Deep Learning for Fast Identification of Bacterial Strains in Resource Constrained Devices

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Abstract. This papers presents a proposal of a deep learning based system for classification of bacterial species, this system is able to work in resource constrained devices by fine tuning the state of the art mobile network called MobileNetV2, which drastically reduces the memory footprint without sacrificing accuracy, this is achieved by using depth wise separable convolutions and a novel layer module, called inverted residual with linear bottleneck. This resource constrained approach aims to provide complementary information to the traditional bacterial identification tests by being a suitable option for deployment in mobile or embedded systems. We measured the performance of several architectures over the original Digital Image of Bacterial Species (DIBaS) dataset and an augmented version of it, the best models achieved 95.53% and 94.22% of accuracy, respectively. We provide comparative tables between the original and augmented data sets as well as the results obtained by different configurations of the architecture, we also made publicly available the code and the pre-trained models with higher scores.

Keywords: Bacterial colony, classification, infectious diseases, mobile networks

1 Introduction

Bacteria are small unicellular microorganisms and are found almost everywhere on Earth. Most bacteria aren't harmful for humans, less than 1% of the different species of bacteria make people sick [18].

Due to the impact of the bacteria in the human life, the recognition of bacterial genera or specie is a very common and important task in many areas like medicine, veterinary science, biochemistry, food industry or farming [30]. The traditional laboratory methods for the identification of bacterial strains commonly require an expert with knowledge and experience in the field, most techniques designed for rapid and automated identification of microbiological samples are based of biochemical or modular biology technologies [15,27]. These traditional approaches are well established, however they are expensive and time consuming since they require complex sample preparation [1].

Automatizing the process of bacteria identification is very promising in the field of bioimage informatics. This field has yielded powerful solutions for specific image analysis task such as object detection, motion analysis or morphometric features [26]. Even so, most image analysis algorithms have been developed for very specific tasks or biological assays.

We propose a general solution to the task of bacterial specie recognition trough a deep learning (DL) approach. The implementation aims to reduce the memory footprint and the computational cost in the training and inference phases, to achieve this, we used a pretrained model of MobileNetV2 over the ImageNet [5] dataset. We fine-tuned the model by redefining the last dense layer of the original architecture. Faster and less expensive computational approaches to bacterial strain classification are necessary for deployment in mobile devices or embedded systems.

2 Related works

Holmberg et al. published of the first attempt to classify bacterial strains in an automated way, they used classification trees to extract the most important features and then classified it with artificial neural networks, achieving an accuracy of 76% in a dataset with 5 species of bacteria [12].

Later, in 2001, Liu et al. presented a computer-aided system to extract size and shape measurements of digital images of microorganisms to classify them into their appropriate morphotype [16]. Their work aimed to classify automatically each cell into one of 11 predominant bacterial morphotypes. This classifier had an accuracy of 96% on a set of 1,471 cells and 97% on a test of 4,270 cells.

Trattner et al. proposed an automatic tool to identify microbiological data types with computer vision and statistical modeling techniques [28]. Their methodology provided an objective and robust analysis of visual data, to automatize bacteriophage typing methods.

Some works consist in the identification of just one specie of bacteria. Forero et al. presented a method for automatic identification of *Mycobacterium tuberculosis* by using Gaussian mixture models [9]. In this work, authors use geometrical and color features of the images.

In 2013, Ahmed et al. published a light scatter-based approach to bacterial classification using distributed computing (due to the computational complexity of their feature extractors). They used Zernike and Chebyshev moments and Haralick texture features, then, the best features were selected using Fisher's discriminant. The classifier was a support vector machine with a linear kernel. The classification accuracy varies from 90% to 99% depending on the specie [1].

More recently, in 2017, Zielinski et al. published a deep learning approach to bacterial colony classification. In this work, authors used Convolutional Neural Networks as feature extractors and support vector machines or random forests as classifiers. Their overall highest accuracy over the Digital Image of Bacterial Species dataset was 97.24%, achieved by Fisher vector [21] encoders combined with VGG-M network.

3 Traditional methods for bacterial identification

Bacterial identification systems, both manual and automated, are in widespread use today in clinical microbiology laboratories.
Biochemical and phenotypic tests usually require a time commitment ranging from a few hours to several days and require large amounts of biological material, which can be a major disadvantage for the identification of microorganisms. Molecular methods have been demonstrated to have a complementary value, but they are not practical for routine use due to their high cost and the level of expertise required for their implementation.
To support the idea that automated and computer-based methods are useful to bring faster or complementary information, in this section we present a short review of the traditional methodologies used in the detection of bacterial agents in the clinical laboratory. A summarize of the methods and some examples are presented in Table 1.

