Viability of bio-based chemicals from food waste

D6.8 Report investigating fuels and chemicals from mixed, post-consumer food waste and the selection of bacterial strains for growth on food waste

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1 Executive Summary

1.1 Objectives of work

REFRESH is an EU H2020 funded research project (running from 2015-2019) taking action against food waste. Twenty-six partners from 12 European countries and China are working towards the project's aim to contribute towards Sustainable Development Goal 12.3 of halving per capita food waste at the retail and consumer level and reducing food losses along production and supply chains, reducing waste management costs, and maximizing the value from un-avoidable food waste and packaging materials.

This piece of analysis sits within REFRESH Work Package 6: Valorisation of waste streams and co-products. One of the key objectives within this work is to identify key valorisation capacities, approaches and technologies for these waste streams and to develop and / or improve technologies for producing food ingredients, feed ingredients, fuels and chemicals. The key valorising route discussed in this report are (bio-)chemicals from mixed, post-consumer waste and the selection of bacterial strains for growth on unavoidable food waste sources (putrescible, post-consumer/retail stage).

This report focuses on 3 aspects as follows:

1. Scientific research into the conversion of mixed post-consumer organic putrescible food waste materials into fuels and chemicals. These are materials left uneaten at the consumer/retail stage, discarded into a general waste or food waste disposal stream.
2. Data sources that can be used to characterise mixed putrescible waste arising and composition that fit with the existing context of composition of post-consumer putrescible food waste in the EU.
3. Experiments conducted to identify key genetic features of bacteria suitable for growing on a mixture of putrescible waste and further steps required to explore candidate chemicals for prospects of production by way of an example with regard to bioplastic PHB production.

The findings of this work provide key insights to both researchers and innovative industrial stakeholders seeking new technological opportunities to create added value from unavoidable food waste.

1.2 Key findings

- Characterising food waste composition indicates that valorising mixed post-consumer putrescible food waste for fuels and chemicals is challenging due to the variability of waste streams.
- Evidence for the potential conversion of consumer putrescible mixed food waste to a selection of candidate fuels and chemicals found in the literature is limited. Research is evident at the laboratory scale with some promising EU pilot scale approaches currently being implemented. Most scaled bio-based fuels and chemicals currently rely on crop-based commodities.
- In this report state of the art microbial genetic technology has been successfully used to select, from a large diversity of many individual clones of a bacterial isolate, mutants that can grow successfully on a characterised putrescible waste.
- All successfully growing clones have been sequenced to identify which genes changed to allow improved growth.
- This is a successful step towards producing commercially viable strains for the valorisation of mixed post-consumer food wastes into bacterial biomass.

The next steps would be to use these findings to investigate the selected strains for possible candidate chemical production in pilot scale research applications. An example how to go about this is given for the biopolymer PHA.

2 Introduction

2.1 Background

2.1.1 Policy background

Waste policy

The EU Waste Framework Directive (WFD) aims to first minimise the volume of putrescible food waste that arises by maximising its use as a food. However, in many countries, a considerable proportion of wasted food and food residues still ends up as a mixed putrescible waste (Stenmark et al 2016). Therefore, taking a pragmatic approach, and acknowledging even with best practice food waste minimisation approaches in modern food retail and consumption there will always be a fraction of mixed putrescible consumer food that is wasted which cannot practically be avoided. So, with this proviso, maximising the use of this putrescible food waste can be justified to recover value. This also adheres to EU Waste Framework Directive priority of recovery before the least desirable option, disposal.

Energy policy

In addition, concerns raised regarding environmental benefits of fuels derived from crops (Searchinger et al 2008) led to some Member State commissioned policy reviews concluding feedstock production must avoid agricultural land that would otherwise be used for food production (Gallagher 2008). Subsequently this led to changes in European policy. Notably amendments to Renewable Energy and Fuel Quality Directives in 2015 limits the cultivation of biofuels on agricultural land to 7%[1] to avert the risk of displacing food production to previously non-agricultural land. This displacement, referred to as indirect land use change, (ILUC) risks negating greenhouse gas savings that result from biofuels. Such changes are associated with conversion of grasslands and forests elsewhere, because of increased demand for crops, releasing existing stores of carbon to the atmosphere and in some cases preventing the potential for further absorption.

Currently specific production land use for Europe's existing biobased products ranges between 0.35 to 0.77 ha per tonne (Spekreijse et al 2019). This is relevant for any notion of expanding the production of sustainable bio-based alternatives where dependence on crops may have consequences for greenhouse gas emissions from indirect land use change. However, there is considerable uncertainty associated with quantifying these consequences (Finkbeiner 2014, Smidt et al 2015). Therefore, taking a precautionary approach, with the proviso that food waste processing impacts is broadly comparable to any crop based feedstock processing, utilising food wastes as a resource may be considered to a relatively lower risk of driving global warming impacts than crop-based commodities. Revised

[1]The so called ILUC Directive.

renewable energy targets for the EU[2] whilst supporting energy from anaerobic digestion of food wastes also now requires a certain percentage of renewable energy to be supplied by advanced biofuels. These may (as one of a number of options) incorporate biomass fraction from unsorted household waste and bio-wastes separately collected from households. This inevitably includes post-consumer food wastes alongside other organic fractions such as green wastes. This concept also links with new EU frameworks for circular economy package[3].

2.1.2 Current waste management processes

Member states can and do segregate wasted food and food residues from households and catering services, either at source or centrally by employing post separation technologies. Statistics for the UK, for example, indicate a sizable proportion of putrescible food waste still ends up in general waste streams from households (84%), but some regions have demonstrated much greater capture rates (WRAP 2016a). There are also major volumes of food wastes that are currently disposed of by UK manufacturing and commercial food retail sectors (WRAP 2016b). Both examples indicate significant potential for further capture of mixed putrescible food wastes.

The result is a mixture of putrescible wasted food and food preparation residues that may be disposed of through dedicated municipal and commercial treatment facilities. Waste recovery technologies such as anaerobic digestion or composting are increasingly employed in Member States[4]. Composting is effective if done well. However, if poorly controlled, there is a risk of fugitive emissions, odour and pest problems. Likewise, producing biogas and methane via anaerobic digestion (AD) plants also has many advantages when energy is efficiently generated, the risk of fugitive emissions is controlled, and digestate quality is suitable for further use (JRC 2017).

2.1.3 The biorefinery concept

The concept of a biorefinery typically includes several integrated mechanical and/or biological process steps that transform a biomass feedstock into a range of products which can support the economic viability of the enterprise.

Holistic or cascading approaches to feedstock exploitation are the conceptual basis for biorefineries. Commercially, however, the businesses closest to the biorefinery concept are seen to be those primarily producing biofuels, initially supported by

[2] The so called RED II, the re-cast Renewable Energy Directive was adopted by the EC on 4 December 2018 and will be mandatory across all member states. This now requires fuel suppliers to supply a minimum of 14% energy content of fuels consumed in road and rail transport to be from renewable sources by 2030, advanced fuels (including fuels from food waste fractions of municipal waste) should constitute 3.5% by 2030.

[3] On 4 March 2019, the European Commission adopted a comprehensive report on the implementation of the Circular Economy Action Plan. The report includes notions of shaping a climate-neutral EU circular economy, where pressure on natural and freshwater resources as well as ecosystems is minimised.

[4] Source: country reports published on the European Composting Network website, accessed May 2018

favourable policy instruments, that have evolved into generating revenue streams from co-product development (Kasnitz 2018, Bauer et al 2017).

Another concept associated with biorefineries has been the sustainable use of resources. The main feature being the use of 'renewable' feedstock, that can be regrown such as forestry, crops or algae, in contrast to the finite fossil resources exploited for petrochemicals and fuels. However, as acknowledged in part by the recent amendments to EU policy (referring to the issue of ILUC in 2.1.1) deeper notions of the sustainable use of resources may also include the dependency of such feedstock on non-renewable fertilisers and fuels, and their impact on soil, water, air quality and climate that are important for sustaining ecosystem services they depend on. In this respect 'renewable' resources can be viewed within the context of sustaining the wider systems on which they depend. Again, this deeper notion of sustainable resource use is also reflected in the EU's recently published circular economy report[3]

2.1.4 Microbial conversion

Bacterial pathways of metabolism are considered the most diverse on the planet; and advances in genetics offers significant biochemical production potential. Though microbial production of chemicals has been established for decades bacteria are now increasingly being exploited by biotechnology companies in developing processes to synthesise products in a more competitive way.

Key examples of existing microbial conversion applications in chemical production processes are given in the next section.

2.2 Key examples of biobased fuels and chemicals in relation to exploitation of mixed post-consumer food waste

2.2.1 Introduction

Post-consumer mixed food waste for generating fuels such as biogas and also upgrading to bio-methane is more widely undertaken in anaerobic digestion plants across Europe[5]. However, the literature concerning waste valorisation into chemicals typically identifies specific (i.e. non-mixed) waste streams and food chain by-products (Galanakis 2012; Tuck et al 2012; Ki Lin et al 2013; Mirabella et al 2014). Valorising mixed post-consumer waste for chemicals is likely to be more challenging, not least because of its variable composition and characterisation (appendix 1). For research this challenge is less tractable since the variable composition of mixed food wastes by geography and season is likely to make it much less amenable for universal approaches but also for practical comparisons across studies in scientific literature.

2.2.2 Fuels and solvents

Ethanol from mixed food waste

Ethanol is the main candidate for the ready fuel market and is commercially distributed in many countries. However, current (*first generation*) industrial bioethanol is made either from starch- or sucrose-derived feedstock such as e.g. cereal grains (corn, wheat) or sugar crops (sugar cane, sugar beet). Pilot second generation biorefineries which convert sugars obtained from less accessible cellulosic sources also rely on commodity residues rather than mixed wastes and currently constitute <0.2% of the EU's 4.35 Million tonnes of ethanol fuel production (IEA 2018).

Unfortunately, putrescible food waste comprises a variable mixture of substrates and sugar types, and thus is not as straightforward to exploit. Nevertheless, it is generally low in lignified tissues and therefore more readily digestible.

There has been some research interest in the conversion of organic municipal solid waste (OMSW) and food waste streams as substrates for production of ethanol as a renewable biofuel or platform for bio-based chemicals (Table 1)

Most of these rely on enzymes to hydrolyse food waste into fermentable substrates. In some cases, research approaches sterilise food waste to prevent bacteria/spoilage impacting yields and recalcitrant fermentation residues are also further hydrothermally treated to maximise overall yield (Matsakas et al 2014). Research shows fermentation may also be phased using different strains of yeasts that utilise different fractions of mixed food waste to improve ethanol production

[5] For example see European country report publications from by the European Composting Network.

(Jeung et al 2012). However, these processes are not translated to commercial scale applications.

In an overview of physical and chemical characteristics of alcohols from fermentation of food chain sideflows, Hegde et al (2018) compare yields in fermentation broth with those from commercial ethanol concentrations from commodity feedstock corn (Figure 1), which is also comparable to commercial ethanol fermentation concentrations of 7–11% (v/v) achieved from cane juice and molasses feedstock (Amorim et al 2011). Though this a step further, it can only serve as a crude indication[6] that ethanol production from food wastes high in sugar and starch (apple pomace, potato waste) compare more favourably with commercial feedstock. This is an indication that is also reflected in other research into ethanol using a high starch retail food waste (Figure 1) formulated from mashed potatoes, sweetcorn, and white bread, rather than samples of actual retail food wastes.

Pilot scale plants

Using unnamed biocatalysts to conduct single stage Simultaneous Saccharification and Fermentation (SSF) Ebner et al 2014 report good ethanol yields from a 1/15th industrial scale (10 wet tonnes per day) pilot plant co-fermenting 2.3 tonnes of mixed supermarket food wastes and 2.4 tonnes of diluted fruit syrup food processing waste. The US company operating the pilot plant from the study is no longer active so it is difficult to substantiate the commercial potential of this approach.

There are few working concept plants demonstrating that putrescible food waste can be used to produce ethanol on a commercial or semi commercial basis; One example is that reported by the company ST1 for its Bionolix plant in Hameenlinna, Finland:

- Producing 1 million litres/year of ethanol from 19 kT of putrescible household and retail wastes,
- It requires energy generated from biogas from onsite anaerobic digestion of processing residues and
- It relies on sharing an external, centralised dehydration plant to upgrade ethanol into fuel from other (non-food waste) decentralised plants.

(Norden 2012)[7].

Another example is the PERSEO demonstration plant reportedly producing ethanol economically with:

- Waste processing capacity of 9 kT per year and

[6] True comparators are likely to be more nuanced given the different subsidies that bio-ethanol may attract in different countries but also factoring logistical considerations into the apparent low cost of waste feedstock.
[7] See http://www.st1biofuels.com/solutions , also 4.2.1 (p32) in Norden 2012

- ethanol yields projected 220kg/tonne, approximating to 2 million litres/year.

It must be noted however that these demonstration plants have not been expanded or replicated at industrial scale.

In summary, whilst it is scientifically possible to convert mixed putrescible food waste to ethanol using various enzymes, it is difficult to gain a clear picture from current research literature on whether these are economically viable at commercial scales. Understanding industrial scale fermentation and it commercial potential also requires the approaches and conditions to be adequately replicated which can be seen as a significant challenge for laboratory-based research (Amorim et al 2011).

