tryptophan auxotroph that was obtained by X-ray mutagenesis (Burkholder & Giles, 1947) and has been used in a multitude of academic and industrial studies. The intensive use of strain 168 has generated various derivative strains, several of which have been sequenced by a joint European–Japanese consortium and later re-sequenced using single strains (Barbe et al., 2009; Kunst et al., 1997; Zeigler et al., 2008). The biofilms formed by B. subtilis have traditionally been studied as complex structured colonies on agar plates or as pellicles formed at the liquid–air interface of static liquid cultures (Cairns et al., 2014; Vlamakis et al., 2013). Branda et al. (2001) were the first to report the various biofilms developed by certain B. subtilis strains, noticing that domesticated laboratory strains derived from strain PY79 formed deficient biofilms. Since then, laboratory strains, including 168, have largely been considered as poor or non-biofilm formers at best (Romero, 2013). Different studies have investigated the genetic differences between this strain and wild-type 3610, reporting that these disparities are responsible for strain 168’s small, unstructured colonies and flat, featureless pellicles (McLoon et al., 2011; Pollak et al., 2015). In particular, a deficiency in the production of exopolysaccharide (EPS) has been highlighted as an important flaw of strain 168 related to biofilm formation (Kearns et al., 2005; McLoon et al., 2011). In B. subtilis, EPS is produced by the proteins encoded in the epsA-O operon (Branda et al., 2004) and is a major component of the biofilm matrix (Flemming & Wingender, 2010). Due to its relevance to biofilm formation, the chemical nature of this polymer has been investigated by various groups. However, these studies have normally used different non-domesticated strains and media, obtaining disparate results (Chai et al., 2012; Jones et al., 2014; Roux et al., 2015). This phenomenon is a testament to the robustness of B. subtilis, a soil bacterium that has evolved the ability to survive on different nutrient sources and therefore can use diverse compounds to produce the polymers that form the backbone of the biofilm matrix (Kohlstedt et al., 2014; Meyer et al., 2014). A problem that permeates B. subtilis research is the plethora of available strains and methods, making it difficult to compare experimental results. Here, we compared various biofilms developed by divergent laboratory stocks of strain 168 originating from various research groups around the globe. We also analysed the expression of the eps and tapA-sipW-tasA operons using fluorescent reporter fusion. We report that the formation of complex colonies varies greatly among the various 168 strains, some of them being able to form architecturally complex colonies similar to those developed by 3610 when grown on complex or supplemented media. In addition, we show that the expressions of P eps -GFP and P tapA -GFP fusion does not necessarily correlate with the formation of architecturally complex structures in these biofilms.

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