

BactEXTRACT: an R Shiny app to quickly extract, plot and analyse bacterial growth and gene expression data

Julien Dénéreáz and Jan-Willem Veening*

Abstract

To streamline the analysis and visualization of bacterial growth and gene expression data obtained by microtitre plate readers, we developed BactEXTRACT, an intuitive, easy-to-use R Shiny application. BactEXTRACT simplifies the transition from raw optical density, fluorescence and luminescence measurements to publication-ready plots. This package offers a user-friendly interface that reduces the complexity involved in growth curve and gene expression analysis and is generally applicable. BactEXTRACT is available at <https://veeninglab.com/bactextract>.

DATA AVAILABILITY

BactEXTRACT source code along with the most recent development version can be found at <https://github.com/veeninglab/BactEXTRACT>, and the online R Shiny application is available with any web browser at <https://veeninglab.com/bactextract>. Example data used in this article can be found at <https://github.com/veeninglab/BactEXTRACT/tree/main/Examples>.

INTRODUCTION

Bacterial growth measurement is one of the most commonly used methods to assess bacterial fitness and is essential for a broad spectrum of applications, ranging from basic biological research to the screening and development of antibiotics [1]. Bacterial growth is typically assessed by measuring the optical density of a culture over time at a specific wavelength through periodic sampling using a spectrophotometer or within a microplate reader [2]. The advantage of using plate readers is that many strains and conditions can be tested simultaneously. However, such high-throughput experiments yield large datasets that require extensive analysis. As a result, several tools have been developed that can dive deeply into quantifying the growth parameters and can perform detailed statistical analysis [2–7]. In order to simplify the process between output data and producing publish-ready plots, we developed BactEXTRACT, an R Shiny application, to expedite the analysis and visualization of bacterial growth data (<https://veeninglab.com/bactextract>). This application stands out for its simplicity and intuitive design, aimed at easing the process of growth curve analysis and plotting.

FUNCTIONALITY

BactEXTRACT is a versatile software tool designed to facilitate the analysis of biological data, particularly optical density (OD) and other related measurements, such as luminescence and fluorescence. It allows for the import of one or multiple Excel or text files, with any amount of sub-table combinations (OD, luminescence, fluorescence), enhancing efficiency in handling extensive datasets. Excel files must be the raw data file produced by common microplate reader such as TECAN or BioTek (Fig. 1a). Currently, supported softwares are TECAN i-control, SparkControl and BioTek Gen5. A settings file can also be uploaded, enabling users to save their analysis parameters, making it possible to consistently apply the same analysis criteria to different datasets without reconfiguring settings each time. Local web browser storage can also be used to save settings that will automatically be set the next time on the same web browser. Customized conditions can easily be entered in the app by the user, allowing the separation of the growth data, for example by strain and treatment (Fig. 1b). In addition, BactEXTRACT allows merging of technical replicates by means of adjacent wells (such as wells A1–A2–A3), allowing, for example, the merging of multiple data files corresponding to biological replicates. The

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Abbreviations: AUC, area under the curve; OD, optical density; RLU, relative light units.