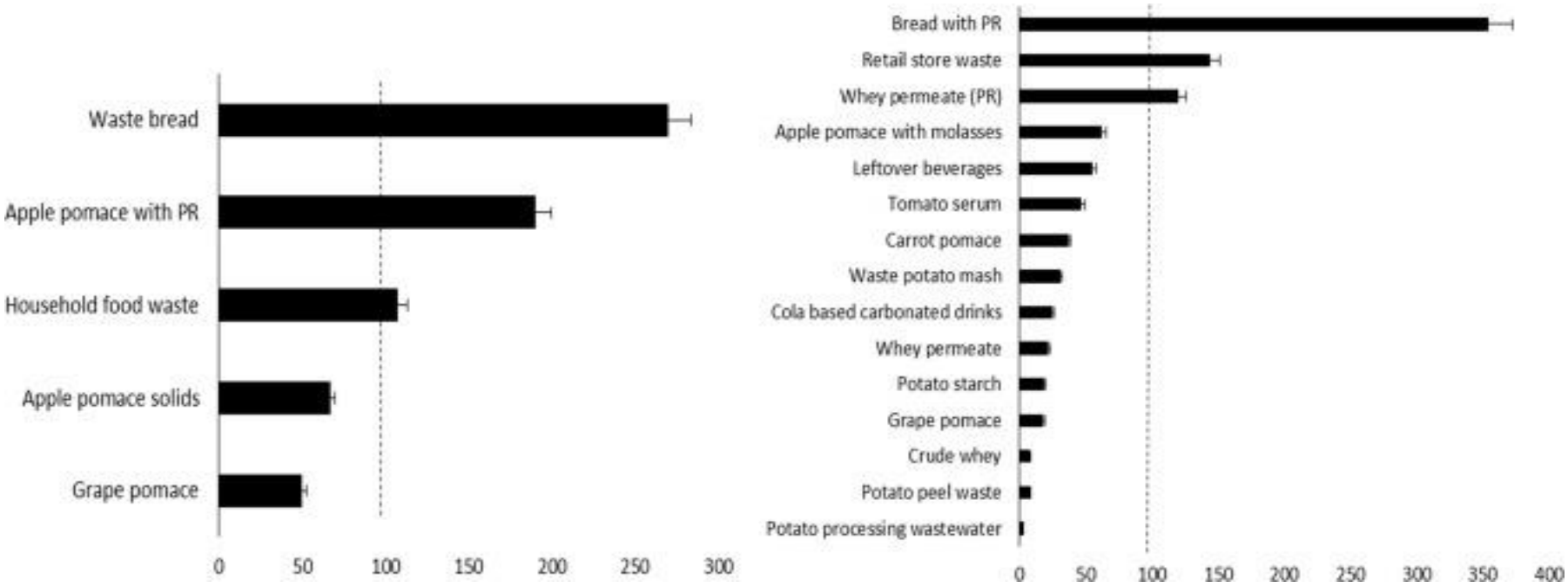


Figure 1 Reported research results on crude ethanol production (left: g/kg substrate, right: g/litre fermentation broth) compared to a commercial benchmark (corn ethanol – dotted line) source: Hegde et al 2018. PR = with a product recovery process employed, error bars represent 5%.

Table 1 Example research published on Ethanol production from food waste (cited in Matsakas 2014 and Jeoung et al 2014).

Food waste source	Pre-treatment	Enzymes	Ethanol yield parameters		
			production (g/L)	Productivity (g/L·h)	Yield (g/g)
Households (dm 45% w/v)	Hydrothermal 200C : only of fermentation residue with acetic acid catalyst	1:5 commercial cellulase and B-glucosidase	42.8	2.85	0.108
Cafeteria	–	Glucoamylase & carbohydrase	n/a	n/a	0.43
Cafeteria	–	Amyloglucoside & carbohydrase	29.1	1.94	0.23
Dinner centre	–	α-Amylases & glucoamylases	8	n/a	n/a
Cafeteria & households	–	α-Amylases, amyloglucosidase, cellulase, & β-glucosidase	32.2	0.55	0.16
Cafeteria	–	Glucoamylases & β-glucanase	48.6	2.03	n/a
Retail store	–	α-Amylase, glucoamylase, & protease	n.a.	n/a	0.36
Dinner centre	–	α-Amylase & glucoamylase	87.9	1.83	n/a
Cafeteria	–	Enzyme solution produced in situ by *A. awamori*	58	1.81	n/a
Leachate	–	–	24.2	0.61	n/a
Modelled organic fraction of MSW	85°C for 1 h	Mixture of cellulase, amylase, protease, hemicellulose, lipase, & pectate lyase	42.8	2.14	n/a
Cafeteria waste	121°C 15 min	Commercial glucan-glucohydrase and carbohydrase enzymes and co- fermentation with two yeast strains	48.6	2.0	n/a

The Acetone, Butanol, Ethanol process

Commercialised bacteria based chemical production began during the first world war using Clostridia for producing Acetone for ordinance (cordite), Butanol and Ethanol known as the 'ABE process' which was superseded by cheaper fossil-based synthesis. Butanol, as well as ethanol, whilst being platform chemicals and solvents may also be used as drop-in fuels for combustion engines.

Various metabolic pathways produce solvents in the ABE process typically in a ratio of 3 parts of acetone, 6 parts of butanol to 1 part of ethanol during a second low pH phase of fermentation (Zhou et al 2018).

In the UK improved microbial ABE production of n-butanol and acetone re-emerged commercially, albeit in a small operation, through genetically selecting from comprehensive library of solventogenic Clostridium strains utilising commodity by-products[8]. These are marketed with a 'renewable' association as a selling point.

Research into the production of butanol from conversion of mixed food waste

In an overview of physical and chemical characteristics of alcohols from fermentation of food chain sideflows, Hegde et al 2018 compare yields in fermentation broth with those of ethanol and butanol concentrations from commodity feedstock corn (maize) and report only conversion experiments using two food wastes (apple pomace and potato starch substrates) that compare favourably with that of commercial feed stocks (Figure 2). A study on ABE production from fermenting hydrolysed fresh and dried domestic garden and food wastes pre-treated by extrusion at 120ºC using a solventogenic *Clostridia acetobutylicum* strain (ATCC 824) indicated incomplete hydrolysis using commercial cellulases and B-glucosidases (Lopez-Contreras et al 2000). 4.2, 1.5 and 0.4 g/litre of butanol, acetone and ethanol, respectively, 6.1g ABE/litre total, were produced from fermentation of a sample of fresh hydrolysed domestic organic waste after 48 hours. The authors indicated the need for genetic construction of strains to enable a wider utilisation of sugars (by degrading cellulosic fractions of domestic organic wastes) to make ABE production economically viable.

Research by Huang et al 2015b utilising model food waste medium high in starch (potatoes, sweetcorn and white bread) achieved higher yields of ABE utilising *Clostridium beijerinckii strain P260* on food waste than on a glucose media control (18.9 and 14.2 g/litre respectively). The food waste yield of acetone, butanol and ethanol, were 5.2, 12.3, 1.4 and g/L, respectively). Again, this was a hypothesises high starch food waste model, which may not be typical of mixed food wastes compositions elsewhere in research (appendix 1). This organism also required no enzyme hydrolysis pre-treatment of the food waste since this strain allows simultaneous saccharification and fermentation (SSF) by secreting enzymes as part of its metabolic process. In addition, the researchers also employed vacuum

[8] http://www.greenbiologics.com/technology.php website accessed May 2018.

stripping to circumvent the inhibitory effect of butanol build up which allowed 20% higher productivities and were also an improvement on similar batch-fed experiments. This approach allowed a near-complete utilisation of converted sugars at higher food waste fermenter concentrations (129g/litre) potentially benefiting economics of processing at scale.

Again, as for ethanol from food waste, these are yields from litre scale bench top experiments. Without recourse to assessments scaling to commercial level processes, and their associated, context specific, production costs it is difficult to make solid conclusions from comparing crude production from different feedstock.

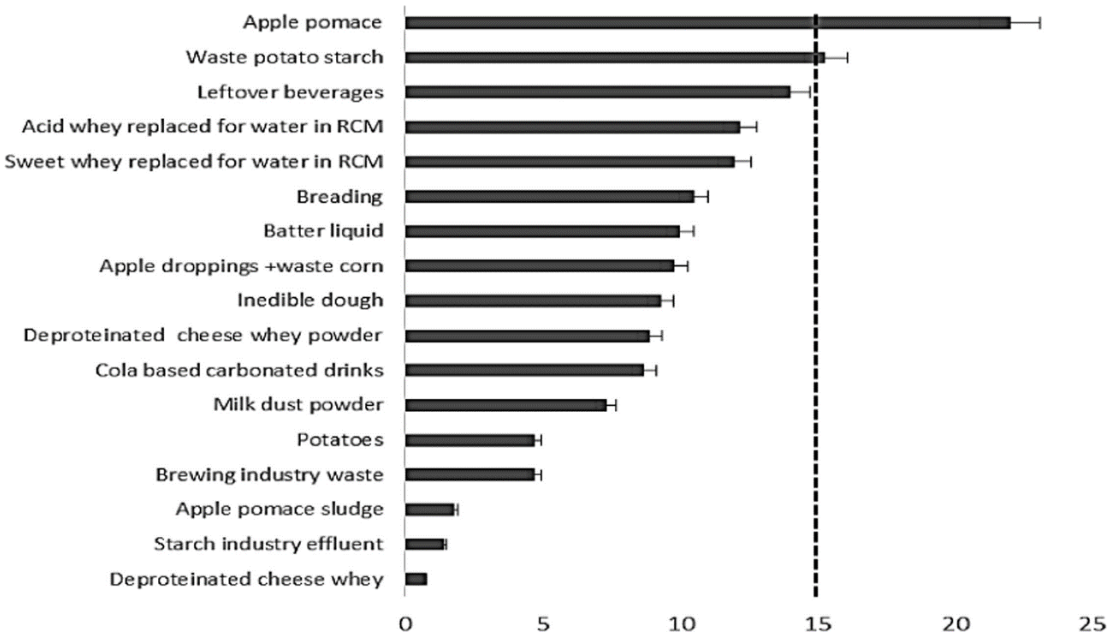


Figure 2 Examples of Butanol concentrations g/litre reported from fermentation of various food waste streams published in research journals (Taken from a review by Hegde et al 2018) *as a crude comparator dotted line indicates fermentation production from commercial corn feedstock.* RCM = Reinforced clostridial medium (substrate typically used for growing clostridia and other anaerobic bacteria).

2.2.3 Organic acids

Succinic acid production

Succinic acid has been identified as one of the top ten near market bio-based platform chemicals with a projected large future market potential as a precursor for the synthesis of high-value products, commodity chemicals, fine chemicals, polymers, surfactants, and solvents (NREL 2016, Figure 3).

Commercial production of bio-based succinic acid already exists. Roquette (Former JV Parent company of Reverdia with DSM, is to be dissolved April 2019) has produced 10,000 tonnes per year of Biosuccinium® at a site in Cassano, Italy using patented recombinant *S. cerevisiae* yeast strains developed by DSM to convert carbohydrates from corn or maize (Nghiem et al 2017; Cok et al 2014). In the US Myriant production in Louisiana is based on conversion of feedstock such as Sorghum and sugar beet using recombinant E.coli ,with a capacity of 13,500 tonnes of Succinic acid per year. Myriant is also producing 1,300 tonnes per year of bio-based succinic acid at ThyssenKrupp Uhde's biotech commercial validation facility in Leuna, Germany.

Also in Germany, Succinity GmbH, a joint venture of BASF and Corbion, produce bio-succinic acid using commodity feedstock sucrose, glucose and glycerol using fermentation by the proprietary microorganism *Basfia succiniciproducens*. Bioamber Inc's plant in Sarnia, Canada has been reported to have produced 30,000 tonnes of Succinic acid per year from corn syrup using yeast strains, with 2017 sales of bio-succinic acid of $14.9 million until bankruptcy filings in 2018 and sale of assets[9].

[9] Monitors, PWC statement: Liquidation of BioAmber's assets will result in little to no residual value for non-secured creditors and no residual value for equity investors. Source: Biomass Magazine.

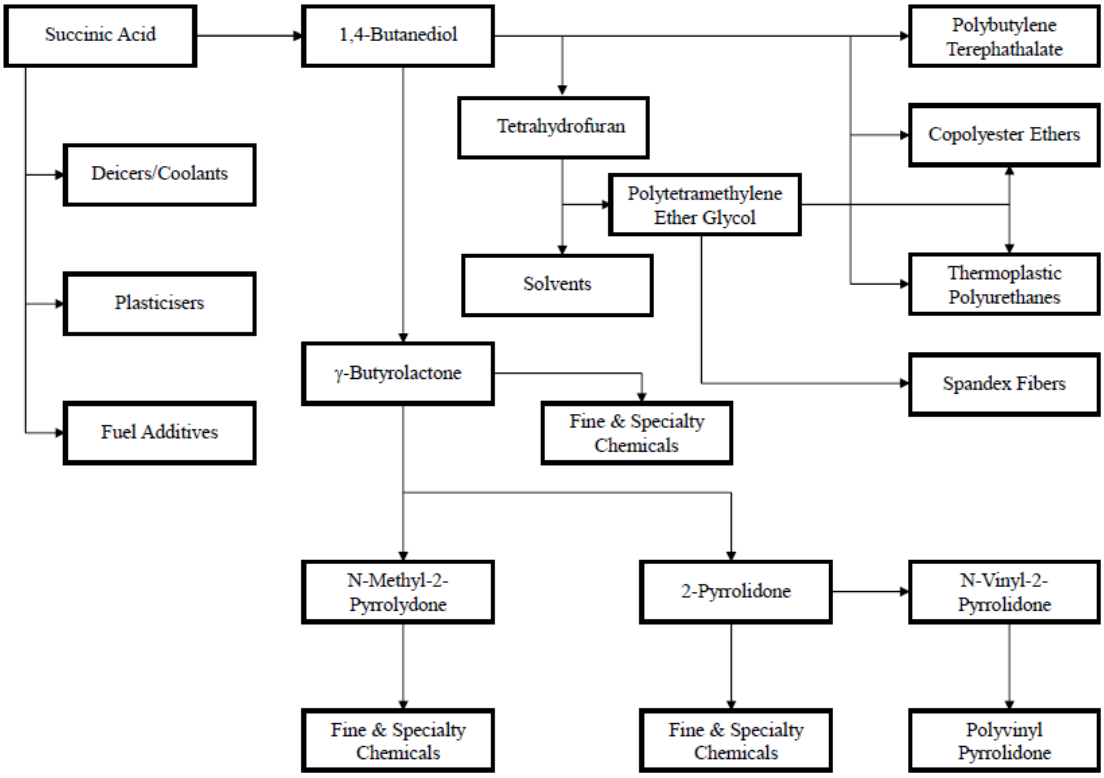


Figure 3 The succinic acid value chain (From Nghiem et al 2017 adapted from IEA Bioenergy–Task 42)

Research into mixed food waste for Succinic acid production

Laboratory scale research into succinic acid from mixed food waste has been published using natural and genetically modified bacteria, (*Actinobacilus succinogene* and a recombinant *E.Coli* respectively*)* to convert a mixed canteen waste substrate (Sun et al 2014). The canteen waste was first converted to a nitrogen and glucose rich hydrolysate using fungi *Aspergillus awamori* and *A. oryzae*. The advantages of using fungal hydrolysis pre-treatment as noted by Dessie et al 2018 are threefold:

1. avoidance of using hazardous chemicals,
2. reduced risks of generating fermentative inhibitors and
3. decrease fermentation cost by avoiding use of commercial chemicals.