3.1 Phenotypic methods

Traditional bacterial phenotypic identification schemes are based on the observable characteristics of bacteria, such as their morphology, development, biochemical and metabolic properties.
Each genus of bacteria has a characteristic protein expression. Although many proteins, including enzymes, are common to most bacteria, a range of unique biochemical pathways define each bacterial genus and the proteins expressed can even differ between species within a genus [29].

Biochemical testing Culture, when feasible, remains the diagnostic method by default, it allows the isolation of the microorganism, its identification and the antimicrobial susceptibility test (AST).
Genus is assigned by a combination of morphological features such as colony size or color, microscopic features such as Gram stain, and rapid biochemical tests such as catalase or oxidase enzyme activity, all are made with simple reagents.
Because of different species within a genus can have different pathogenicities and resistance profiles, identification at the specie level is performed by specific biochemical or serological tests.
These traditional approaches, based on the pure culture of the microorganism, require at least 36–48 hours [2].
Manual systems or multi-test galleries These are isolated cells with lyophilized substrate that are inoculated individually, the test results are expressed numerically and each specie is defined by a numerical code.
Biochemical test kits include tests for carbohydrate fermentation, methyl red, citric acid utilization and hydrogen sulfide production. The most representative are the Minitek identification system with paper substrates, API-20A system with dry powder substrates, PIZYMAN-IDENT rapid enzyme activity assay system and RaPID-ANA systems. The aforementioned microbial biochemistry reaction plate includes 30 biochemical matrices and their related biochemical test indicators, it also includes phosphate

buffered saline (PBS), bacterial turbidity standard tube, and eight identification series [7].

Automated systems The automated microbiology analysis provides results by capturing images with a high-definition digital camera and then analyzing them using its built-in software. Microbiology analyzers can be used for automatic colony counting, inhibition zone measurement to test antibiotic sensitivity test, and measurement of the hemolytic zone [7]. The first automated identification system to become available for clinical laboratories was the Vitek system (bioMerieux, Inc., Durham, NC) [3].

Mass spectrometric methods show promise for rapid identification, particularly Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry. This offers the analysis of whole bacterial cultures for unique mass spectra from charged macromolecules by rapid, high-throughput testing with a rapidly growing database [22].

3.2. Molecular methods

Molecular diagnostic methods rely on the analysis of genomic markers corresponding to nucleic acid sequences. The taxonomy and phylogeny of bacteria is based on the sequences of conserved genes, especially those coding for ribosomal ribonucleic acids (rRNA) [29].

Nowadays, several molecular methods are available for microorganism identification, such as polymerase chain reaction (PCR) and related PCR-based methods including random amplification of polymorphic DNA (RAPD), amplified ribosomal DNA restriction analysis (16S-ARDRA) and DNA/DNA hybridization. For successful inclusion in the species, 70% similarity to the consensus sequence based on DNA-DNA hybridization and more than 97% similarity to the consensus sequence of the 16S rRNA genes are required [6].

PCR techniques PCR is a technique used to amplify small and targeted segments of DNA to produce millions of copies of a specific gene fragment. This technique was developed in 1983 by Kary Mullis. Each cycle of PCR have 3 main important steps such as denaturation, alignment of specific primers, annealing and final extension [23].

Real-time PCR Real-time PCR has catalyzed wider acceptance of PCR because it is faster, sensitive and reproducible, while the risk of carryover contamination is minimized. There is an increasing number of chemicals used to detect PCR products as they accumulate within a closed reaction vessel during real-time PCR. These include the non-specific DNA-binding fluorophores and the specific, fluorophore-labeled oligonucleotide probes [17].

This testing method combines PCR chemistry with fluorescent probe detection of amplified product in the same reaction vessel. In general, both PCR and amplified product detection are completed in an hour or less, which is considerably faster than conventional PCR detection methods [8].

Multiplex-PCR Multiplex polymerase chain reaction (Multiplex PCR) is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction. Since its first description in 1988, this method has been successfully applied in many areas of DNA testing, including analysis of deletions, mutations and polymorphisms, or quantitative assays and reverse-transcription PCR [11].

