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M.S. Thesis

CNN's microbiome feature  
extraction and utilization for host  
prediction

– Using small datasets to CNN models –

Host prediction을 위한 CNN의 microbiome  
feature 추출 및 활용방법

August 2022

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# CNN's microbiome feature extraction and utilization for host prediction

– Using small datasets to CNN models –

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이 논문을 이학석사 학위논문으로 제출함

2022 년 8월

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# Abstract

This study aimed to compare the performance, strengths, and weaknesses of machine learning models based on convolutional neural networks and models not based on it; and analyzed the performance of various machine learning models according to the type and purpose of the given data. As a large number of data can be used with the continuous development of hardware, the possibility of machine learning using large datasets has already been sufficiently verified. Therefore, this study confirmed that using a relatively small gut microbiome dataset, machine learning models that predict a host could be designed with significant accuracy with appropriate tuning and loss function setting.

In this study, the operations of machine learning models were compared using a fecal microbiome dataset(4108 samples, 672 species). The training and validation dataset is a small subset of entire microbiome data(871 samples, 34 species). And it was shown that there was a difference in performance depending on the problem situation settings like the complexity of the data and the prediction purpose of ML models.

As a result of the study, the convolutional neural network-based models had the disadvantages of using more resources and taking a long time to learn. However, they maintained high accuracy compared to other discriminative models that were lumpy-labeled or more complex. Conversely, the models that did not use the convolutional neural network showed similar performance to the neural network-based model in discriminating simple data and accurately labeled data, with simple construction and learning. In addition, it was confirmed that the machine learning model could be used sufficiently even on a small dataset through appropriate design adjustments and function settings.

Summarizing the results, machine learning methods can verify data labeling of large datasets using a relatively small number of accurately labeled data. This can be used to check the labeling accuracy of large datasets that have been published as open-source before use in research.

**Keyword: Machine learning, Small dataset, Gut microbiome, Host prediction, Convolution network**

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# Chapter 1. Introduction

The basic meaning of microbiome is a group of microorganisms living in a specific body site of a host or environment. Several microorganisms constituting the microbiome community may have a symbiotic, commensal, or parasitic relationship with each other or with a host.[1]

Recently, the meaning of the microbiome has been expanded more than the conventional meaning, including interactions between microbiomes constituting a community, interactions with hosts, and relationships with the surrounding environment.[2] In other words, current studies using big microbiome data deal with high throughput omics data covering numerous classification criteria such as various environments, life stages, body sites, and diets. In addition, the sharing of open-source data has been increasing in general academic fields, and usable data is gradually accumulated, forming massive datasets.

In this situation, machine learning, which has strong

performance in finding patterns with meaningful information in data,[3] is attracting attention. To analyze massive datasets with complex structures, machine learning methods are gradually being used in research in microbiome analysis, and significant results are being published. That is to say, the performance of the ML model trained on a large dataset has been sufficiently verified from various experimental results.[4, 5]

It has been found that a CNN model using a small dataset in a multi-classification problem can also show good performance if there is a suitable loss function.[6] Suppose these results can be applied to the microbiome dataset. In that case, it is expected that CNN-based ML methods can be used to examine whether the labeling of the data constituting the large dataset is accurately performed using a small number of accurately labeled data.

Some previous studies that used machine learning methods for microbiome dataset analysis were reviewed in this study.[7, 8] The research result that a CNN model trained on a small dataset can show adequate performance was also analyzed.[6] Afterward, five



machine learning models were created to predict hosts by learning microbiome data. The created machine learning models are divided into non-CNN models and CNN-based models. Non-CNN models used include KNN(K-Nearest Neighbor), multinomial logistic regression, FFNN(Feedforward Neural Network), and 1D CNN and 2D CNN models are used as CNN-based models. The performance between CNN-based ML models and non-CNN models was compared using a small dataset in a host prediction problem. The strengths and weaknesses of the CNN-based model compared to the non-CNN model were confirmed in microbiome host prediction.

Accurate classification and labeling of data used in research using such large datasets are very important.[9] Therefore, in research using an open-source dataset, it is necessary to review whether the labeling of the data to be used is accurate. As a result of the experiment, it is expected that ML can help solve these problems.

## Chapter 2. Prior Research Review

ML models, data, and analysis methods using microbiome data as training data for machine learning models in previous studies were briefly summarized to investigate how actual ML methods were used in microbiome research.

### **2.1. The reasons that ML methods are used to analyze complex and enormous datasets.**

One of previous research[7] investigated the possibilities of ML for developing cancer therapeutics and approaching the analysis of large amounts of complex healthcare information.

It is known that specific microbial signatures are associated with cancer in several ways, including promoting cancer development and affecting the safety, tolerability, and efficacy of treatments.[10] As the microbiome dataset grows and becomes more complex, the 'omics' technologies used to identify biological molecules involved in a cell or organism's structure, function, and dynamics have several

difficulties extracting the full potential of numerous microbiome data.[11]

Regulation of the gut microbiome is known as an innovative option for improving medication reactions in cancer patients.[12] However, a primary issue needs to be addressed to identify a direct relationship between the gut microbiome and clinical practice. The gut microbiome is affected by many factors, including the patient's health status, disease progression, and the type of medications and diet used.[13] So, it is difficult to ascertain the entire interaction between the microbiome and drug metabolism.