The yields were shown to be broadly comparable to other non-mixed wastes using fungal hydrolysis. Table 2 shows a summary of research results for microbial succinic acid yields using various food chain related materials and wastes substrates – all of which are from laboratory scale experiments.

For comparison, industrial succinic acid production relies on proprietary or selected strains in optimum conditions using commodity feedstock to produce competitive fermentation concentrations of 50 g succinic acid per litre (Nghiem et al 2017).

Limited research has been found specifically addressing the economic viability of approaches using *mixed* putrescible food wastes to produce succinic acid. The nearest research, using *Actinobacilus succinogenes* fermentation, applies to glucose and amino acids from a pilot scale model theoretically using 1 tonne/day bakery waste hydrolysed with industrial grade enzymes (Lam et al 2014). The study indicates conversion into succinic acid could be economically viable where yields are assumed to be directly scalable from those found in laboratory experiments[10]. However, more recently Dessie et al.'s (2018) review indicates that fermentative production using natural *Actinobacilus succinogenes* is unable to demonstrate effective use for industrial scale commercial production.

Genetic modification is proposed as a way of circumventing many of the limiting factors, such as unwanted by-products, which has been reported to make the downstream processing cost less competitive for the natural producer of succinic acid, *A. succinogenes (*Dessie et al 2018). This is also echoed by Pleisnner et al 2016 who indicate that whilst lactic acid production is possible with native microbial strains, the successful and efficient conversion of organic residues into succinic acid or fatty acids likely depends on the use of engineered microbial strains. This can be seen also in recent research where the use of engineered strains of aerobic, oleaginous yeast *Yarrowia lipolytica (*Li et al 2018), shows relatively high yields converting fruit and vegetable wastes (Table 2).

This obviously has socio-political implications where popular opinion may be against the proliferation of genetically modified organisms, especially where product chemicals are to be used in the food chain, even if the scientific community generally considers this to be safe or of minimal risk to wider ecological systems.

[10] Yield of 0.55 g succinic acid/ g of bread extracted from a broth concentration of 47.3 g succinic acid /L taken form Lueng et al 2012, cited in Lam et al 2014)

Table 2 Published laboratory scale research on succinic acid yields from microbial conversion of various food chain substrates (*A. succinogenes* source and citations in Dessie et al 2018, except *Y. lipolytica*, Li et al 2018).

Substrate	Micro-organism	Pre-treatment	Nitrogen source (g/L)	Fermentation type	Concentration (g/L)	SA productivity (g/L/h)	Yield (g/g)
Corncob	*A. succinogenes*	H_2SO_4	Yeast Extract	Batch	23.64	0.49	0.58
Wheat milling by-products	*A. succinogenes*	Fungal autolysis	Hydrolysate	Batch	50.6	1.04	0.73
	A. succinogenes		Yeast Extract	Batch	62.1	0.91	1.02
Mixed food waste	*A. succinogenes*		Hydrolysate	Batch	24.1	0.29	0.87
	Recombinant E.coli (MG1655)		Hydrolysate	Batch	26.4	0.20	0.98
Pastry waste	*A. succinogenes*		Hydrolysate	Batch	17.1	0.24	0.85
	Recombinant E.coli (MG1655)		Hydrolysate	Batch	26.5	0.20	0.79
Waste bread	*A. succinogenes*		Hydrolysate	Batch	47.3	1.12	1.16
Cake waste	*A. succinogenes*		Hydrolysate	Batch	24.8	0.79	0.8
Pastry waste	*A. succinogenes*		Hydrolysate	Batch	31.7	0.87	0.67
Fruit and vegetable waste	*A. succinogenes*		Hydrolysate	Batch	27.0	1.28	1.18
	Recombinant Y. lipolytica PSA02004	Enzymes	Hydrolysate	Batch	45.6	0.69	0.46
Whey	*A. succinogenes*	None	Yeast Extract and Peptone	Batch	21.3	0.44	0.57
Rapeseed meal	*A. succinogenes*	H_2SO_4 and pectinase	Yeast Extract	Fed-batch	23.4	0.33	0.115
Citrus peel waste	*A. succinogenes*	H_2SO_4, Enzymes	Yeast Extract	Batch	8.3	-	0.7

Lactic Acid

Lactic acid is produced industrially via fermentation, with dominant applications in the food industry and also the pharmaceutical industry as well as other industrial uses. In 2004 the US DoE concluded that Lactic acid (LA) would be one of the dozen most promising value-added building blocks that can be derived from sugars for the production of various commodity and specialty chemicals (Werpy and Peterson 2004).

Recently an increased demand for lactic acid has been driven in part by the market for polylactic acid used for biodegradable plastics (Nova Institute 2018). Industrial scale production of polylactic acid provides over 10% of the bioplastics market and is second only to starch blends in the biodegradable market sector (Nova Institute 2018). PLA based products are able to replace key fossil plastics such as polystyrene and polypropylene. Though PLA can be chemically synthesised, 95% of production is through bacterial fermentation[11]. Selected Lactobacillus bacteria species are used to ferment simple sugar-based substrates from crop feedstock such as maize or corn, although research is now being carried out to implement commercial-scale methane to lactic acid fermentation technology (Nova Institute 2018).

The major manufacturers of PLA include:

- NatureWorks LLC, US 150,000 tonnes per year.[12]
- Novamont SpA, Italy 150,000 tonnes per year.
- BASF, Germany.
- PTT MCC Biochem Co., Ltd.[13]
- Corbion/Total-Corbion, Netherlands[14].

[11] Corbion website accessed Sept 2018

[12] A joint venture of Cargill and PTT Global Chemical.

[13] A joint venture of PTT and Mitsubishi Chemical Corporation, Source: Bioplastics Magazine: *Biodegradable polymers market forecast to rise sharply by 2023*
Published online 27.07.2018, website accessed Sept 2018.

[14] More recently a joint venture between TOTAL and Netherlands company Corbion announced production from its 75,000-ton-per-year plant in Rayong, Thailand using sugar cane. Accessed Feb 2019.

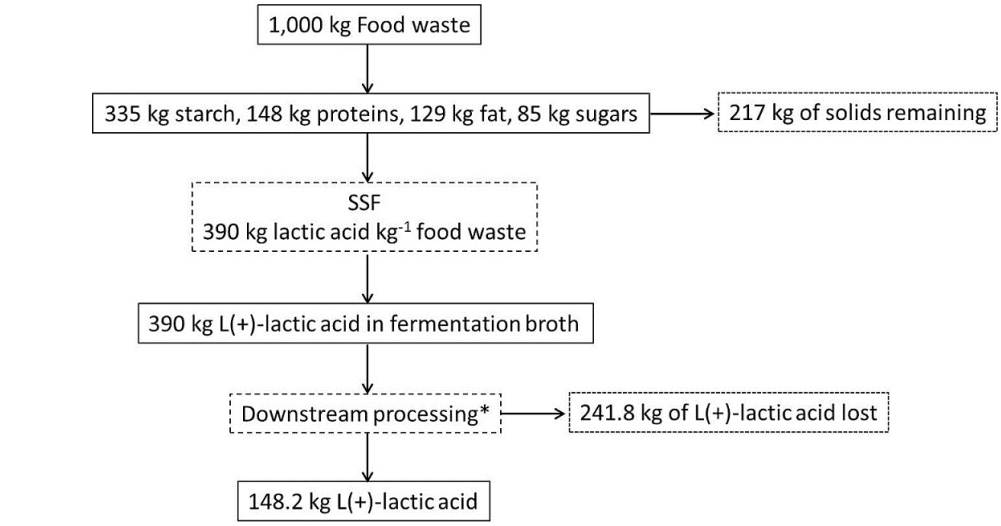
Food waste for lactic acid /polylactic acid production

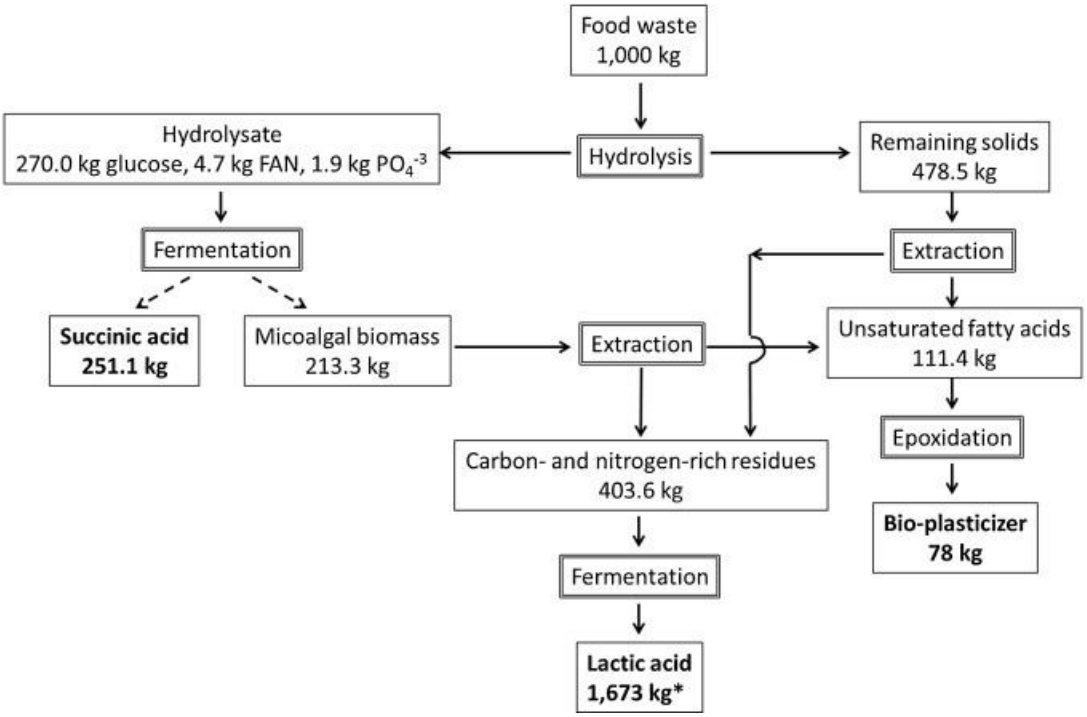
Producing lactic acid through microbial conversion of organic food processing wastes has generated interest recently with 22 Million Euro funded AgriChemWhey investing in industrial-scale bio-refinery to prove the techno-economic viability of converting whey permeate (WP) and delactosed whey permeate (DLP) into lactic acid. However, this is at an early stage of development having only started in 2018[15].

The application of microbial conversion of *mixed, post-consumer* food waste substrates to produce lactic acid has been reported at laboratory stages in published research literature using various fermentation approaches (Table 3). Again, whilst it is feasible to demonstrate yields at this scale in laboratories it is challenging to assess whether these are viable at commercial scales. No studies were found with production yields at technical pilot scales or greater. The laboratory based study using simultaneous saccharification and fermentation by Pleissner (2017) reports only 38% of the initial lactic acid could be recovered from fermentation broth due to downstream processes required to remove salts, including a subsequent evaporation step to concentrate lactic acid by 13 (Figure 4 top).

Also, management of the resulting solids residues may have cost implications. In previous work a conceptual biorefinery approach has been considered to further utilise the solid residues from succinic acid production in a cascading approach (Figure 4 bottom). However, the authors indicate lactic acid would still require an additional 1917.5 kg glucose from other sources to make up the feedstock carbon requirement. These indicate some of the potential challenges in translating laboratory research and conceptual processes and into a commercially viable enterprise.

[15] https://www.agrichemwhey.com/news/agrichemwhey-kick-off-press-release/





*requiring additional 1.9 tonnes of glucose feedstock sourced from elsewhere

Figure 4 Above: Lactic acid production from a single stage simultaneous saccharification and fermentation (SSF) of mixed restaurant food waste (Pleissner et al 2017), **and below:** as part of an integrated biorefinery concept producing succinic acid, bio-plasticisers and lactic acid (Pleissner et al 2016).

Kwan et al. 2016 showed overall conversion yields of 0.23–0.27 grams of lactic acid per gram of different kinds of food waste (dry basis) at laboratory scale. These yields were assumed to be appropriate for scaling to 10 tonnes *food waste powder* per hour in a techno-economic assessment indicating the approach is commercially viable (Kwan et al 2018). Food waste powder results from small decentralised Government funded food waste treatment facilities in Hong Kong which grind and dry food waste at source. The revenue from lactic acid reported by the authors also constituted only half of total revenues, with animal feed making a sizeable proportion of the remaining income.