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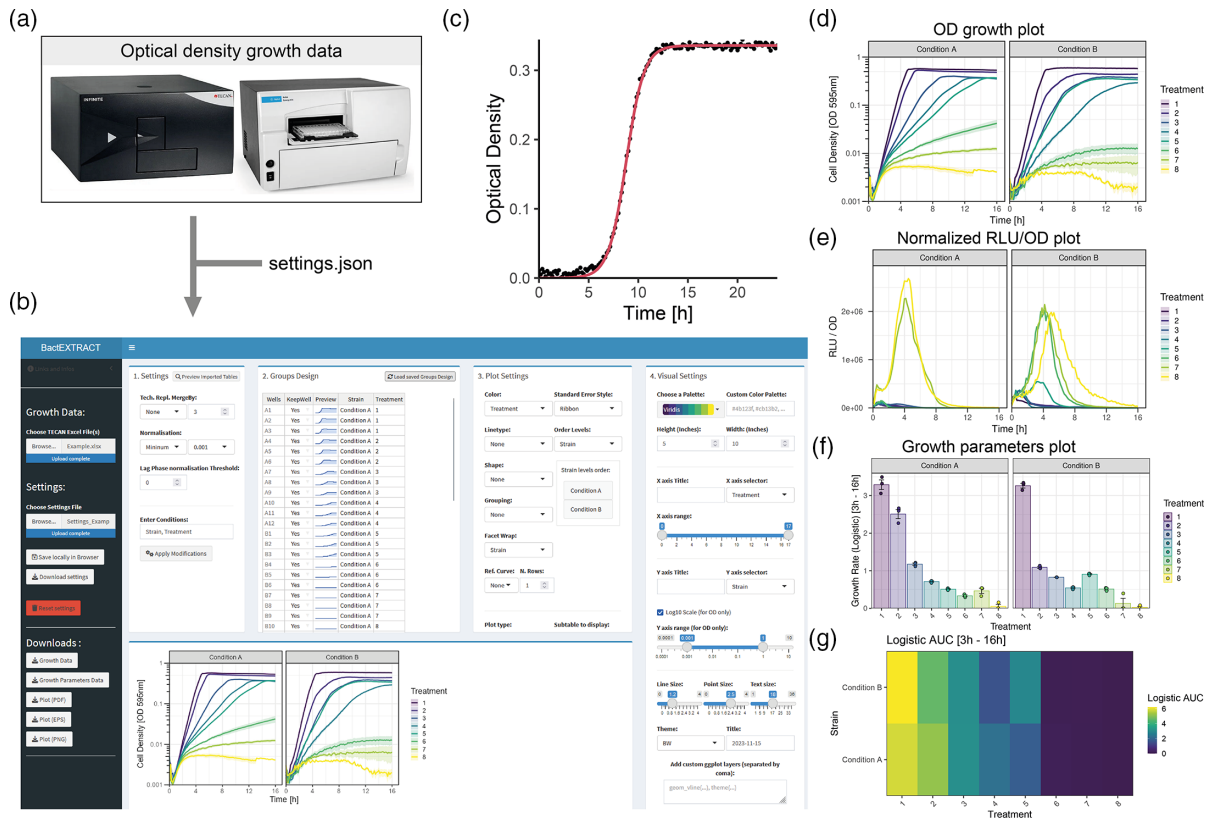


Fig. 1. BactEXTRACT R Shiny application. (a) Raw data from common plate readers and simple text files can be input into the software. Multiple files can be uploaded at the same time. (b) The web interface of BactEXTRACT allows for the quick editing of any parameters and customized theming. (c) BactEXTRACT fits a logistic curve to every sample. Different graphical layers such as colour, linestyle and facet can be chosen to allow for clear separation of all groups present in the data. (d) Standard OD growth plots on logarithmic or linear axes can be plotted. (e) Luminescence and fluorescence sub-tables (if any) can be selected and plotted as well, either normalized by OD or as a standalone plot. (f, g) Each growth parameter output from the logistic fitting can be plotted as either a bar plot (f) or as a checkerboard plot, which is particularly handy when combinations of antimicrobial compounds are being tested (g).

application provides useful normalization settings, either by time or by OD. Normalization by OD enables the use of specific wells as background to use for normalization, and time normalization allows the standardization of lag phases over a dataset. Customization options are abundant and are based on the conditions set by the user (Fig. 1b), affording users the ability to tailor the thematic presentation of their data. It also supports the creation of combination plots that display OD with relative light units (RLU) or with any other fluorescence/luminescence data present in the Excel file normalized by OD, a crucial requirement for reporter growth assays (Fig. 1e). To analyse growth parameters, BactEXTRACT relies on Growthcurver [2], a simple R package fitting a logistic equation to each growth curve using:

$$N_t = \frac{K}{1 + \left(\frac{K - N_0}{N_0}\right) e^{-rt}}$$

where N_t describes the population size at time t , as described in the Growthcurver package (Fig. 1c) [2]. The user has the flexibility to select the desired time range for the fitting process. Standard parameters are available for plotting, such as area under the curve (AUC), growth rate and all single parameters of the fitted logistic equation. Visual representations of these parameters include either a bar plot or a checkerboard plot, which is particularly beneficial for contrasting two different conditions, such as in antibiotic combination assays (Fig. 1f, g). Finally, BactEXTRACT allows for the export of both visual plots in various file formats and computed data, facilitating easy sharing and further examination of the results.

DISCUSSION

BactEXTRACT offers several features that can facilitate scientific research and does not require the acquisition of programming skills that can be time consuming. While BactEXTRACT is very powerful at plotting data, it has a few limitations. For example, currently statistical analysis between groups has to be performed by the user. However, as the data can be downloaded in the 'tidy' format,

further processing in R is facilitated. Despite offering fewer complexities in mathematical modelling than other bacterial growth curve analysis softwares, such as AMiGA and QurvE [7, 8], BactEXTRACT distinguishes itself with its simplicity and straightforward approach and has become one of the favourite tools used in our group and by our collaborators for analysing and plotting growth curves and we thus expect it to be of general use to the microbiology community. Future developments will allow more features to be added, such as different parametric models to fit bacterial growth curves, or quality of life improvements for better customization plots and faster processing.