DNA sequencing In the 1980s, a new standard for identifying bacteria began to be developed. In some laboratories, it was shown that phylogenetic relationships of bacteria, and, indeed, all life-forms, could be determined by comparing a stable part of the genetic code [1]. Genes such as; the ribosomal, 18S rRNA, 16S rRNA, 23S rRNA and 16S-23S rRNA internal transcribed sequences, rpoB (encoding β sub-unit of RNA polymerase), groEL (encoding heat-shock protein), gyrB (encoding β sub-unit of DNA gyrase) and recA (involved in the homologous recombination of DNA) are found in almost all bacteria and have been used for identification using conserved sequences as universal primers in PCR [14].

Table 1. Summary of some bacterial detection methods. The table presents advantages and disadvantages of the example systems.

Detection method	System examples	Advantages	Disadvantages
Phenotypic methods			
Culture on microbiological media and identification by biochemical tests	API (bioMérieux) Enterotube (BBL) RapID systems and MicroID (Remel) Biochemical ID systems (Microgen)	Sensitive. Inexpensive.	Lengthy and time-consuming process [25]. Might require 24–48 h [25]. Some species cannot be distinguished by morphology and cultural characteristics [22].
MALDI-TOF MS	Vitek MS Biflex III Autoflex III Microflex LT Biotyper	Fast. Accurate. Less expensive than molecular detection methods. Trained laboratory personnel not required.	High initial cost of the MALDI-TOF equipment [25]. Detection is not direct from clinical samples.
Molecular methods			
Real-time PCR Multiplex-PCR	MagNA Pure LC (Roche Applied Science) BioRobot EZ1 (Qiagen) BI Prism 6100 (Applied Biosystems) NucliSens Extractor (bioMérieux)	Culturing of the sample is not required. Specific, sensitive, rapid, and accurate. Closed-tube system reduces the risk of contamination. Can detect many pathogens simultaneously.	A highly precise thermal cycler is needed. Trained laboratory personnel required for performing the test.
DNA sequencing	Databases available for 16S rRNA: GenBank MicroSeq RDP-II RIDOM	Can identify fastidious and uncultivable microorganisms. 16S rRNA sequencing is the gold standard.	Trained laboratory personnel and powerful interpretation softwares are required. Expensive. Not suitable for routine clinical use. Contamination of a sample by post-amplification products [22].

4 Materials and methods

This section describes the methodologies we used to generate two versions of the dataset (the class distribution of the samples is also presented) and the details about the implementation of the system (transfer learning and fine tuning).

4.1 Digital Images of Bacteria Species dataset

The original version of DIBaS dataset contains a total of 33 species of microorganisms, approximately 20 RGB images (of 2048x1532 pixels) per specie. We remove the set of images of *Candida albicans* colonies as it is considered fungi [19]. The dataset was collected by the Chair of Microbiology of the Jagiellonian University in

Krakow. The samples were stained using the Gramm's method. All images were taken with an Olympus CX31 Upright Biological Microscope and a SC30 camera with a 100 times objective under oil-immersion [30]. The DIBaS dataset is publicly available[1].

Data augmentation We perform some steps to augment the quantity of samples in the dataset, the following list enumerates the steps we have taken:

1. Obtain 10 crops of the original image. Each crop with a size of 224x224 pixels. Four crops from the corners and one from the center, each was flipped horizontally to obtain the other five crops. Add the 10 crops of each image to the augmented dataset.
2. Add the resized versions of the original images (of 2048x1532 pixels) to the augmented set. Each image was resized to 224x224 pixels using Lanczos resampling.
3. Rotate every sample in 90, 180 and 270 degrees. Add the resulted images to the augmented dataset.

Images in both datasets are RGB. For a graphical example of the resulting images, see Figure 1.

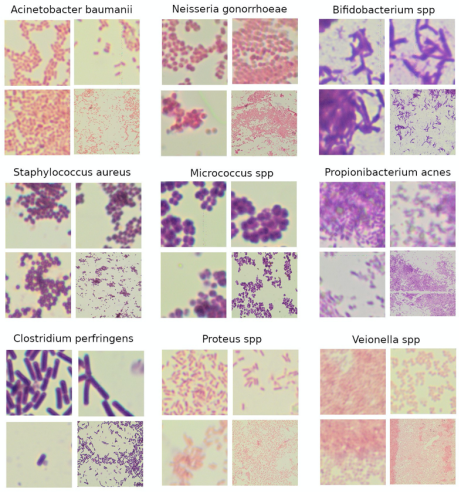

Fig.1. Augmented samples for 9 of the 32 classes in the dataset.

Dataset versions and splits Two different versions of the dataset were used in the experiments: the original version (after *Candida albicans* removal) with a total of 669 samples and an augmented version with a total of 26,524 samples. Each dataset was divided into train, validation and test sets, with 80% of the total samples for the training set and 20% for validation and test sets (10% each). The samples for each set were selected randomly.