Unfortunately, these conclusions are not amenable to the situation in Europe, where limitations may relate to wet waste transport costs omitted from this study and, importantly, the EU's current ban on using food waste likely to contain animal residues as animal feed.

Other organic acids

Examples exist of nascent industry initiatives such as the French company Afyren[16] which reports successful pre-industrial scale batch conversion using non-sterile anaerobic fermentation to produce platform chemicals such as acetic and butyric acid. Winning various innovation awards it has secured 60 Million Euros investment for scaling to industrial production of seven organic acids, for use as bio-based platform chemicals. Websites indicate these processes are applicable to both agri-food waste feedstock and catering wastes, however, specific information on yields and other processes specifically related to the utilisation of mixed food wastes were not available.

[16] http://afyren.com/en/

Table 3 Laboratory scale research into microbial conversion yields of Lactic acid from various food wastes (Studies cited in Pliessner 2018 and Kwan 2016)

Substrate	Microorganism	Process	Scale	LA Conc. (g L−1)	Yield g g−1 (dry food basis)	Productivity (g L−1 h−1)
Mixed food waste	Lactobacillus casei Shirota	Fungal hydrolysis & fermentation	Bench-top (2 litres)	94	0.94	2.61
Bakery waste	Lactobacillus casei Shirota	Fungal hydrolysis & fermentation	Bench-top (2 litres)	82.6	0.94	2.5
Bakery waste	Thermoanaerobacterium aotearoense LA1002	Fungal hydrolysis & fermentation	Bench top	78.4	0.85	1.63
Kitchen waste	Bacillus coagulans NBRC12583	Enzymatic hydrolysis & fermentation	Bench top	86	0.98	0.72
Kitchen waste	Mixed culture	Enzymatic hydrolysis & fermentation	Bench top	34.5	0.54	0.21
Defatted food waste	Bacillus coagulans	Proteolytic pre-treatment & fermentation	Bench top	37	0.84	–
Kitchen waste	Lactobacillus TY50	Direct fermentation	Bench top	36.3	0.7	1.01
Kitchen waste	Lactobacillus manihotivorans LMG18011	Direct fermentation	Bench top	48.7	–	0.75
Kitchen waste	Lactobacillus sp. TH165 &175	Direct fermentation	Bench top	33.8	0.73	0.47
Kitchen waste	Lactobacillus rhamnosus 6003	Simultaneous enzymatic hydrolysis and fermentation	Bench top	45.5	–	0.75
Apple pomace	Lactobacillus rhamnosus CECT-288	Simultaneous enzymatic hydrolysis and fermentation	Bench top	36.6	0.88	2.78

Food waste (a)	Lactobacillus rhamnosus	SSF	Bench top	–	0.45	0.9
Food waste	Indigenous microbiota	SSF	Bench top	–	0.46	0.3
Food waste (b)	Lactobacillus delbrueckii	SSF	Bench top	–	–	0.7
Potato slurry (c)	Lactobacillus plantarum	SSF	Bench top	–	0.7	1.2
Food waste (20% w/w solid)	Streptococcus sp. strain A620	SSF (sterile)	72 litre	–	0.27	2.16
Food waste (20% w/w solid)	Streptococcus sp. strain A620	SSF (non-sterile)	72 litre	–	0.25	2.12

2.2.4 Diols

Diols form a family of various platform chemicals which has attracted interest as an avenue for bio-based chemical research using microbial conversion of glucose, corn molasses or glycerol (Zeng and Sabra 2011).

2,3-butanediol (BDO)

BDO is a precursor to 1,3-butadiene, one of the top 12 biobased chemicals listed to have near market potential (NREL 2016, Table 8 p10) and also the solvent methyl ethyl ketone. It is considered to have applications in printing inks, and fumigants, perfumes and cosmetics, plasticisers and food additives (Zeng and Sabra 2011).

Others have shown that BDO can be microbially converted from enzymatically hydrolysed food processing industry wastes such as apple pomace, beet molasses and potato pulp using a Baccillus strain (Sikora et al 2016). However, the only research found with specific reference to conversion of mixed food wastes was that by Liaku et al (2018) who found that a selected strain of Enterobacter *ludwigii* was able to produce 50 and 17.6 g/litre of BDO in cultures of both mixed fruit and sulphuric acid pre-treated vegetable waste substrates, respectively.

2.2.5 PHA applications as plastics

Whilst recent media attention and associated public interest in reducing plastic pollution in the marine and aquatic environment may renew interest in low impact marine biodegradable plastics recycled from post use materials, bioplastics in total currently represent only 1% of global plastic production. Of this less than half are currently biodegradable.

However, production capacity is forecast to increase from around 2.1 million tonnes in 2018 to approximately 2.6 million tonnes in 2023 and the main increase has been related to two microbial production based biodegradable polymers poly lactic acid (PLA) and the class of polyhydroxyalcanoates (PHA's)[17]

Commercial PHA production

PHA's have similar properties to fossil-based olefins used in plastics (such as ethylene and propylene). PHA's are intracellular carbon and energy storage polymers that are naturally synthesized by many species of bacteria and as such are marine bio-degradable. Some of these have been harnessed for the commercial production of PHA for use as a bioplastic (Box 1).

[17] European Bioplastics Website accessed Feb 2019.

Box 1 types of PHA and their commercial applications

Short chain length PHA's (scl - 3–5 carbon atoms):

PHB is the most commonly cited PHA with typically a brittle crystalline material causing some disadvantages for processing.

PHVB – includes the incorporation of 3-hydroxyvalerate (HV) with PHB, to achieve copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate - PHBV. It is tougher, more flexible, and has a wider thermal processing tolerance. With increased molar fraction in the copolymer.

Typical applications for scl-PHAs food packaging materials.

Medium chain length PHAs (mcl **-** 6-14 carbon atoms) are more flexible and elastic, and with suitable purity are suitable for implantable medical uses.

Currently 40 companies are developing PHA polymer applications, but there are challenges, with currently only very small, specialist markets (Ravenstijn 2018) such as high purity, high value medical use (Koller 2018). Table 4 shows some examples of these, the bacteria used, the scale of production and the feedstock on which they are (or were) grown. Most of which are dedicated crop materials, rather than industrial by-products. PHA's currently represent 1.4% of the bioplastic market although commercial production has been forecast to quadruple by 2023 (Nova Institute 2019).

More recently US based commercial start-ups Newlight and Danimer Scientific report production licence agreements with large plastic using retailers[18,19] for PHA production processes. The former using sequestered carbon dioxide and methane as the microbial carbon source.

[18] Newlight Signs 10 Billion Pound Production License with IKEA and 15-Year Production License Agreement With Paques Holdings BV. Newlight Website. Accessed Oct 2018. Danimer Scientific report

[19] Danimer Scientific report product development agreements with Nestle and PepsiCo.

Table 4 Examples of commercial and semi-industrial PHA products (adapted from Koller 2017 and Dietrich et al 2017)

Company	Production period	Brand name	PHA type	Microbial producer	Substrate/ feedstock	Scale tonnes /year	Product applications
Telles (Joint venture with Metabolix, USA and ADM US)	2007-2012	Mirel^{tm} and Mvera^{tm}	P3HB	Not reported	Corn glucose	50,000	Raw material for Injection moulding; Cosmetics Micropowders; Packaging coating for paper and cardboard; Denitrification for Aquariums; Plasticizers for PVC and PLA.
ICI, UK (later Zeneca, Monsanto)	1976-1998	Biopol®	P3HB	Cupriavidus necator	Glucose from carbohydrate feedstock	800	
Danimer Scientific, USA	Planned	Nodax™	Medium chain length PHA (unclear)	Not reported	Rapeseed (canola) oil	In development	Raw materials (resins) for production of plastic bags, bottles, hygiene, mulch sheeting.
PHB Industrial SA /Copersucar, Brazil	Ongoing	Biocycle®	P3HB, P3BHV	Cupriavidus necator, Burkholderia sacchari Alcaligenes sp	Cane sugar	5000	Raw materials (pellets) for production of plastic sheet extrusions, injection, coating paper
Biomer Germany	1993-present	Biomer™	P3HB	Cupriavidus necator	Corn glucose	1000	Raw materials (pellets) for production of plastic sheet extrusions, injection mouldings.
Bio-On, Italy (planned)	Planned	Minerv™	PHA (unclear)	Not reported	Sugar production co/by-products	10,000	Raw material for cosmetics, sun cream, automotive, electronics & packaging components.
TianAn Biopolymer, China	Ongoing	Enmat™	P3HB, P3HBV, Ethyl 3-HB	Cupriavidus necator	Starch based sugars	100-1000	Raw materials (powder, pellets) for thermoplastics: injection molding, extrusion, thermoforming, blown films; Fiber & Nonwovens; Denitrification: water treatment. Ethyl 3-HB for Fine chemicals

Company	Production period	Brand name	PHA type	Microbial producer	Substrate/ feedstock	Scale tonnes /year	Product applications
Tianjin GreenBio (+DSM)	2004 onwards	Sogreen™	P(3,4HB)	Not reported	Starch based sugars	Not reported	Raw materials (resin, pellets)
Shenzhen Ecomann Biotechnology Co. Ltd., China	Not reported	AmBio™	Not reported	Not reported	Sugars	5000	PHA pellets, resin, microbeads
Tepha, US	Ongoing	TephaFLEX®	P4HB, P3(3HB-*co*-4HB)	Not reported	Sugars	Not reported	Medical material for sutures, meshes & surgical films
Newlight Technologies (newlight.com)	Ongoing	AirCarbon™	PHA (unclear)	'9*x* microorganism-based biocatalyst'	Sequestered methane	In development	Raw material for extrusion, blown film, cast film, thermoforming, fiber spinning, and injection moulding applications. Cosmetic packaging - R&D partnership with The Body Shop

P3HBV = poly(3-hydroxyvalerate)

Polyhydroxyalkanoates (PHA's) production from food wastes

As outlined previously, over the last two decades various microbial processes for producing polymers have been developed for commercialisation using commodity feedstock as substrates. Research into microbial conversion from *mixed* retail and post-consumer food waste streams as a substrate for this kind of direct polymer production has not been observed in the literature. The nearest research to this has been the use of types of food related substrates and food processing effluents utilising mixed microbial cultures listed in Table 5.

A recent review concluded that PHA production process yields reported in the research literature have not advanced significantly over the last 20 years (Blunt et al 2018). In this respect cost assumptions from 20-year-old research used to model the conversion of food processing waste streams into PHB (Choi & Lee 1997) has been updated in recent theoretical techno-economic modelling showing possible competitiveness at scale (Broeze & Mooibroek 2018).

However, in selecting particular processing waste streams or by-products, (beet processing wastes, fatty acids etc.) this example does not directly represent the kind of composition observed in mixed, post-consumer waste outlined in appendix 1. Other approaches therefore have been used to first convert food wastes into viable sugar rich feedstock prior to further microbial conversion (Kwan et al 2018). Others have indicated the potential for strategies for more varied feedstock such as food wastes and effluents using mixed microbial cultures (Table 6Table 6). Unfortunately, these have not been proven at pilot scales and indicate lower yields compared to pure strain approaches (Kourmentza et al 2017, Table 5).

The literature indicates downstream separation (cost of digestion agents) has the most significant impact on the economics of production, with substrate/feedstock costs also being a key factor (Choi & Lee 1999). The reported potential environmental benefits of microbial polymers, such as their global warming impact, also may vary, with downstream processing to separate PHA from cellular material being an important factor (Dietrich et al 2017).

Microbes able to maximize the desired individual cellular PHA content and attributing less substrate feedstock on which they are grown to production of other cellular materials or metabolites, are considered the most beneficial for improving the economic potential.

Therefore, selecting for new microbial strains could offer a potential pathway to improving the economic and sustainability case for food waste based PHA bioplastic production if these could: -

a) Allow microorganisms to grow on a low or zero cost waste food chain substrate and;

b) be able to tolerate conditions of continuous production processes

c) offer higher concentration of cells to reduce the bioreactor and equipment costs and;

d) produce much higher cellular content of a desired PHA polymer whilst also allowing easier (low cost) extraction from the unwanted fraction

(based on Koller 2018).

Table 5 Selected research results on PHA production from food waste substrates (adapted from a review by Kourmentza et al 2017)

Strain	Food/waste substrate	%HB %HV	Cultivation scale/type	Dry Cell Weight (g/litre)	PHA (g/Litre)	PHA (%)	Yield per substrate
C. taiwanensis	Cassava starch + Val e	(87–13)	Flask	2.8	1.88	67	Not reported
	Corn starch + Val e	(80–10)		3.3	2.14	65	
	Potato + Val e	(80–10)		2.6	1.43	55	
	Sweet potato + Val e	(80–10)		1.6	0.83	52	
	Wheat starch + Val e	(80–10)		4.1	1.72	42	
H. mediterranei DSM 1411 (Halophile)	25% pre-treated vinasse	(86–14)	Flask		19.7	70	0.87
	Stillage	(85–15)			16.4	71	0.35
	Hydrolysed cheese whey	(98.5-1.5)	Batch	7.54		54	0.78
	15% v/v olive mill wastewater	(94-6)	Flask		0.2	43	
Halomonas campaniensis LS21	Mixed substrates (mostly comprised of kitchen waste)	PHB	Continuous pH-chemostat			26	

Table 6 Examples of research into Mixed Microbial Cultures (MMC) for PHA production from food processing effluents (adapted from a review by Kourmentza et al 2017).