Machine learning includes algorithmic methods to solve problems without specific computer programming. Data analysis using ML receives input data, trains a model, and makes precise predictions on new data. In the above process, ML connects between a specific class of AI that includes a learning mechanism and a large dataset. It finds complex patterns in big datasets[3] or turns input data into more valuable and interpretable information. Also, ML can be used for data integration.[14] Therefore, ML can analyze massive

datasets from various forms (demographic, laboratory, and image data)[15] and combine them into predictions about cancer, such as disease risk, prognosis, diagnosis, and appropriate treatment. Using ML in conjunction with substantial dataset approaches can help discover which microbial characteristics are consistently and reproducibly effective in predicting or treating cancer in a patient.[16]

The most crucial point to be aware of when using ML to analyze these diverse and complex large datasets. To use ML methods for hypothesis testing, it is necessary to design a model suitable for a given experimental environment, which is context-specific.[17] A good model at solving one type of problem is unlikely to perform well at solving a different kind of problem. Researchers who use ML for data analysis need to know how to use ML algorithms in various situations.

## **2.2. Example of an ML model for host prediction.**

A prior study[8] created an ML model that predicts objective

IBS (Irritable Bowls Syndrome) based on gut microbiome analyses. The dataset and clinical data obtained by sequencing the fecal samples with 16S rRNA were used.

It is difficult to distinguish whether differences in gut microbiome from normal individuals are the cause or consequence of IBS. This is difficult to address as the gut microbiome profile and IBS pathophysiology are influenced by shared environmental factors like diet style, various stresses, and hormones.[18] However, it is known that gut microbes exert effects on the host immune system and gut barrier function by the brain-gut axis.[19] Also, IBS patients have dysbiosis in the gut microbiome, showing lower microbial diversity than healthy controls.[20, 21]

Bacteria constituting the microbiome were analyzed at the genus level. The microbiome data consisted of 689 taxa, and feature-taxa was extracted using LASSO(L1 regularized logistic regression; Least Absolute Shrinkage and Selection Operator).[22] IBS were identified using the random forest technique included in python scikit-learn.[23] After that, the accuracy was measured

through cross-validation(10-fold, 100 repeats). The inverse parameter of regularization strength for logistic regression was optimized by internal 5-fold cross-validation. This model could distinguish IBS patients with more than 80% sensitivity and more than 90% specificity.

There are some limitations to the ML method used in this paper. The first limitation is that there was no functional investigation into the microbial community, and the number of subjects used as a control group was small(26 individuals). For example, it was thought that better results could be obtained if an approach using various meta-omics was added to the dataset[24], such as metagenomics, metatranscriptomics, metaproteomic, and metabolomics. The second limitation was that there was no dietary-related information in the dataset. Therefore, the prediction model may have missed the diet's possibility of affecting the gut microbiota profile dataset.

However, the ML method using gut microbiome data to classify IBS patients showed promising results despite limitations.

Further analysis suggested that it is possible to classify IBS patients into more diverse subtypes.

### **2.3. Performance of CNN model trained on small datasets.**

Previous studies have confirmed that the ML models used microbiome analysis using extensive datasets.[7, 8] However, one study[6] indicates that CNN models can show high accuracy even in small dataset training by setting an appropriate loss function.

Six datasets(CUB, NAB, Cars, Flowers-102, MIT Indoor, CIFAR-100)[25, 26, 27, 28, 29, 30] were used in this paper. Although the number of classes and the number of samples are different for each dataset, the number of samples per class is 4 to 80 except for one dataset, CIFAR-100. (CIFAR-100 has 500 samples per class) When using datasets with up to 100 samples per class, the cosine loss shows better performance than cross-entropy. For example, when the ML model is trained with the smallest test set of only 10 and 25 samples per class, the cosine loss performs 17% and 26% better than cross-entropy, respectively.

Figure. 1 shows the models' validation accuracy using AG news datasets[31]. This accuracy test's purpose is to show the change in performance difference according to subsample sizes in text classification. CNN models trained on small datasets showed that the cosine loss function outperformed the cross-entropy loss after SoftMax activation in the multi-classification tasks. This result means cosine loss helps train CNN classifiers from scratch on small, limited data. On the other hand, if the size of the training dataset is large enough or if the network weight parameters trained on the extensive dataset are used, the model's performance is not significantly affected regardless of which of the two loss functions was used. BERT(fine-tuned)[32] is the pre-trained model with huge text corpora.



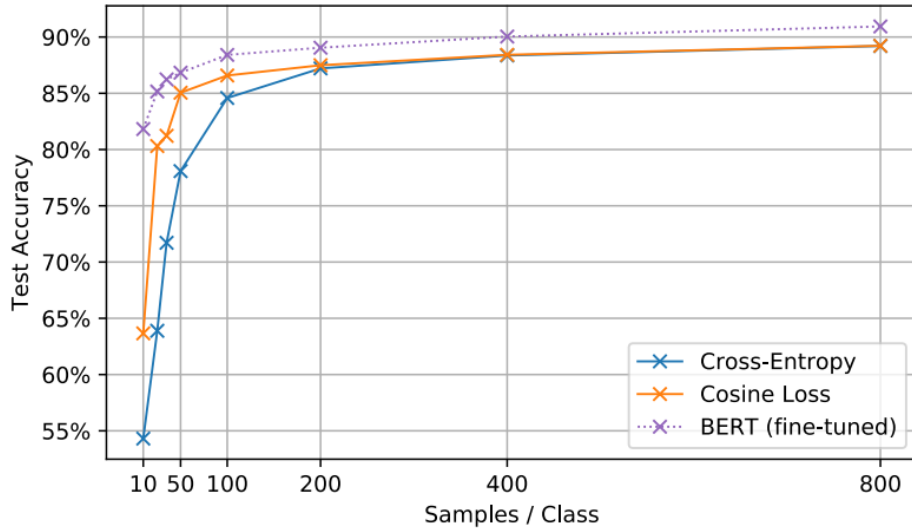


Figure. 1 Validation accuracy achieved using the cross-entropy and the cosine loss on sub-sampled versions of the AG News dataset, averaged over 10 runs.[6]