Table 2 describes the distribution of the samples per each genera and specie, for both versions of the dataset.

[1]http://misztal.edu.pl/software/databases/dibas/

Table 2. Quantity of examples per each specie in the two versions of the DIBaS dataset.

Genera	Specie	Original Samples	Augmented Samples
Acinetobacter	baumanii	20	712
Actinomyces	israelli	23	860
Bacteroides	fragilis	23	960
Bifidobacterium	spp.	23	896
Clostridium	perfringens	23	868
Enterococcus	faecalis	20	784
	faecium	20	680
Escherichia	coli	20	880
Fusobacterium	spp.	23	924
Lactobacillus	casei	20	856
	crispatus	20	724
	delbrueckii	20	592
	gasseri	20	820
	jehnsenii	20	788
	johnsonii	20	880
	paracasei	20	840
	plantarum	20	784
	reuteri	20	820
	rhamnosus	20	820
	salivarius	20	840
Listeria	monocytogenes	22	936
Micrococcus	spp.	21	600
Neisseria	gonorrhoeae	23	892
Porfyromonas	gingivalis	23	984
Propionibacterium	acnes	23	932
Proteus	spp.	20	880
Pseudomonas	aeruginosa	20	832
Staphylococcus	aureus	20	780
	epidermidis	20	776
	saprophiticus	20	816
Streptococcus	agalactiae	20	848
Veionella	spp.	22	920

4.2 Model architecture

MobileNets are efficient models for mobile and embedded vision applications. These models uses depth-wise separable convolutions to build light weight deep neural networks [13]. MobileNets are capable to perform a wide range of tasks such as object detection, classification, feature extraction, semantic segmentation and large scale geolocalization [24].

In this paper, we used the mobile architecture called MobileNetV2, published by Sandler, M. et al. [24], which is mainly based on the MobileNetV1 [13], but improves the accuracy and efficiency by inserting *linear bottleneck layers* to the separable convolution blocks, they also use an inverted residual block instead the "traditional" residual block [10]. The main contribution of the MobileNetV2's paper is the introduction of a new layer module called: *inverted residual with linear bottleneck*, this module takes

a low-dimensional compressed representation as an input, which is first expanded to a high dimension and then filtered with a lightweight depth-wise convolution, features are subsequently projected back to a low-dimensional representation with a linear convolution, the inverted residual with linear bottleneck module allows to reduce the memory footprint needed in the inference phase by never fully materializing large immediate tensors [24].
We strongly recommend to read the full MobileNetV2's paper to get deeper into the theory and intuition behind this architecture.

4.3 Implementation

The system is written in a Jupyter[2] notebook with the 3.8.2 version of Python and PyTorch [20] in version 1.5.1, full details of the system are well explained in the paper repository[3]. We propose two different implementations; both are fine-tuned versions of the MobileNetV2 architecture. The first is the simplest and aims to achieve good accuracy without scaling the computational power needed. The second aims to achieve a higher accuracy but also increasing the computational cost. The description of both architectures is presented in this section.
Preprocessing Both models use 3 channel (RGB) images of 224x224 pixels as inputs. As the pre-trained models were trained with a specific pre-processing and in order to achieve consistency, the input images were normalized in the same way: the dataset was loaded in a range of 0-1 and then was normalized using a mean of 0.485, 0.456, 0.406 and standard deviation of 0.229, 0.224, 0.255 (for each RGB channel). At the end of the pre-processing, each input to the network is a tensor of shape 3x224x224, where each element is normalized as mentioned previously.
Model with one dense layer This version of the architecture is the result of fine tuning the dense layer of the original MobileNetV2 (which has 1280 inputs and 1000 outputs). The new classifier is composed by a dense layer with 1280 inputs and 32 outputs, which is preceded with a dropout layer with probability of 0.2.
Model with two dense layers To achieve a higher accuracy, we decided to increment the model's capacity by adding a dense layer to the classifier with a Rectified Linear Unit (ReLU) as activation and a dropout layer (with probability of 0.2) for regularization. Table 3 contains the blocks of the new classifier. As we mentioned above, this version is computationally more expensive.

Table 3. Blocks in the new version of the classifier.