Pilot Plant, Location	Feedstock	Origin of MMC and Enrichment Strategy	*Yield* (g/g)	PHA % (%mol HB: %mol HV)	Production rate mg PHA/ g /h
Nagpur, India	Pre-fermented milk and ice cream processing wastewater	Activated sludge	0.425 *	39–43	
Lucun WWTP in Wuxi, China	Hydrolysed and acidified raw excess sludge	Activated sludge/synthetic mixture of VFA, ADF feast famine with carbon limitation and inhibitor of nitrification	0.044–0.29 *		2 –39
Eslöv, Sweden	Beet process water, (including volatile fatty acids)	PHA producing MMC from pre-fermented effluent of Procordia Foods		60% (85:15 HB:HV)	
Brussels North WWTP (Aquiris, Belgium)	Pre-hydrolysed and fermented WWTP sludge	Sludge fed with municipal WW under aerobic feast famine	0.25–0.38	27–38% (66–74:26–34 HB:HV)	100–140
Leeuwarden WWTP, Friesland, Netherlands	Fermented residuals from green-house tomato production	Sludge fed with municipal WW under anoxic feast/aerobic famine	0.30–0.39	34–42% (51–58:42:49 HB:HV)	28–35
Mars company, Veghel, Netherlands	Fermented wastewater from confectionary processors	Activated sludge from a WWTP fed with the fermented wastewater under aerobic feast/famine with inhibitor of nitrification	0.3	70–76% (84:16 HB:HV)	

HB= Hydroxy Butyrate, HV = Hydroxy Valerate

2.2.6 Food additives

There may be significant potential in harnessing microbes such as lactic acid bacteria, capable of growing on specific food waste substrates that are able to produce interesting compounds such as sweeteners, flavourings or bacteriocin preservatives, etc. that can be extracted and processed for higher value food grade uses[20] .

However, few research publications were evident applying this concept within the scope of this report; namely restricted to the utilisation of unsegregated mixed post-consumer food wastes as substrates for their production. Examples are mainly found in research for the extraction of compounds from separated specific fruit or vegetable processing residues with examples such as phenolic acids, flavonoids, carotenoids, anthocyanins, hydroxycinnamic acids given in Pleissner et al 2016, rather than post-consumer or retail mixed wastes.

Key challenges foreseen in producing food additives from food wastes subject to risk of spoilage, pathogen contamination and other logistical cost considerations will be traceability and processing assurances for industry users of compliance with EU regulations, novel food status, and food purity /safety criteria for food additives.

2.2.7 Enzymes

Though there may be a crossover with obtaining industrial enzymes from food wastes where their use may allow economic conversion of mixed food wastes into fuels and chemicals, the central scope of this report concerns fuels and chemicals from post-consumer waste rather than microbiological products such as enzymes. In this respect, using mixed food waste as a substrate to produce high value enzymes readers are referred to reviews such as that given by Uçkun et al 2014. This review indicates the dominance of research which focuses on specified waste streams from defined sources in the industrial food chain, rather than post-consumer mixed food wastes. The authors conclude that research (contemporary to 2014) shows these food waste to be a potential, (rather than commercially demonstrated), substrate for producing industrial enzymes such as amylases, cellulose, pectinases, proteases, and lipases.

[20] Professor Jeroen Hugenholtz, Wageningen University and Research, personal communication July 2018

2.2.8 Summary

Sustainable aspirations of bio-based industries may be related to key criteria, namely:

1. To utilise feedstock and processes that can consistently compete commercially with fossil fuel equivalents but also;
2. Have relatively low direct environmental impacts over the full product lifecycle;
3. Have no effect (directly or indirectly) on the capacity of existing cropland for commodities, such as food production, and so have a low causal risk of driving indirect land use change impacts which may potentially negate some of their GHG benefits.

Currently bio-based chemical and fuels often rely on crop-based feedstock so there is an uncertainty in how their expansion would affect the last criteria.

Using lower or zero cost, hard to avoid and underutilised putrescible wastes as feedstock is one strategy that seeks to offset this. The challenge is to harness modern technology able to utilise this potential feedstock which is compositionally heterogeneous to create specific, higher value platform chemicals.

Researchers using microbial conversion approaches indicate that improving the commercial potential for food waste valorisation would require genetic engineering of microbial strains. This may have socio-political implications regarding public acceptance.

Most research to date is laboratory based at the bench top scale and it is challenging to translate whether these approaches would be commercially viable without realistic models or the necessary investment to replicate conditions, yields and necessary processes empirically at or near to commercial scale.

Current research projects would appear to be now engaging with this issue and attempts are being made to bridge this gap with multi-million Euro funded demonstration pilot projects and planned techno-economic analyses (examples of EU funded projects are given in Table 7).

Table 7 Examples of public funded R&D projects investigating conversion of organic fraction of municipal solid waste (OFMSW) to platform chemicals and fuels

Product(s)	Size	Pretreatment	Processes	Investment	Research output	Reference	timeline
Ethanol	25 T/d OFMSW infeed	Thermochemical hydrolysis	SSF, AD (stillage)	No information	Demonstration plant	PERSEO (Interreg)	2003-2006
Ethanol	As above	Not stated	SSF, AD	€1.7 Million	Optimising demonstration plant & business models	WASTE2BIO	2017-2020
Ethanol, Volatile fatty acids, polyhydroy-alkanoates	10 T/d OFMSW infeed	Not stated	AD	€15 Million	techno-economic viability	URBIOFIN	2017-2020
Lactic & Succinic acid & Biosurfactants.	n/a	Enzyme mediated hydrolysis	CM/MM, ME, SSF, MED.	€3.4 Million	Feasibility of cascade valorisation from MSW	PERCAL	2017-2020
Butanol and hydrogen	'Pilot scale'	Thermal	Hydrolysis via fermentation	Up to €6 million	technical and economic feasibility	BESTF2-project	2016-2019

SSF = Simultaneous Saccharification & Fermentation, AD = Anaerobic digestion, CM= Chemical modification, MM= Microbial modification, ME = membrane electrolysis, MED= membrane electrodialysis

2.3 Key challenges

2.3.1 Compositional and logistical challenges

The first challenge concerns the variability in waste composition. Putrescible MSW may vary both regionally and seasonally, depending on the sources of raw (food) material purchased by the consumer. To some degree, this may be overcome by combining waste on a scale that generates a uniform chemical composition, partitioning wastes and/or targeting microbial conversion approaches that are able to tolerate wider variation in feedstock composition. This would require further research into variations in putrescible waste composition over time at different collection scales.

However, the second (and probably most difficult) challenge concerns the high water content. This will have a number of implications. For example, water provides an environment suitable for rapid microbiological deterioration and contamination so costs for stabilisation to prevent rapid spoilage is a key implication. In addition, the moisture content of bulk putrescible wastes adds to transport costs. Any dewatering and drying to prevent spoilage and reduce transport costs will also be an energy intensive processes. However, putrescible MSW is widely collected, so it may be possible to develop decentralised approaches similar to ST1's ethanol plants in Finland.

2.3.2 Market related challenges

Near-term market assessments of the potential for bio-based chemicals identify 12 key chemicals based on publicly demonstrated pilot scale or greater production processes (Table 8). These are dominated by the use of crop-based commodity feedstock such as maize or corn and plant-based lipids rather than food chain wastes.

Key challenges are competing for investment in markets dominated by established petrochemical industries firstly, or secondly those relying on large scale production using crop-based feedstock as indicated in Table 8 and associated cost prices shown in Table 9.

Recent research (Table 9) indicates that the prices of bio-based products are below €10/kg with bulk platform chemicals and solvents, generally around €1-2/kg. Therefore, new entries into this market also have to compete with the current cost prices that the market has come to expect for similar or equivalent material functionalities.

Bio-based chemicals	Typical uses
1,3-butadiene	butadiene rubber in car tyres
1,4-butanediol	building block for polymers, solvents and chemicals
Ethyl lactate	made from ethanol and lactic acid - replacing industrial solvent
Fatty alcohols	longer chains used in domestic detergents, personal care products, and industrial applications. Shorter chains as plasticisers.
Furfural and Furfurylic acid	broad industrial applications in plastics, pharmaceuticals and agro-chemical products.
Glycerin	Low and stable cost makes it a candidate for wider bio-based product markets
Isoprene	Building block for polyisoprene and butyl rubbers and styrene co-polymers.
1,3-propanediol	Polymers, personal care products, solvents, and lubricants
Propylene glycol	Existing markets in a wide variety of applications from foods, pharmaceutical and cosmetics to industrial, de-icer, coolant and solvent uses
Succinic acid	Projected large future market potential as a precursor for the synthesis of high-value products, commodity chemicals, polymers, surfactants, and solvents.
Para-xylene	Used for precursors (terephthalic acid and dimethyl terephthalate) for production of polyethylene terephthalate (bio-PET) bottles with interest from large soft drink markets

Table 8 Top 12 near market bio-based chemicals (NREL 2016)

More specialised non-essential luxury products such as cosmetics and personal care products alongside with plasticisers, may attract more lucrative prices. In addition, as technology applications improve and markets change some R&D companies have been seen to be moving from applications for bulk platform chemicals into utilising bacterial processes to exploit emerging and potentially more lucrative markets in the high value smaller volume pharmaceutical and biologics sector[21] where the benefit of a consistent higher quality substrate is less cost prohibitive in relation to the value of the product and therefore preferred over waste sources.

[21] E.g. Bactevo and Chain Biotech in the UK.

Product category	Price (EUR/kg)	Turnover (EUR million/yr)
Platform chemicals	1.48	268
Solvents	1.01	76
Plastics	2.98	799
Paints,coatings, inks dyes	1.62	1623
Surfactants	1.65	2475
Cosmetics/personal care	2.07	1155
Adhesives	1.65	391
Lubricants	2.33	552
Plasticisers	3.6	241
Man-made fibres	2.65	1590
Total	1.94	9167

Table 9 Biobased product market (Spekreijse et al /JRC 2019)

In general, the bio-based chemicals market is very small compared to current market and economy of scale and prior investment makes competition with standard products a likely barrier. Recent example[22], demonstrating the challenges of creating new markets or competing with current markets is the case Canada based Bioamber Inc. now filing for bankruptcy after receiving public funds of $50 Million for investment in a $140 Million bio-succinic acid production plant and floated on the stock market.

2.3.3 Technical challenges.

Moving from publication oriented laboratory scale research to proof of concept and to a commercially profitable industrial scale technology are also well-

[22] Bioamber Inc's plant in Sarnia, Canada has been reported to produce 30,000 tonnes of Succinic acid per year from corn syrup, with 2017 sales of bio-succinic acid of $14.9 million until bankruptcy filings this year. Myriant has commercial bio-succinic acid plant in Lake Providence, USA with a capacity of 13,500 tonnes of Succinic acid per year. Myriant is also producing 1,300 tonnes per year of bio-based succinic acid at ThyssenKrupp Uhde's biotech commercial validation facility in Leuna, Germany. Websites accessed June 2018.

known technical challenges. For food waste conversion into chemicals the technical challenges are very much dependent on the products required. More generally the key technical challenge is that of obtaining a consistent yield from a potentially variable feedstock.

2.3.4 Policy challenges

As outlined in section 2.1.1, the EU Waste framework directive sets out a hierarchy for how member states should prioritise waste prevention and recovery processes. An assessment on where putrescible waste valorisation for chemical production would rank compared to other recovery operations in the Waste Hierarchy priority is not within the scope of this report. However, this also may be a challenge to determine objectively. This can depend on lifecycle assessment based evidence[23] which may depend on context but also methodical assumptions applied in comparing processes, especially where indirect consequences are concerned. This includes the choice of reference systems, study boundaries and if and how substitution of products, such as national energy supplies, are factored into the assessment (JRC 2011). Therefore, this may also be considered a challenge in respect to existing policy assumptions regarding re-use and recovery.

Rex et al 2017 concluded that with the hypothetical example of utilising mixed food waste for conversion to succinic acid, lack of policy support and actor commitment can also be considered as key barriers.

Exploiting genetic engineering to modify metabolic pathways of bacteria for chemicals production can be subject to requirements for the environmental risk assessment under Directive 2009/41/EU and 2001/18/EC regarding containment and release of genetically modified (micro) organisms. According to Annex 1 B (1) Mutation that is induced by mutagenesis techniques does not constitute a genetic modification. However, legislation may evolve with regards to technical working groups views on advancing techniques and application of synthetic biology (Bluhk 2014).

[23] Article 4 of the Waste Framework Directive 2008/98/EC states: When applying the waste hierarchy, Member States shall take measures to encourage the options that deliver the best overall environmental outcome. This may require specific waste streams departing from the hierarchy where this is justified by life-cycle thinking on the overall impacts of the generation and management of such waste.

3 Producing bacterial strains for chemical production from mixed food waste residues

This section of the report describes experimental work investigating genetic strains of bacteria suitable for growing on putrescible waste with potential for chemical production.

3.1 Aim and Objectives

3.1.1 Aims

The aim of this work has been to successfully grow and characterise the genetic traits of a strain of bacteria that could successfully utilise food waste. This can be used to provide a blueprint for creating an organism with the optimal genome for the breakdown of putrescible waste into bacterial biomass. The overarching aim is to either use this to produce specific natural precursors of fine chemicals processed from either the biomass as a feedstock or by genetically engineering the organism to process chemicals itself.

3.1.2 Objectives

The key objectives of this research have been to:

1. Produce growing media or broth that adequately characterises that which may be obtained from putrescible food wastes.

2. Identify relevant genes that allow bacteria to best exploit [a characterised version of] putrescible food waste to enhance their potential for producing chemicals.

3. To utilise state of the art microbial genetic technology to generate massive diversity through the production of many individual clones from one bacterial isolate.