# Chapter 3. Host Prediction Using ML Models

## 3.1. Host Prediction Pipeline

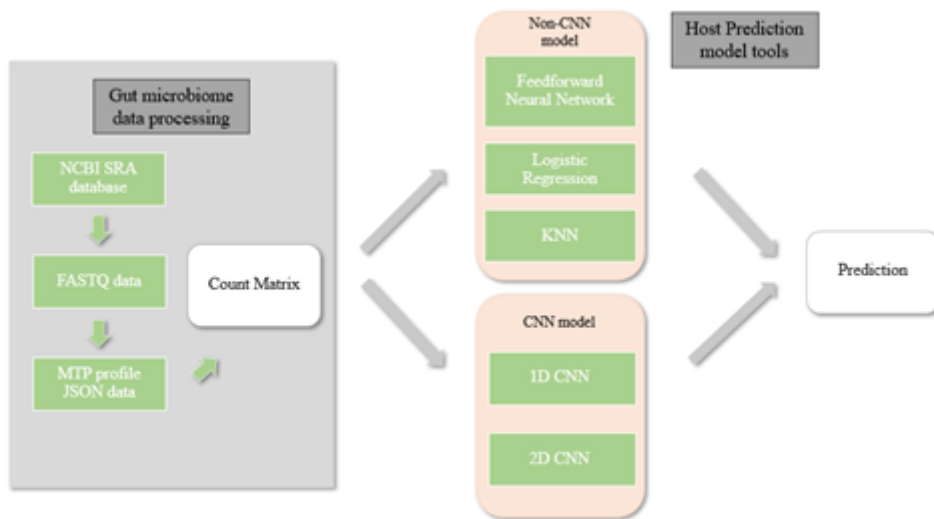


Figure. 2 Pipeline of gut microbiome data processing and host prediction

Gut microbiome data stored from the NCBI SRA database used as training, validation, and test dataset. The entire microbiome dataset used in this experiment consisted of 4108 samples, 672 species(Supplementary Table 1). The SRA study numbers of the fecal samples used are shown in Supplementary Material 1. Microbiome FASTQ files were processed into JSON format by

Microbiome Taxonomic Profiling(MTP) with QIIME using the EzBioCloud 16s database.[33] Using MTP products, the composition ratio of each individual constituting the microbiome was calculated to form a count-matrix CSV file. The CSV file parsing data of one JSON file was entered in each row. The percentage of microbiome composition according to parsing level was displayed in each column. The count-matrix files were generated per one fecal sample data by analyzing the microbiome composition at the genus and species level, respectively. Count-matrix CSV files were used as input data of ML models, and trained models predicted the host species( or genus) of fecal samples data.

## 3.2. ML Tools

The three models were classified into the non-CNN models' group. Multinomial logistic regression model, K-nearest neighbor model, and Feedforward neural network model were constructed using the Python Scikit-learn library.[27]

Python Keras library constructed CNN models, 1D CNN and

2D CNN.[34] The CNN model structures can be seen in Figure 3. Two CNN models with cosine loss and cross-entropy as loss functions were created in the experiment using CNN. Accuracy measurements of all ML models were repeated ten times, and the average of the highest observed values was obtained.