Block type	Inputs	Outputs	Probability
Dropout	-	-	0.2
Linear	1280	1280	-
ReLU	-	-	-
Dropout	-	-	0.2
Linear	1280	32	-

[2]https://jupyter.org/
[3] https://github.com/gallardorafael/bacterialidentification

4.4 Transfer learning

To compensate the lack of training examples (given the difficulty of collecting them), transfer learning is a very good option to achieve high accuracy in classification while reducing the training time. Both of our proposed models were trained after being initialized with a pre-trained version of the weights. The pre-trained weights are from a model trained over the ImageNet database [5], the pre-trained model is available in the *model zoo* [4] of PyTorch. As mentioned above, all the implementation details are available in the paper repository.

5 Results

As detailed in the section about the implementation, we proposed two different architectures, which mainly vary in the dense layers at the end of the network. In this section, we present the accuracy scores for both architectures, trained and tested over the two versions of the dataset and varying the quantity of epochs.
After several experiments with different configurations of the hyper parameters, we realized that the best accuracy scores were achieved by models trained with batch size of 32. Also, the increase of the training time with respect to the input size was not worth it and we decided to fix the input shape to 224x224 pixels.
Table 4 summarizes the results of each trained model, with different hyper parameters and for both versions of the dataset. The first column indicates the number of fully connected layers in the classifier block of the network, the second column is for the version of the dataset, the third column shows the number of training epochs and the fourth presents the accuracy score obtained by the model in that row. We determine the number of epochs by analyzing the training and validation loss.

Table 4. Summarize of accuracy scores by training epochs and dataset version.

Dense layers in classifier	Dataset version	Training epochs	Test accuracy
1	Original	10	0.8874
		30	0.8785
		50	**0.9553**
	Augmented	10	0.9375
		15	**0.9382**
		30	0.9229
2	Original	10	0.8642
		30	**0.9232**
		50	0.8839
	Augmented	10	**0.9422**
		15	0.9364
		30	0.9410

Accuracy scores in bold are the best for each model architecture trained over one version of the dataset. The highest accuracy for the original dataset was achieved by the

[4] https://pytorch.org/docs/stable/model zoo.html#module-torch.utils.model_zoo

architecture with just one dense layer in 50 epochs, the highest accuracy for the augmented dataset was reached by the architecture with two dense layers in just 10 epochs. Validation and training loss plots indicates that both architectures begin to overfit after the 10th epoch when training over the augmented dataset.
Some output examples are presented in Figure 2. From left to right, the species are: *Staphylococcus epidermidis*, *Lactobacillus paracasei*, *Neisseria gonorrhoeae* and *Porfyromonas gingivalis*. Top five most probable classes are shown below each sample.

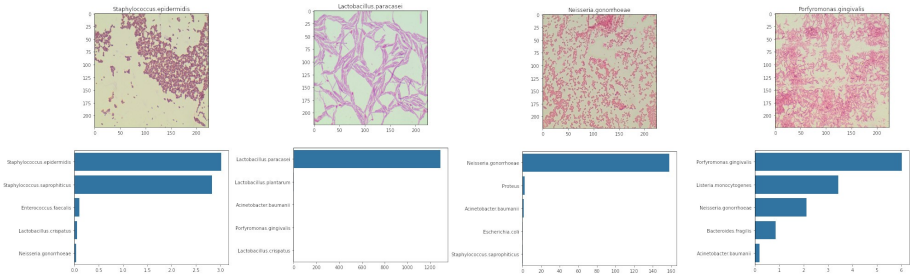

Fig.2. Top-5 most probable species for 4 samples in the test set.

6 Conclusions

The overall highest score (0.9553) was achieved by the architecture with one fully connected layer, trained in 50 epochs over the original dataset. It is important to consider that the test set of the original dataset consists of an average of 2 images per class (in experiments, we extract 10 samples per test image using ten crops), which can reduce the difficulty of the test set. On the other hand, the architecture with two dense layers achieved a 0.9422 over a test set with more than 4000 samples, which indicates that the model had a good generalization, increasing the certainty of the predictions.
For real world applications, we strongly recommend to use the model trained and tested over the augmented dataset. The lack of test samples in the original dataset may cause a high accuracy score, even if the model is not good enough. In a sensitive area such as microbiology, false positives and false negatives are a big problem. To reduce the impact of errors in identification, researchers can rely on probability graphs such as the showed in Figure 2, to see the *n* most probable classes for each sample.
In future work, we could include an analysis of the false positives and false negatives for each class in the dataset, so that researchers can have a measure of certainty about the predictions for each specie. The repository of the paper contains the code and instructions to prepare new species to increment the dataset and re-train the models if needed. A mobile application could be launched, in this app, the users could identify bacterial strains by just connecting the camera of the mobile devices to the microscope.

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