4. To experimentally select mutants that can grow successfully on characterised putrescible waste from the diverse population of clones.

5. Sequence successful clones to identify a list of genes that allows improved growth as the first step towards producing a suite of commercially viable strains for the valorisation of putrescible waste.

3.2 Background

3.2.1 Glossary

Chassis organism - A bacterial strain in which engineered heterologous (non-native) genetic constructs can be hosted to engineer desired traits such as manipulating metabolic pathways to improve generation of specific chemical molecules (‘*cell factories*’).

Genome – the total nucleic acid complement of a living organism.

Whole Genome Sequencing – the nucleic sequence of the total genetic complement of an organism.

Transposon – a sequence of DNA that can move to new positions within the genome of a single cell, or can translocate (be transposed) between bacterial cells.

Transposon Mutagenesis – the process of inserting a transposon into the genome of a host bacterial genome.

Mutant library – a collection of different mutants

TarGET library – a mutant library (containing several million different mutants) prepared using transposons with an outward facing promoter.

Outward facing promoter – a DNA sequence placed at the end of a transposon which points out from the transposon activating the adjacent gene.

Activated gene – a gene in a TarGET library cell with an insertion just in front of the gene, in the promotor region. These genes are expressed (make the protein continuously) regardless of environment.

Inactivated gene – a gene which is disrupted by the presence of a transposon on the gene body.

Recombination systems – everything that allows nucleic acids to recombine to form new combinations, or a new order, of genes in a genome.

Transposon end reads – The region where the transposon and the host bacterial genome are next to other. This represents the site at which the transposon has inserted.

Insertion site – a unique site, designated by a single location reference, at which a transposon is inserted into a genome.

Short read sequencing – a sequencing technology (most commonly Illumina) which provides lengths of contiguous DNA sequence a few hundred base pair in length.

Long read sequencing - a sequencing technology (most commonly Illumina) which provides lengths of contiguous DNA sequence a few thousand base pair in length.

Regulatory cascades – the process of gene regulation which involves more than one step. E.g gene product A regulates gene B, and gene product B which regulates genes C and D and E.

Phenotype - an observable biological characteristic of an organism that is a result of environmental stimulus triggering expression of its genetic code

3.2.2 Traditional genetic engineering of bacteria

Traditionally genetic engineering is carried out by using techniques to introduce random mutations (changes) into the bacteria's genetic instructions that controls its traits. Experimental conditions are set up to select the bacteria with mutant combinations which exhibit the desired trait(s). In this case, the desired traits are those that allow it to grow best in putrescible waste whilst able to be engineered to produce certain fine chemicals. Then the genetic mutations that allow them to do so are identified using analytical techniques. This approach is dependent on finding the desired phenotype by creating massive random variation, including unwanted as well as wanted mutations. The main advantage is that the whole genome is covered and no prior knowledge of the bacteria's metabolic pathways are required.

Even with the advent of whole genome sequencing, linking the genetic mutation involved to a successful phenotype (the desired, environmentally induced, genetic trait) is difficult. This is because the random mutations cause the linkage of useful mutations with non-useful mutations in one cell (Figure 5a). A large sample size is required to deconvolute the association between phenotype and mutation. More modern methods use a more sophisticated approach with what are called mutant libraries. These libraries are used to direct evolution towards the required genetic traits, and importantly introduce known mutation into each cell.

3.2.3 Directed evolution method

Modern directed evolution methods involve incorporating variants of known genes into a bacterium genome using natural recombination systems. This may be single or multi-targeted and can now be automated by a process called multiplex automated genome engineering (Wang et al 2009).

The single target methods are laborious and require serial manipulation of single genes and cannot be used for parallel and continuous directed evolution of gene networks or genomes. With the multi-target automated methods, it is possible to engineer dozens of targets simultaneously but as the number of targets increases, (there are about 5,000 genes in a single genome), it becomes technically impossible to engineer the defined library because, as with all of the directed evolution methods, knowledge of the site within the pathway to be engineered is essential. It is therefore only possible to optimise well characterised bacterial pathways.

3.2.4 TarGET technology

TarGET stands for Targeted Genomic Evolution Technology and is a proprietary genetic technique developed by Bactevo[24]. Traditional transposon mutagenesis only inactivates genes. The unique property of TarGET is the use of **outward facing promoters** which over expresses (activates) every gene in the chromosome, one gene per cell (Figure 5b). TarGET both activates and inactivates genes, thus combining the coverage of the original random mutagenesis methods with whole genome sequencing techniques to precisely locate each mutation. Once located experimental selection can be used to identify essential bacterial genes or genes involved in environmental adaptation. This allows the association of phenotype and genotype through the selection of single mutations per cell.

TarGET utilizes **short read sequencing** to map the **insertion sites** of the individual **transposons** to a specific location in the bacterial chromosome. This is achieved by comparing the **transposon end reads** and the known sequence of a bacterium under investigation. This allows the unambiguous mapping of transposons to genes and so we can generate a list of genes involved in growth in the conditions tested – here growth in putrescible waste. Because there is only one mutant per cell and the whole population is sequenced it is possible to map several million reads to the genome and identify several thousands of different insertion sites simultaneously. Which in turn allows us to characterise the importance of every gene in the genome for growth in putrescible waste. Because there is only one mutation per cell, (Figure 5b) the linkage of random mutations with a successful phenotype (Figure 5a) is minimised. Bactevo uses this technology to assess the genetics of bacterial growth under specific test conditions with specific growth media (Figure 6). In this case, putrescible food waste is the growth media of interest.

Analysis for gene mapping is proprietary software but in brief: Machine learning algorithms have been trained on curated databases and these are used to classify genes as protected (conditionally essential), disrupted (conditionally costly) or activated (growth enhancer).

The number of transposons expected in a region of the chromosomes is calculated as the number of total insertions divided by the length of the region under investigation. A million mutants in a million base pairs of DNA would, if evenly distributed, give one insertion per base pair. If only one insertion is present across a whole gene (about one thousand base pairs of DNA) then this region is clearly protected from insertion and is labelled "conditionally essential". If there are 1,000 transposons in a region only 50 base pairs long then this is considered to be a "hotspot" for insertion. If insertions are only adjacent to a gene, then the gene may still be activated.

[24] (US Patent).

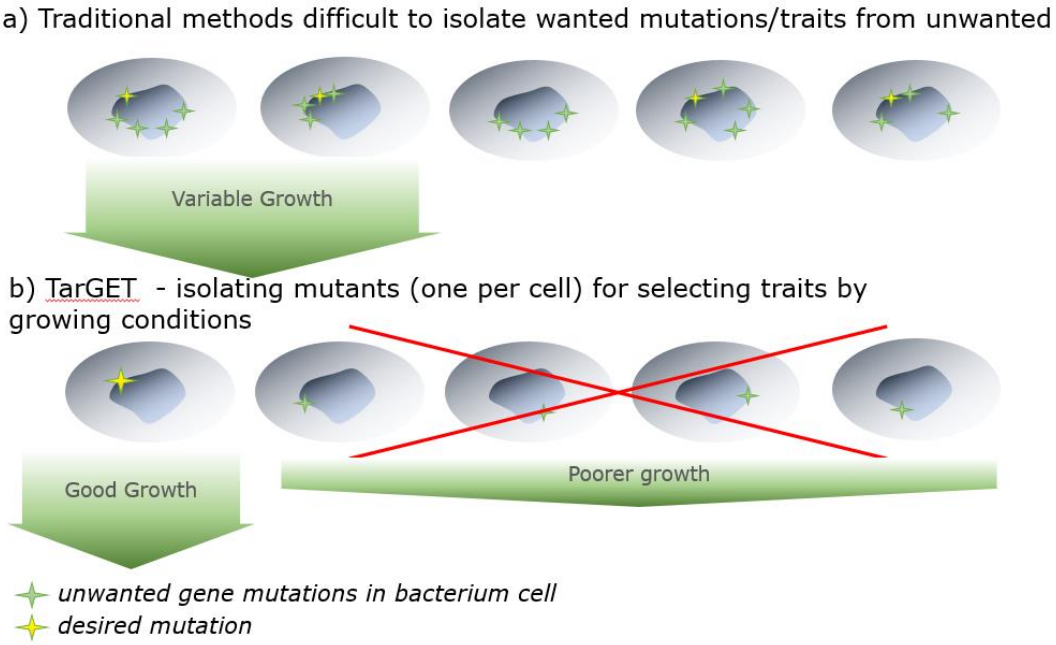


Figure 5 simple schematic distinguishing traditional genetic mutagenesis compared with TarGET activating and deactivating mutant genes

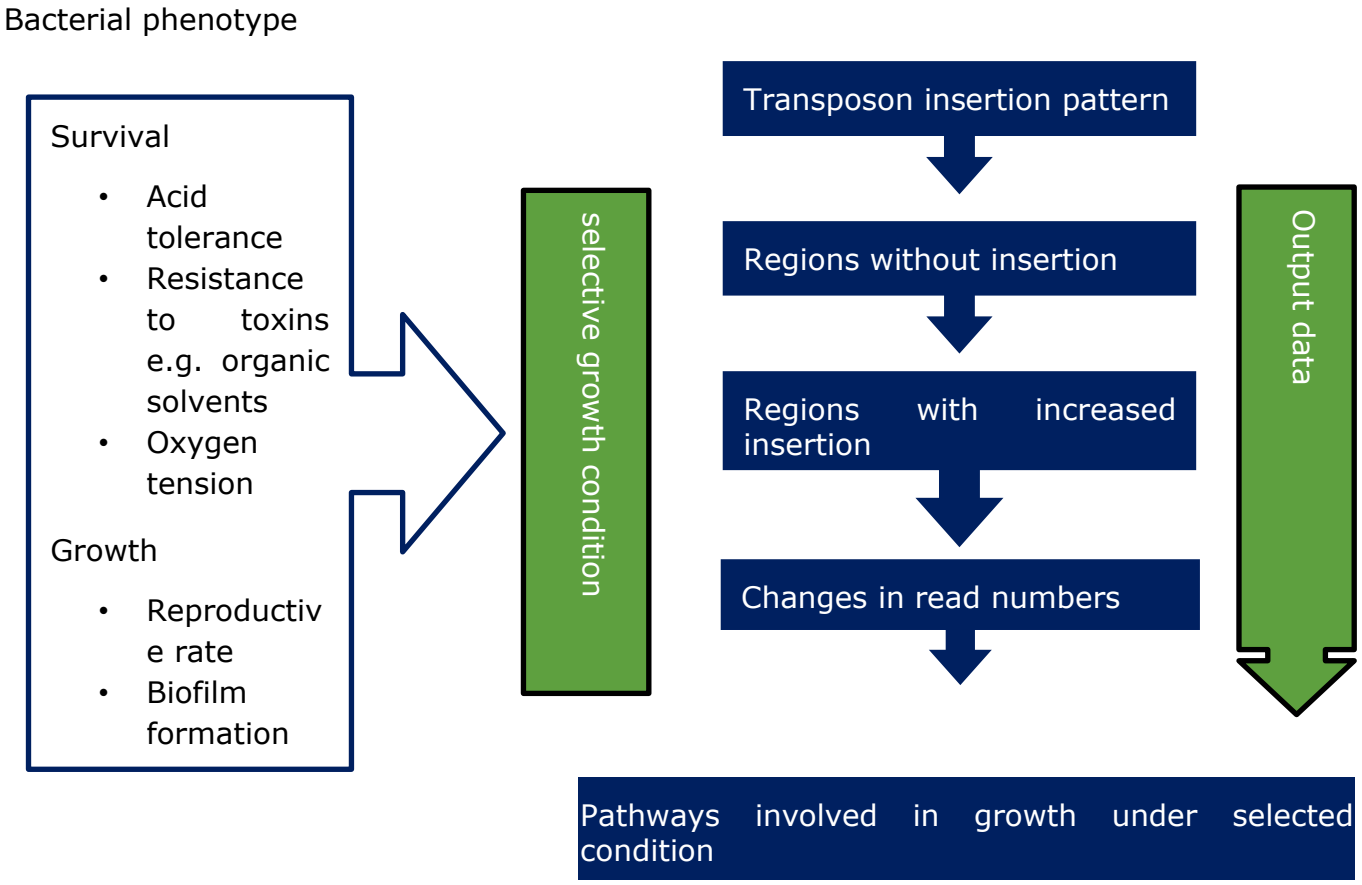


Figure 6 Schematic showing how Target technology maps genetic characteristics (using transposon insertion patterns) of bacteria phenotypes that grow successfully in selective experimental conditions.

3.3 Experimental overview

3.3.1 Feedstock characterisation

Food waste broth was made up by adding water (40% by volume) to a theoretical mix of food waste shown in Table 10. Due to the variation of mixed putrescible wastes from municipal sources, a hypothetical mixture of food wastes has been characterised based on a review of data sources, (see review of studies in Appendix).

Food item	Weight (g)	Energy (kcal)	Fat (g)	Carbo-hydrate (g)	(of which sugars)	Fibre (g)	Protein (g)	Salt (g)
Peach, stoned	107.2	40.7	0.5	8.1	8.1	1.6	1.1	0.0
Potato, diced, unpeeled	147.8	119.7	0.7	25.9	1.2	2.4	2.7	0.0
Carrot wedges	74.1	31.1	0.4	5.7	5.3	2.2	0.4	0.0
Cherry tomatoes	35.4	7.1	0.2	1.1	1.1	0.4	0.2	0.0
Cucumber, sliced	30.4	3.3	0.2	0.4	0.4	0.2	0.2	0.2
Lettuce	81.7	11.4	0.4	1.1	1.1	1.1	1.0	0.0
Carrot strips	23.4	6.8	0.1	1.0	1.0	0.7	0.1	0.0
Bread	100.4	243.0	0.9	48.3	3.2	2.7	9.1	0.9
Quiche	100.0	274.0	18.0	19.0	2.1	1.6	8.9	0.6
Ham	100.2	113.2	3.1	2.7	2.7	0.5	19.0	1.8
Yogurt	100.6	150.9	8.3	16.1	16.1	0.5	2.9	0.1
Drink, squash	100.0	4.7	0.0	0.3	0.3	0.0	0.0	0.1
Total	1001.2	1006.0	32.9	129.7	42.6	13.8	45.8	3.7

Table 10 The composition of the modelled food waste substrate

Water was added to this waste at 40% w/v and the broth was homogenised prior to samples being drawn for use in growing test bacteria cultures.