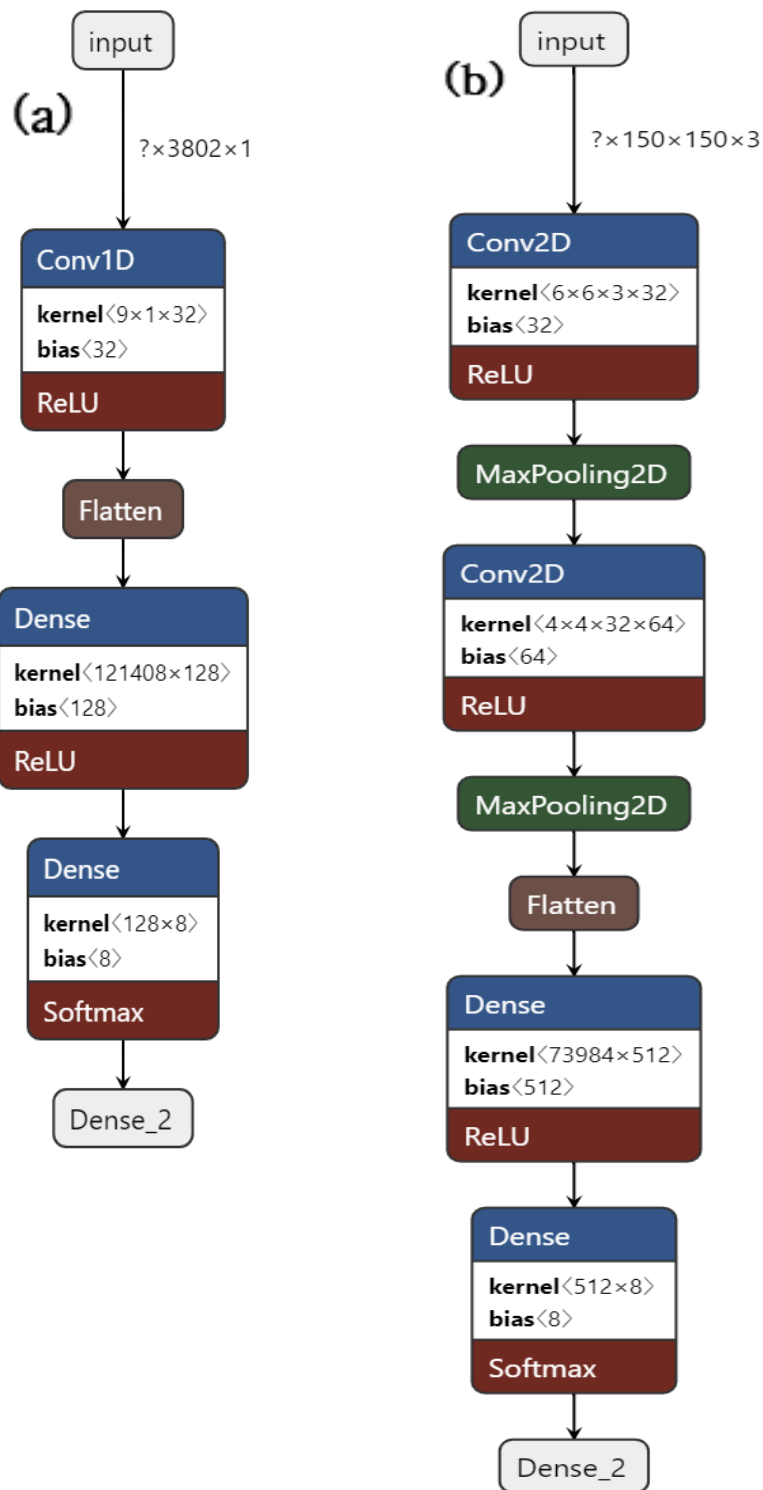


Figure. 3 (a) Basic 1D CNN model structure. (b) Basic 2D CNN model structure. Netron[35] was used to draw images.

### 3.3. Situation Settings

There were some unique terminologies used in this experiment's problem situation settings. 'Genus/Species-level data' means that when parsing JSON mtp profile data to make Count Matrix, the microbiome data was collected at the genus/species level. 'Labeled Species' (648 samples, 7 species: *Acinonyx jubatus*, *Ailuropoda melanoleuca*, *Bos taurus*, *Canis lupus familiaris*, *Canis mesomelas*, *Mus musculus*, *Sus scrofa domesticus*) means labeled data that the model trained on. 'Lumpy-labeled Species' means the species not named as their scientific names. 223 data of 37 species were grouped into 'etc\_group'. The models used this data group of various species as one 'etc\_group' dataset. Figure 4 shows a brief representation of each case.

'Labeled Species Prediction' situation means that the purpose of the model is to classify the labeled species used for training. In the 'Lumpy-labeled Species Prediction' case, the purpose of the model was to classify the data of the species not used as labeled data into etc\_group. Examples of each situation can be

found in Figure 5. The experiment was conducted in four situations:  
1) Genus-level Labeled species prediction, 2) Species-level Labeled species prediction, 3) Genus-level Lumpy-labeled species prediction, 4) Species-level Lumpy-labeled species prediction. The model used what level of data and what the models wanted to predict became the standard for setting the situation.

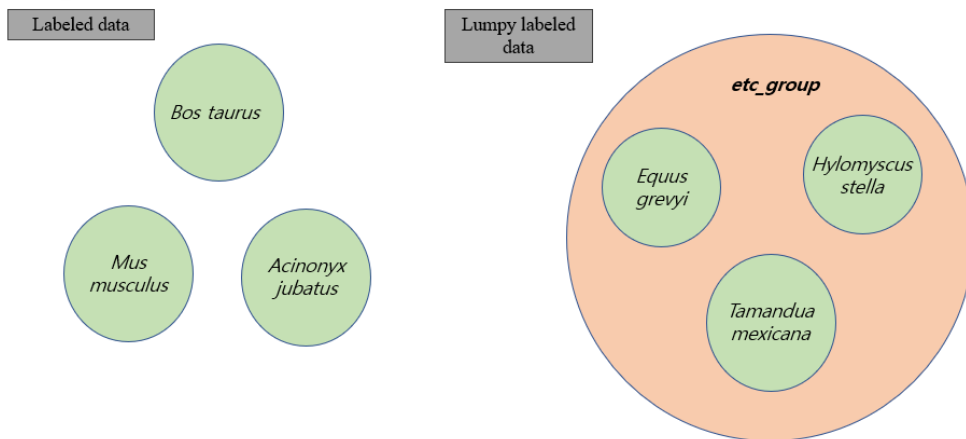


Figure. 4 Simple examples of 'Labeled data' and 'Lumpy-labeled data.' For simplicity, only three species are shown in each case. In this case, *Bos taurus*, *Mus musculus*, and *Acinonyx jubatus* were labeled as their own scientific name while *Equus grevyi*, *Hylomyscus stella*, and *Tamandua mexicana* were labeled as 'etc\_group' equally.