Funding information

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Author contributions

J.D.: conceptualization, code development, software, writing – original draft preparation. J.W.V.: writing – review and editing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Peer review history

VERSION 2

Editor recommendation and comments

<https://doi.org/10.1099/acmi.0.000742.v2.3>

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John Munnoch; University of Strathclyde, SIPBS, UNITED KINGDOM, Glasgow

Date report received: 08 January 2024

Recommendation: Accept

Comments: This study would be a valuable contribution to the existing literature.

SciScore report

<https://doi.org/10.1099/acmi.0.000742.v2.1>

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iThenticate report

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Author response to reviewers to Version 1

We would like to thank the referees for their thoughtful and insightful comments and suggestions and were happy to read they appreciated our work. We address each comment point by point below.

Reviewer #1:

1. Line 26 - 'very deeply' should be changed to 'deeply'

This has now been corrected.

2. Line 34 - 'amounts' should be changed to 'amount'

This has now been corrected.

3. Line 36 - Refers to figure 1A, a microplate reader. However, the context is that it is referencing microplate software. Either the figure or reference should be as not to make it appear the authors are referencing software while showing a figure of the hardware.

We clarified the text to separate the hardware reference to Figure 1A, and added the specific softwares supported in the following sentence.

4. Line 41- 'Strain' and 'Treatment' shouldn't be capitalised

This has now been corrected.

VERSION 1

Editor recommendation and comments

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John Munnoch; University of Strathclyde, SIPBS, UNITED KINGDOM, Glasgow

Date report received: 05 December 2023

Recommendation: Minor Amendment

Comments: The work presented is clear and the arguments well formed. This study would be a valuable contribution to the existing literature. This is a study that would be of interest to the field and community. Thank you for your submission, reviewers raised only minor amendments, once dealt with, we will be happy to accept. The manuscript was well written and the tool will be valuable. I would like to raise an issue with the webtool on low resolution or small screens (or when the window is tiled on screen). While resolvable by zooming, initially there are some layout issues. No requirements to resolve but raised in case this is an easy fix for you.

Reviewer 2 recommendation and comments

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Paul Hoskisson; University of Strathclyde, SIPBS, Royal College, 204 George Street, Glasgow, UNITED KINGDOM

<https://orcid.org/0000-0003-4332-1640>

Date report received: 01 December 2023

Recommendation: Accept

Comments: The manuscript of Dénéreáz & Veening describes the development and implementation of an incredibly useful R based software tool called BactEXTRACT, which is implemented as a Shiny to allow real time web-based plotting of data. The application also has a very helpful storage facility to enable reproducible plotting of data. The provision of example data is also useful to users to enable data to be formatted correctly, should they not be using certain brands of plate reader. There is also the likelihood that that this app could be used for the analysis of data from Cell Growth Quantifier systems at scale too. The software uses the Growthcurver R app to analyse the data, which provides robust analysis framework and through BactEXTRACT is easier to implement without the requirement for R skills from the user. All data is available, the software works and the Github links function. Overall the software will be very useful to the community and will find wide potential applications across microbiology. The manuscript is well written and I have no changes for the authors to make and I look forward to seeing it published.

Please rate the manuscript for methodological rigour

Very good

Please rate the quality of the presentation and structure of the manuscript

Very good

To what extent are the conclusions supported by the data?

Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?

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Is there a potential financial or other conflict of interest between yourself and the author(s)?

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If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?

Yes

Reviewer 1 recommendation and comments

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Anonymous.

Date report received: 01 December 2023

Recommendation: Minor Amendment

Comments: Line 26 - 'very deeply' should be changed to 'deeply' Line 34 - 'amounts' should be changed to 'amount' Line 36 - Refers to figure 1A, a microplate reader. However, the context is that it is referencing microplate software. Either the figure or reference should be as not to make it appear the authors are referencing software while showing a figure of the hardware. Line 41 - 'Strain' and 'Treatment' shouldn't be capitalised Otherwise a sound paper and a nice to use package

Please rate the manuscript for methodological rigour

Good

Please rate the quality of the presentation and structure of the manuscript

Good

To what extent are the conclusions supported by the data?

Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?

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