3.3.2 Bacterial selection

The initial selection criteria were for a bacterium that is able to grow on putrescible waste and also may be genetically engineered for improved growth. *Pseudomonas* parent strains were chosen for screening because *Pseudomonas* are metabolically diverse and are used in the biotechnology sector as chassis organisms for many processes. A Pseudomonas strain adapted for growth in putrescible waste would be suitable for further adaptation into fine chemical production. Parent strains for TarGET libraries (over one million independent mutants, or clones) in *Pseudomonas fluorescens* and P. *putida* were screened for growth at a range of dilutions of putrescible waste as supplied by the Quadram Institute Biosciences.

This screening selection has been applied to determine the genetics involved in bacterial growth on putrescible waste by comparing cultured mutant bacteria that do and do not grow in the medium. The mutants able to grow in the higher concentrations have been related to isolatable genetic changes made during the generation of each clone.

3.3.3 Experimental controls

Based on previous experience, a range of concentrations including non-exposed controls is necessary to allow effective interpretation of the results. The following growth conditions were therefore used:

LB – rich laboratory media - maximum growth, no selection expected.

MMG – Minimal media plus glucose – glucose as the sole carbon-energy source, no selection expected

SW1, SW20 or SW40 - Putrescible waste at 1, 20 or 40% diluted in water. Selection expected at 40%

SWM1, SWM 20 or SWM 40 - Putrescible waste at 1, 20 or 40% diluted in Minimal media. Selection expected at 40%

3.3.4 Scale up to 1 litre

The ability of the mutants to grow at 1L has not been performed because transfer of the libraries from Bactevo to the Quadram Institute, as originally planned, has not been possible due to staff/structural changes during the project.

3.4 Results

3.4.1 Growth curves

Growth curves for the selection of the TarGET library were completed using a maximum of 40% putrescible waste in water. Both *P. fluorescens* and *P. putida* parent strains (background strains in which libraries were created) grew in 40% (w/v) putrescible waste broth for at least 72 hours.

Direct comparison of parent strain with library showed that 72 hours of culture allowed the library to gain an advantage over the Parent strain (WT = wild type) at 20%, and, to a lesser extent, at 40% waste concentrations. for both organisms.

However, *P. putida* library culture was less dense than the parental strain (demonstrating no selection) culture at 48 hours but at 72 hours the library had outgrown the parental strain. *P. putida* was therefore not a useful tool for investigating the genetics of growth. However, *P. putida* may make a good chassis organism and could be manipulated using the data from the *P. fluorescens library. P. fluorescens* library at 48 hours outgrows the wild type in 40% putrescible waste broth and at 72 hours the *P. fluorescens* library had outgrown the wild type at both 40% and 1% putrescible waste broth.

The 48hr incubation at 40% was carried forwards in repeat experiments as the optimal conditions (the strongest selection pressure) needed to enrich for mutants capable of growth on putrescible waste.

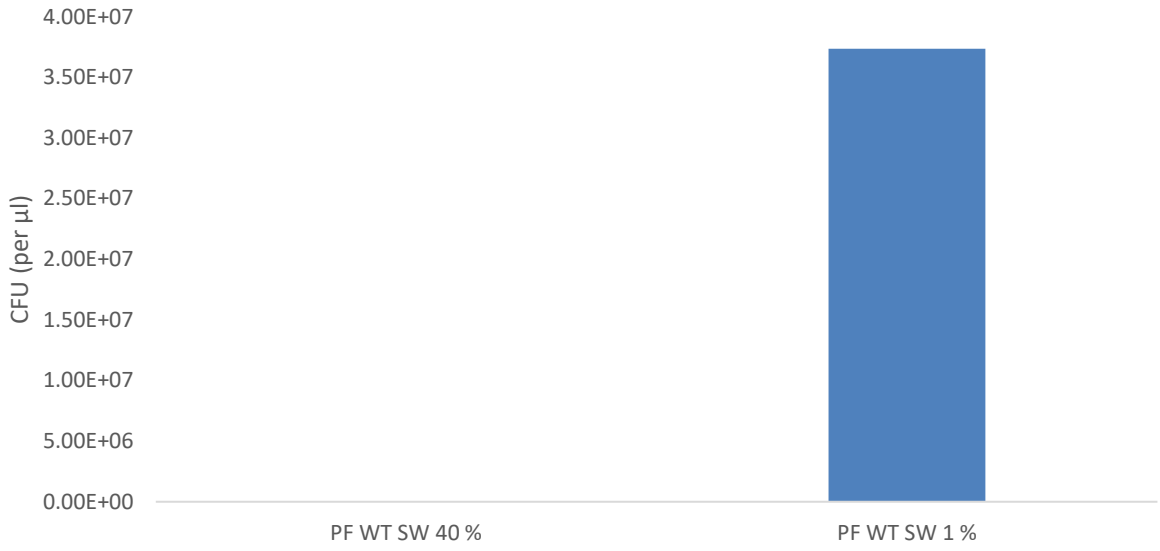


Figure 7 Colony forming units of parental strain *P. fluorescens* bacteria (PF WT) after 48h growth in test putrescible sandwich waste (SW) at 40% and for comparison 1% (w/v, SW/water.)

Figure 8. Colony forming units of parental strain (WT) P. fluorescens compared to mutant library after 48h growth in test putrescible sandwich waste (SW) at 40%.

3.4.2 Sequencing results

Initial sequencing results for optimisation of the protocol.

Initial experiments of the putrescible waste TarGET experiment demonstrated that most transposons were located at either ends of the chromosome for both libraries. Very few insertions were located in the central part of the genome. This is commonly seen when the diversity in the exposed mutant library is too small and since insertion is more frequent at the ends this biases results. The experiments were repeated with higher concentration of bacteria, and so larger transposon library. The results were processed using Bactevo's proprietary bio-informatics approach.

The expected reduction in the diversity within the transposon library was seen at 40% putrescible waste diluted in water (Figure 9).

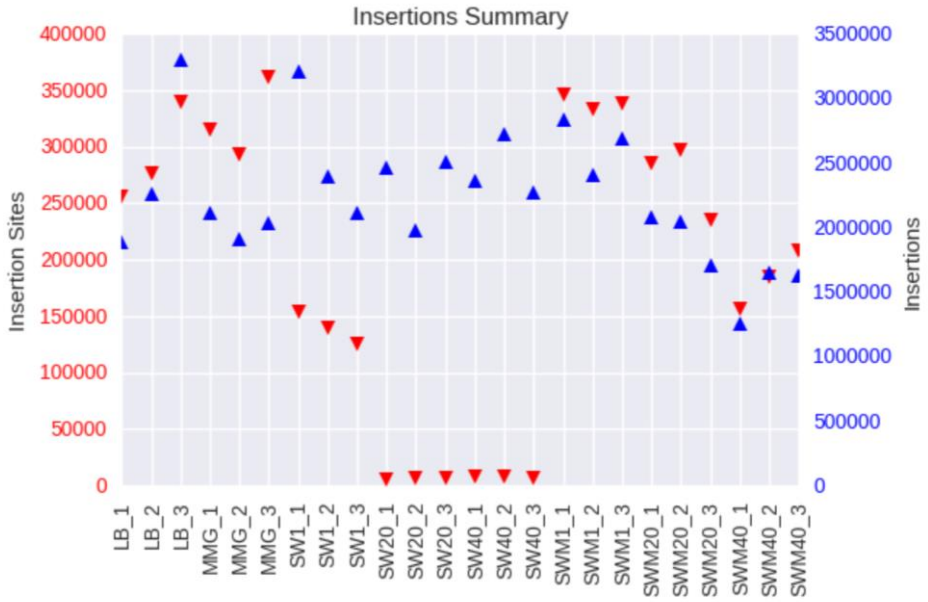


Figure 9: Insertion sites (V, left Y-axis) corresponds to the number, or diversity, of mutants grown in the population. Insertions (Δ, right Y-axis) corresponds to the number of cells in the culture.

A low number for insertion sites (red inverted triangles) indicating low diversity, but with a high population (blue triangles) represents selection of smaller number of successful mutants thriving and replacing the less successful mutants. An enrichment of mutants is observed in SW40 (40% putrescible sandwich waste) but not in the control (LB). Therefore, the distinguishing genetic characteristics of these mutants were of interest.

3.4.3 Gene lists

1,598 out of the 6,144 total genes in the bacterium's genome have been identified as conditionally essential for growth; mutant bacteria strains were only successful if these genes were not inactivated by transposon insertion.

The list of genes identified is in the appendix II

In addition, genes have also been identified as conditionally costly; mutant bacteria strains grew better in the putrescible waste with these genes being inactivated by transposon insertion, yet still able to grow in the non-waste control media without inactivation.

The most interesting results were the genes which, if activated, improve growth rate on putrescible waste. These growth enhancing genes are listed in Table 11.

Gene locus	Gene name	Function
PFL2_1389	rpsB	30S ribosomal protein S2 / translation control linked to *tsf*
PFL2_1391	pyrH	ridylate kinase
PFL2_2617	acrD	efflux pump
PFL2_2618	acrA	acriflavine resistance

Table 11 Genes identified that improve bacteria growth on test food waste

3.4.4 Essential metabolic and regulatory pathways for associated nutrients

Also of interest are genes which are essential for growth in putrescible waste but not essential for growth in the control (glucose based medium).

By combining the results, it has been possible to identify pathways which could be manipulated to increase growth in putrescible waste (Table 12).

Pathway	Associated nutrients/ processes	Interpretation
Glycolysis/ glyoxylate bypass	Aerobic respiration not being used	*glk*, *pgm*; inactivated in waste Anaerobic respiration as a result of flux through PEP pathways
CBH metabolism	Sugars other than glucose	Annotation to be resolved
Two component regulators and secondary messengers	Unknown regulators linked to c- di –GMP levels	Lap – inactivated in waste *bifA, wspF* – conditionally essential– controls the switch away from biofilm formation to motility

Table 12 Metabolic pathways associated with bacteria growth in the putresible waste substrate but not in controls

In putrescible waste *P. fluorescens* uses carbohydrate metabolism via anaerobic respiration. This is not classic glucose metabolism and suggests that other sources are being effectively processed.

This indicates that a build-up of succinate removed via the glyoxylate shunt may be a characteristic of the *P. fluorescens* metabolic pathway grown in these experiments.

TarGET data indicates that the bacterial secondary messenger cyclic-di-GMP and motility are also important for growth in putrescible sandwich waste. This indicates cyclic-di-GMP levels switching from biofilm formation to sessile or planktonic growth could be an important factor for successful biomass growth. Cyclic-di-GMP also regulates virulence, antibiotic production and heavy metal resistance, which may be linked to growth in putrescible sandwich waste. However further investigations are required to understand

fully mechanisms required for bacteria growth associated with this messenger.

Genetic locus	function	Comment	Putrescible Waste
bifA	Cyclic-di-GMP phosphodiesterase	Inversely regulates biofilm formation	Conditionally essential
lapA	Large adhesive protein	Required for biofilm formation	Inactivated
Wsp	Signal transduction system	Increases levels cyclic-di-GMP	Conditionally essential
PFL2_3842	EAL domain response regulator	Phosphodiesterase responsive to c-di-GMP	Conditionally essential

Table 13 Conditionally essential genes identifed that relate to strains with succesful growth in the putrescible waste broth.

3.4.5 Summary of findings

The major bacterial response for successful growth in putrescible waste is to switch from biofilm formation to sessile/planktonic growth regulated via c-di-GMP levels. These can be altered by single gene manipulations.

Engineering the four genes in Table 11 for constitutive expression will enhance the rate at which bacteria grow on putrescible waste. Inactivating 15 genes identified will enhance the rate at which bacteria grow on putrescible waste.

This is easily manageable with standard GM technology and could be used to control biomass production, either increasing or decreasing it.

Thousands of genes are not required for growth in putrescible waste but many of these are required for growth in other conditions. This leaves considerable scope to engineer a chassis organism which can grow on putrescible waste and heterologous expression of production pathways should be possible.

Growth to extinction of the carbon energy source should be tested using similar experimental design to allow all energy rich chemical matter in the putrescible waste to be utilised.

Succinate is used as a major intermediate in pseudomonas metabolism in putrescible waste and so blockage of the removal mechanisms would allow accumulation of this platform chemical.

3.5 Next steps: prospects for candidate chemicals: an example exploring PHB production potential

Potential for ***polyhydroxybutyrate*** production by *Pseudomonas fluorescens* on putrescible waste

Background

As outlined in 2.2.5 certain bacteria are able to produce PHA's with applications for bio-based plastics, and the use of food waste as a feedstock for microbial production of biodegradable polymers could be a sustainable pathway for utilising waste for making these materials. In this context PHB was selected as an example somewhat arbitrarily, although plastics pollution is a current and topical concern requiring sustainable, biodegradable solutions.

Building on the approach from Bactevo Ltd (now Nanna Therapeutics Ltd) transposon mutagenesis technology for the generation and screening of bacterial variants to identify genetic changes in *P. fluorescens* that improve growth on putrescible waste - A further step can be to assess the feasibility of bioplastic production by *P. fluorescens*.