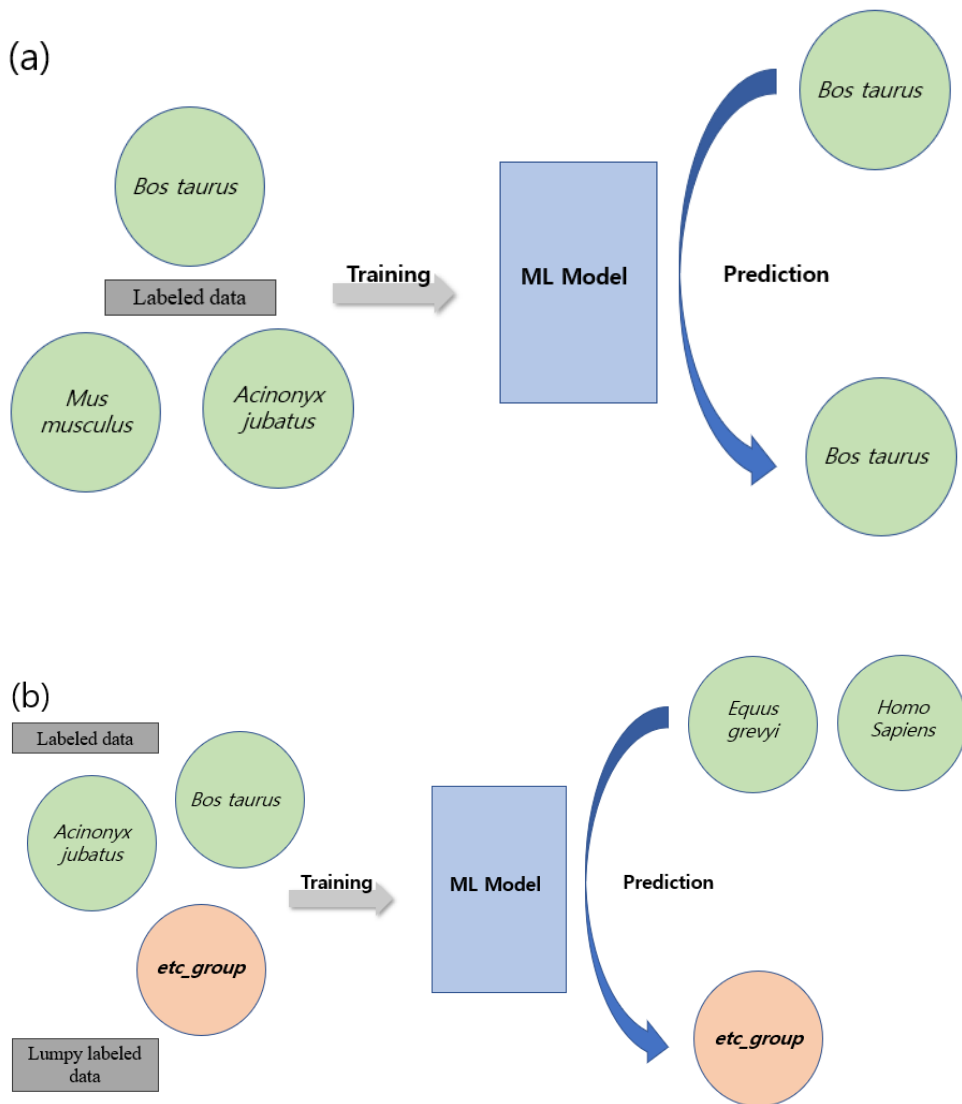


Figure. 5 (a) Labeled species prediction situation. (b) Lumpy-labeled species prediction situation. In (a), the labeled data was used for training, and the model determines the data of the species used for training, for example, *Bos taurus*. In (b), both labeled data and lumpy labeled data were used for model training. In this case, the model determines which species have been trained with *etc\_group* (like *Equus grevyi*) or have not been trained at all, like *Homo sapiens*.



## Chapter 4. Results

### 4.1. Accuracy Comparison

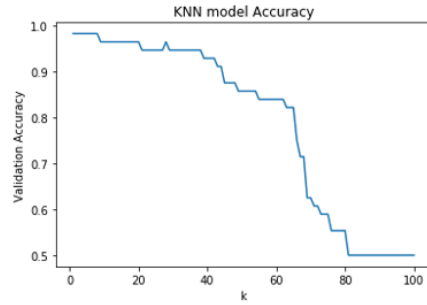
The average accuracy value was obtained by repeating ten times for each model. A random state value was assigned to none when training and validation split in an 8:2 ratio. A multi-layer perceptron(NLP) classifier was used in the NN models. While testing CNN ML models, cosine proximity and categorical cross-entropy were used as loss functions. The one showing the better accuracy of the two results was selected in each test result.

Figures 6, 7, 8 show one random example of each model among the validation tests repeated in the process of finding the average accuracy. The accuracies of the models used in all situations are summarized in Table 1. Figure 9 shows the model accuracies in lumpy-labeled prediction situation using the test dataset(3237 samples, 628 species). The test dataset was made by removing the data used for training from the entire microbiome dataset.

(a)

Feedforward Neural Network  
Average Accuracy : 0.981928

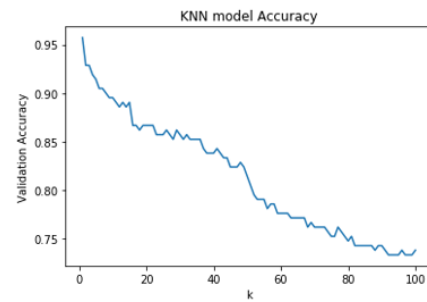
Logistic regression  
Training accuracy : 0.9333333333333333  
Validation accuracy : 0.9156626506024096



(b)

Feedforward Neural Network  
Average Accuracy : 0.912243

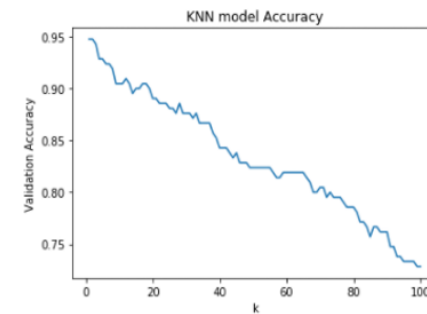
Logistic regression  
Training accuracy : 0.8724672228843862  
Validation accuracy : 0.8523809523809524



(c)

Feedforward Neural Network  
Average Accuracy : 0.910124

Logistic regression  
Training accuracy : 0.8688915375446961  
Validation accuracy : 0.8857142857142857



(d)

Feedforward Neural Network  
Average Accuracy : 0.876848

Logistic regression  
Training accuracy : 0.8736591179976162  
Validation accuracy : 0.8238095238095238

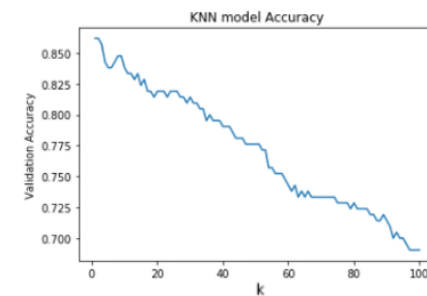


Figure. 6 (a), (b), (c), (d) show the validation accuracies of non-CNN models in four situations. Genus-level labeled species prediction, Genus-level

lumpy-labeled species prediction, Species-level labeled species prediction, and Species-level lumpy-labeled species prediction are indicated in the order.

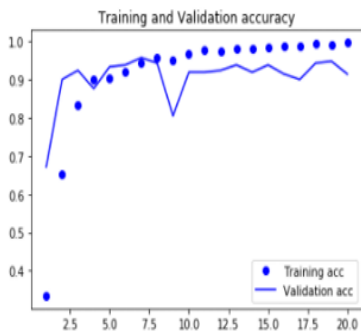
(a) 1D CNN



(b) 2D CNN



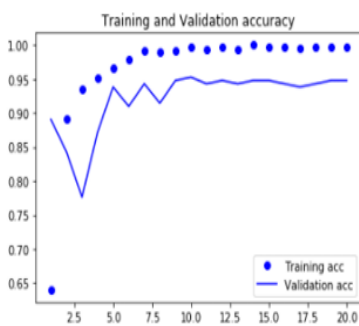
(c) 1D CNN



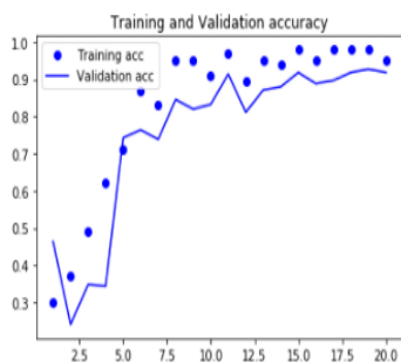
(d) 2D CNN



(e) 1D CNN



(f) 2D CNN



(g) 1D CNN



(h) 2D CNN



Figure. 7 (a), (c), (e), (g) show the training and validation accuracies of 1D CNN in Genus-level labeled species prediction, Genus-level lumpy-labeled species prediction, Species-level labeled species prediction, and Species-level lumpy-labeled species prediction, respectively. (b), (d), (f), (h) show the training and validation accuracies of 2D CNN in Genus-level labeled species prediction, Genus-level lumpy-labeled species prediction, Species-level labeled species prediction, and Species-level lumpy-labeled species prediction, respectively.

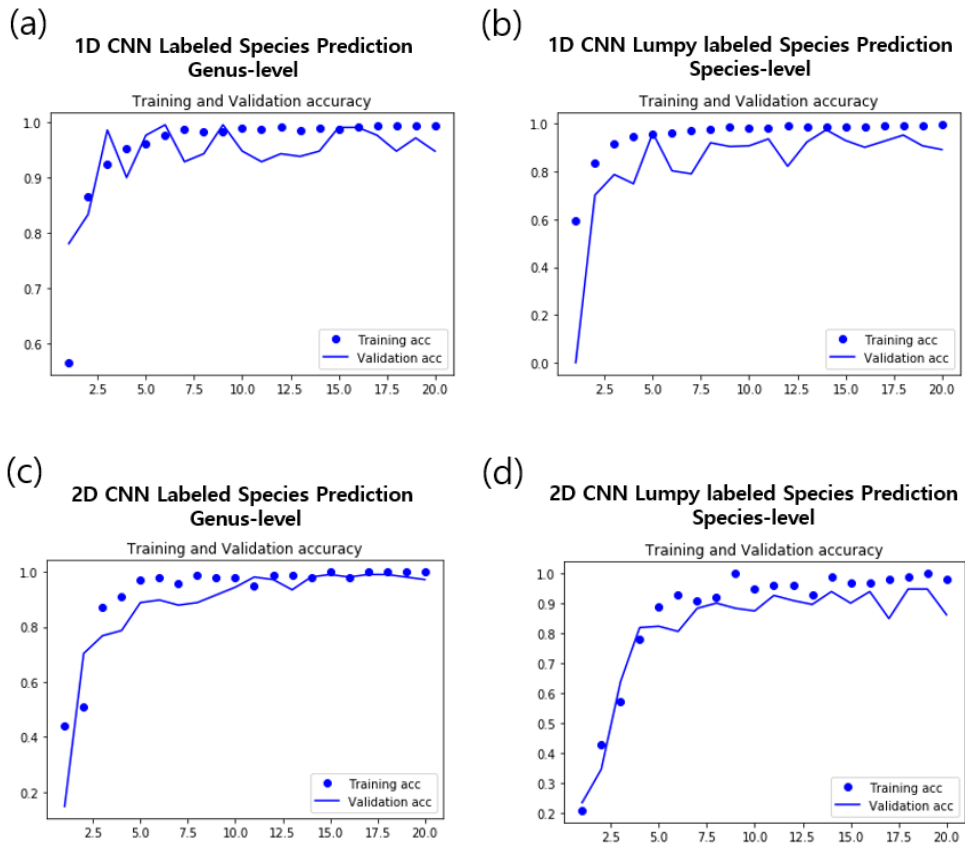


Figure. 8 Training and validation loss of CNN models using cosine-loss as loss function.

	Genus-level Labeled species prediction	Genus-level Lumpy labeled species prediction	Species-level Labeled species prediction	Species-level Lumpy labeled species prediction
KNN	0.9734	0.9047	0.9238	0.8619
LR	0.9156	0.8523	0.8857	0.8238
FFNN	<b>0.9819</b>	0.9122	0.9101	0.8768
1D CNN	0.9810	0.9524	<b>0.9510</b>	0.9374
2D CNN	0.9814	<b>0.9824</b>	0.9373	<b>0.9825</b>

Table. 1 Validation accuracy of ML models for each situation.

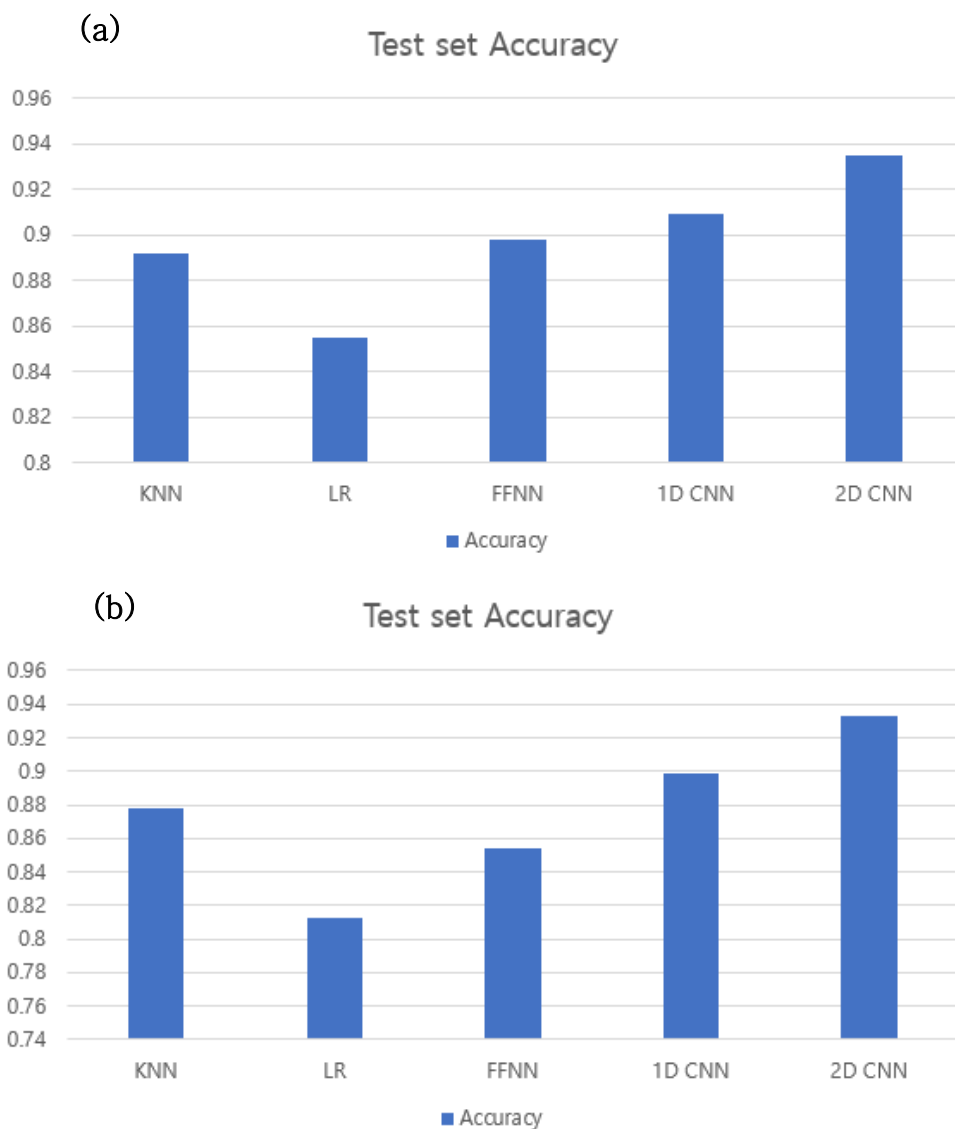


Figure. 9 ML models' accuracies using the test dataset in lumpy-labeled species prediction situation. Prediction results using a genus-level dataset were presented in (a). (b) shows the results of models using a species-level dataset.

## 4.2. Discussion

When using genus-level microbiome data and predicting labeled species, there was not much difference in accuracy between the two types of models (non-CNN and CNN). Therefore, simple non-CNN models were more competitive than CNN models, consuming many resources when analyzing genus-level data and predicting labeled species.

While predicting lumpy-labeled species, the accuracies of the models using CNN were relatively higher than that of other models. It was worthwhile to use the CNN models to discriminate and judge the data of rare species among the data of already labeled species.

Genus-level data had about 3800 vectors, and Species-level data had more than 14000 vectors. While using non-CNN models, the accuracy decreased as the data structure became more complex. However, CNN models were relatively unaffected by the complexity of the data.

Many factors influence the microbiome.[13, 36] Therefore, even samples collected from the same body site of the same species show significant differences in microbiome composition due to variables such as diet, health status, and life stage. For example, the C57BL/6 mouse, often used as an experimental mouse, has a different microbiome if the vendor is different.[37] Therefore, necessary data are often mixed with unnecessary data in large open-source datasets. In this situation, when labeling inspection is performed using ML methods, it is essential to classify numerous types of data that are not subject to analysis in the study. Lumpy-labeled species prediction is suitable for this situation and will classify data not needed in 'etc\_group.' On the other hand, suppose the open-source datasets to be verified are predicted to consist only of samples with the same labeling as the dataset to be used in the study. This case corresponds to the labeled species prediction situation. In this situation, non-CNN ML models with a simpler structure can examine the dataset at a lower cost than the CNN models. However, there is a possibility that data with inaccurate labeling that may be mixed in the dataset to be inspected cannot be identified. This occurs because the result of labeled species



prediction is necessarily concluded as one of the learned species. It is expected to be solved to some extent by introducing an appropriate cut-off according to decode-prediction in the lowest classifier of the ML model.

The reason for the phenomenon that non-CNN models such as KNN cannot make precise predictions as the number of dimensions of data increases is expected that the generated count-matrix is a sparse matrix. When using datasets with a high sparsity level, KNNs have difficulty forming reliable neighborhoods.[38] The data used in this study are high-dimensional, and if the model tries to classify more than seven species, the data structure becomes more complex. The generated count-matrix is highly likely to become a sparse matrix. The random forest model used in previous studies is also expected to show similar results. The random forest has the advantages of simple model structure, less overfitting, and good generalization to new data. However, the structure of the model - due to the use of multiple decision trees, memory usage is high, and there are problems that it does not work well for high-dimensional data or sparse data.[39]

## Chapter 5. Conclusion

As a large number of data can be used with the continuous development of hardware, the possibility of machine learning using large datasets has already been sufficiently verified in the microbiome field. To properly utilize the large open-source datasets, it is necessary to check the correct labeling of the data. Therefore, this study examined that host predicting machine learning models could be designed with sufficiently significant accuracy with appropriate tuning and loss function setting when using a small gut microbiome dataset as a training dataset.

As a result of the study, the convolutional neural network-based models had the disadvantages of using more resources and taking a long time to learn. However, they maintained high accuracy compared to other discriminative models that were lumpy-labeled or more complex. Conversely, with simple construction and learning, the non-CNN ML models showed similar performance to the CNN-based models in discriminating genus-level data and accurately labeled data. In addition, it was confirmed that the machine learning

model could be used sufficiently even on a small dataset through appropriate design adjustments and function settings. Therefore, researchers can check whether the data is appropriately labeled by using ML models that have been trained on a small number of data that have been reliably investigated before using a large public dataset.

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## 초 록

본 연구는 합성곱 신경망에 기반한 기계학습 모델들과 기반하지 않은 모델들의 성능과 장단점 비교를 목적으로 하며, 주어진 데이터의 종류와 목적에 따라 다양한 기계학습 모델들의 성능을 분석했다. 계속되는 하드웨어의 발달로 다수의 데이터를 활용할 수 있게 되면서 미생물 군집 분석에 매우 큰 데이터셋을 활용한 기계학습의 가능성은 이미 충분히 검증되고 있다. 분석결과에 의도치 않은 노이즈가 포함되지 않기 위해서는, 오픈소스 거대 데이터셋을 사용하기 전에 사용할 데이터셋이 정확히 라벨링이 되어있는지 확인하여야 한다. 본 연구는 상대적으로 작은 장내 미생물 군집 데이터 셋을 사용하여 호스트를 예측하는 기계 학습 모델이 적절한 조정 및 손실 기능 설정으로 상당한 정확도로 설계될 수 있음을 확인했다.

본 연구에서는 분변 미생물 군집 데이터셋(샘플 4108개, 672종)를 이용하여 머신 러닝 모델의 성능을 비교하였다. 훈련 및 검증 데이터 셋(871개 샘플, 34종)은 전체 미생물 군집 데이터셋의 작은 하위 집합으로 구성되었다. 그리고 데이터의 복잡도와 ML 모델의 예측 목적 등 문제 상황 설정에 따라 성능에 차이가 있는 것으로 나타났다. 연구 결과, 합성곱 신경망 기반 모델들은 사용하는 리소스가 많고 학습에 필요한 시간이 더 오래 걸린다는 단점들이 있었다. 그러나

데이터의 복잡도가 증가하고 레이블이 정확하게 지정되지 않은 데이터들을 판별함에 있어 다른 모델들에 비해 높은 정확도를 유지하였다. 반대로 합성곱 신경망을 사용하지 않은 모델들은 구성과 학습이 간단하고, 단순한 데이터들과 정확하게 레이블이 지정된 데이터들을 판별함에 있어 신경망 기반 모델과 비슷한 성능을 보였다. 또한 적절한 구조설계와 함수 설정을 통해 기계학습 모델이 작은 데이터셋을 기반으로도 충분히 사용될 수 있음을 확인하였다.

간단한 기계 학습 방법으로 적은 수의 정확하게 레이블이 지정된 데이터를 사용하여 대규모 데이터 세트의 데이터 레이블을 검증할 수 있다. 이는 연구에 사용하기 전에 오픈 소스로 게시된 대규모 데이터 세트의 레이블 지정 정확도를 확인하는 데 사용할 수 있다.

**주요어 :** 기계학습, 작은 데이터셋, 장내 미생물 군집, 숙주 예측, 합성곱 신경망

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