As outlined in section 2 Polyhydroxyalkanoates (PHAs) are a class of bacterial biodegradable biopolymers and polyhydroxybutyrate (PHB) is a widely used short chain polymer which could be the target bioplastic. *Pseudomonas* sp. often produce longer chain length polymers (PHAs) and there is precedent for PHA production by *P. fluorescens* strains A2a5, S48 and a Nigerian local strain on sugars, waste frying oil and cassava respectively (Jiang *et al*., 2008; Gamal *et al*., 2013; Aremu *et al*., 2010).

To establish the potential for PHB production by *P. fluorescens* with genetic modifications for improved biomass on putrescible waste identified in this organism requires: -

1. Identification of putative PHB pathways in *P. fluorescens* strains.
2. Identification of genetic targets (provided by Bactevo Ltd) to improve growth of putative PHB producers on putrescible waste.

3.5.1 Genetic modification and chassis organisms

As outlined by Nikel et al (2016) bacteria as *Chassis organisms* can embody at least four different aspects:

1. The physical container of the genetic constructs;

2. the genomic skeleton and the cellular machinery necessary for heterologous (*non-native*) gene expression;

3. the biochemical network in which the activities encoded by the gene implant are nested;

4. the spatial scaffold for the genetic and metabolic graft to take place in space and time.

Examples of bacterial application are given in Box 2. Biotechnology companies have developed the tools to manipulate these bacteria. Opportunities may rely on ensuring freedom to operate. So, choosing species that are not currently being used to develop new routes to the production of fine chemicals can be advantageous.

Box 2. Examples of bacteria uses employed by the biotech sector (Source: J Wain, Bactevo)

- ***Escherichia coli* K12** – a general biotechnology candidate used for the production of several bio-chemicals including 1,4 butanediol. Genetic tools for this organism are well defined
- ***Pseudomonas aeruginosa*** – an environmental organism used for the production of silver nanoparticles from aqueous silver and in many bioremediation processes. Not as well defined as *E. coli* but more versatile in its ability to grow on different feed stocks
- ***Acinetobacter baumannii*** - an environmental organism also used in many bioremediation processes and one familiar to the biotech industry. Not well defined, but versatile and contains many genes not found in other organisms.
- ***Rhodopseudomonas palustris*** – a photosynthetic bacteria that can break down both lignin and cellulose feed stocks and produce hydrogen from glycerol. It is the most versatile of the bacteria used in biotechnology applications.

3.5.2 Identification of putative PHB pathways in *P. fluorescens* strains

Pseudomonas species can produce both long and short chain PHAs or a mixture of polymers. Separate genetic pathways may exist for the production of different polymers e.g. medium chain length PHAs versus PHB and the proposed aim is to identify genes likely to be specific to PHB production.

Pseudomonas sp. 61-3 is known to produce both PHA and PHB and separate pathways have been reported for each product. The PHB locus consists of *phbR*, *phbB*, *phbA* and *phbC* and this pathway has also been reported for several other confirmed *Pseudomonas* PHB producers indicating that a distinct PHB pathway exists (Table 14).

To assess the similarity between the core synthases involved in PHB and PHA production, the protein sequences for the PHA and PHB synthases (*phbC* and *phaC*) were compared and only moderate sequence similarity was observed (52-53% for the two proteins from *Pseudomonas* strain 61-3). This indicates that the PHB pathway is easily distinguished from the PHA pathway (example *phaC* sequences listed in Appendix 3ii).

The next step, using the identified PHB genes (Table 14, Appendix 3i) to search the NCBI sequence database, identifies other *Pseudomonas* strains that could potentially produce PHB. This reveals that ~ 10 *Pseudomonas* strains may have the capacity to produce PHB. These pathways display high similarity to the confirmed pathway in *Pseudomonas* 61-3 (88-94 % amino acid similarity). Commercially available strains are listed in Table 15 and additional sequences for PHB genes provided in Appendix 3iii. The identified ***P. fluorescens* NCIMB-11764** strain is a candidate for PHB production on putrescible waste.

Table 14 Identification of PHB genes in confirmed *Pseudomonas* PHB producers.

Strain	PHAs produced	PHB genes	Experimental work	Yield	Authors
Pseudomonas strain 61-3	**PHB** and co polymer of PHB-co-3HA	**PHB:** phbC, phbA, phbB, phbR	Genes cloned and expressed. PHB production assessed in nitrogen limited salt medium. PHBs were extracted with chloroform and acetone and analysed by GC.	0.9 g/L DCW, 20 % PHB (with gluconate)	Matsusaki, *et al.*, 1998
P. extremaustralis	Mainly **PHB**	PHB: 1) phaR, phaB, phaA, phaC 2) *phbF, phbP, phbX*	Two *P. extremaustralis* strains were assessed; *P. extremaustralis* DSM 17835 uses octanoate and *P. extremaustralis* DSM 25547 uses both glucose and octanoate. PHB production was performed in 0.5 NE2 medium containing sodium octanoate.	38 % PHB as a % DCW (with 0.25% octanoate)	Catone *et al.*, 2014
Pseudomonas sp. USM 4-55	MclPHA and **PHB**	**PHB:** *phbB*, *phbA*, phbC, *phbR*	PHBs extracted by methanolysis with acid methanol/chloroform and then analysed by GC.	Genes cloned and PHB production performed in *E. coli*: 40 % DCW (with glucose).	Tan *et al.*, 2010

Table 15 Commercially available *Pseudomonas* PHB producers.

Strain	Provider	Culture collection reference	Link
P. aeruginosa extremaustralis 14-3	DSMZ	DSM-17835 and derivative DSM-25547	https://www.dsmz.de/
Pseudomonas strain 61-3	JCM	JCM-10015	http://jcm.brc.riken.jp/en/
P. fluorescens NCIMB-11764	NCIMB	NCIMB-11764	https://www.ncimb.com/
P. arsenicoxydans strain CECT 7543	DSMZ	DSM-27171	https://www.dsmz.de/

3.5.4 Potential to optimise a P. fluorescens PHB producer for improved growth on putrescible waste

Bactevo Ltd have identified a number of mutations that enhance growth of *P. fluorescens* NCIMB 9046 on putrescible waste. It is possible that these mutations could be engineered into the candidate *P. fluorescens* PHB producer NCIMB-11764 to improve biomass (and potentially PHB yields) on putrescible waste.

Activation of the following genes provided a conditional advantage on putrescible waste:

• rpsB (30S ribosomal protein S2, translation control linked to c-di-GMP regulation which promotes swimming motility),

• pyrH (pyridylate kinase – energy metabolism, phosphoenol pyruvate (PEP) control of phosphate pathways – linked to motility and membrane potential),

• acrA, acrD (efflux pump – response to toxic stress through extrusion of substrates).

These genes were identified in the putative PHB producer P. fluorescens NCIMB 17764 (Appendix 3iv) and there is potential to engineer (activate) these targets to enhance growth.

Note: There is some evidence that overexpression of the PHB regulatory gene (phbR) improves yields (Matsusaki et al., 1998).

Inactivation of the following genes provided a conditional advantage on putrescible waste:

Inactivation of 15 genes was beneficial for growth and Bactevo Ltd have provided details for 3 of these genes (glk, lapA and pgm).

The lapA gene was not identified in the putative P. fluorescens NCIMB-11764 PHB producer which is beneficial since inactivation would not be required. The pgm and glk genes were both identified in P. fluorescens NCIMB-11764 (Appendix 3iv) and could be inactivated using genetic engineering technology.

Approximately 1500 genes are conditionally essential on putrescible waste:

Conditionally essential genes for growth of P. fluorescens NCIMB 9046 on putrescible waste have been identified but these are likely to vary between P. fluorescens strains. The presence of these genes in the target P. fluorescens PHB producer could be evaluated upon provision of a gene list. Incorporation of this information into a metabolic model of P. fluorescens (e.g. Borgos et al., 2013; Timm et al., 2015) could provide an insight into the genes important for growth on putrescible waste and production of PHB. Metabolic modelling is established within the Wain group.

3.5.5 Limitations to next steps

The large number of genes that are essential for growth on putrescible waste make manual searching of genetic databases for strains capable of converting putrescible waste into valuable chemicals virtually impossible. To reduce the complexity we suggest starting with *P. fluorescens* and with production of PHAs. For data reduction, to facilitate searching genomic databases for suitable strains a genome scale model (GSM) of the metabolic process could be constructed and then searching could be carried out at the pathway level rather than at the gene level. To generate an improved GSM would not be straight forward but would require improved annotation of *P. fluorescens* genomes and the involvement of a skilled mathematical modeller. Once established however the model could be modified and extended for other similar bacteria, or different chemical products, if necessary.

3.6 Conclusions

It is possible to select for strains of *P. fluorescens* that show improved biomass growth on a characterised putrescible food waste.

P. fluorescens is also suitable as a potential chassis organism for optimisation to grow on putrescible waste. It is now possible to design a bacterial cell for maximum growth on putrescible waste using the gene list resulting from this work.

Succinate is used as a major intermediate in pseudomonas metabolism in putrescible waste and so blockage of the removal mechanisms would allow accumulation of this platform chemical.

The example approach given for investigating prospects for PHB production indicates that there are Pseudomonas strains available that may produce PHB and these could provide positive controls for such a project. The approach also indicates that PHB genes are distinct from the PHA genes which should assist in the identification of PHB producers. A PHB pathway was identified in one commercially available P. *fluorescens* strain (NCIMB 11764) which could provide a good starting point for optimisation of PHB production on putrescible waste. Beneficial mutations, identified by Bactevo Ltd, could be engineered into putative PHB producers for improved growth on putrescible waste.

3.6.1 Limitations and barriers

The experiments only utilised a fixed characterised version of food waste as a test subject. This may not be representative of different sources of food waste as outlined in the appendix.

The sensitivity of selected bacterial strains to the composition of the food waste is not determined by these experiments. Further work could look at the efficiency of bacterial degradation in variations of putrescible food waste.

As well as using variations in the compositional make up of a mixed food waste substrate, experiments selecting mutants with individual food waste components e.g. bakery products, meat waste, dairy waste, etc. could be investigated.

Utilising the biotechnology approaches for genetic modification of bacteria to enable exploitation of food waste for bio-based plastic production may be a significant social and environmental concern for various interest groups and wider society. This may be the case even if production is within a closed system with measures to control any potential for release of GMM.

Finally, further experiments moving from a laboratory to pilot scale would be necessary to indicate the potential for commercial product yields to allow any techno-economic assessment to be conducted.

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5 Appendix 1

5.1 The quantity and composition of wasted post-consumer food and food residues

5.1.1 Volumes

The current best available dataset for the EU-28 as a whole indicates that 46.1 ± 4.9 million tonnes of food waste occurred in households with a further 10.5 ± 1.5 million tonnes from the food service sector in 2012 (EU FUSIONS).

For the majority of individual EU Member States the quality of data on the quantities and composition of wasted food and food residues that are collected is poor.

For the purposes of this report, we shall consider only putrescible (food) residues created only by the consumer and catering sectors in the EU-28.

Putrescible food wastes here concern those that are *liable to become putred or rotten*; in that after minor damage or spoilage from discarding they may start to decompose by micro-organisms within days and of a nature and character that could give rise to obnoxious odours and attract vermin. By definition, organic wastes occurring throughout the food supply chain may therefore be putrescible although the terms are not necessarily the same. Coconut husk or hazelnut shells are clearly organic but would not be described as putrescible.

5.1.2 Consumer (household) sector

Existing data from the UK can give an insight into the composition of household food waste. Whilst the disposal route for food waste will vary between local authorities and between individual countries, the most common disposal routes used in the UK are shown in the following table. The individual components of the waste will depend on the chosen route for disposal from the household.

Table 16 Routes for disposal of food waste from UK households

Route for disposal	Description of food items potentially disposed of via route	Description of food items unable to be disposed of via route
Food waste caddy	Raw food - vegetables, fruit, meat and fish Cooked food - vegetables, fruit, meat and fish Meat and fish bones Teabags and coffee grounds Dairy products - eggshells and cheese Bread, cakes and pastries Rice, pasta and beans Out of date food Plate waste	
Home composting	Vegetable trimmings, egg shells, tea-bags, coffee grounds	Cooked food
Garden waste collection		Kitchen waste (e.g. fruit and vegetable peelings, meat, egg shells, tea bags, coffee grounds)
Mixed food & garden waste collection	Kitchen waste (e.g. fruit and vegetable peelings, meat, egg shells, tea bags, coffee grounds) Raw & cooked food	
Mixed general waste (non-recyclables)	Plate waste, cooked food	
Liquid waste via sewer	Milk, sauces, dressings, juices, gravy, soft drinks	Oils and fats

The exact system for collecting, sorting and recycling household waste will vary between local authorities. Typical destinations may include anaerobic digestion or Animal By-Products Regulations compliant commercial composting (in-vessel composting). It is noted that mixed food and garden waste is not a preferred system since all the collected organic material would need to be treated at significant cost at a composting site compliant with the Animal By-Products (Enforcement) (England) Regulations 2011. Garden waste treated separately would not be subject to this restriction.

The FUSIONS project found that compositional data could not be obtained on an EU-wide basis for food waste. However, there are several key studies at member state level which give good insights into the composition of food waste. These studies are briefly described below with regard to their implications on characterising putrescible food waste for this work. It is important to note that the methodology used for the waste compositional analysis varies between the studies particularly in relation to mixed/composite foods as well as the inclusion or exclusion of liquid wastes which are frequently disposed of via the sewer.

A report by WRAP, Household Food and Drink Waste in the United Kingdom 2012 provides detail on the types of food and drink waste split by food group. It can be seen that most of the food waste arises from six key food groups: fresh vegetables & salads, drink, fresh fruit, bakery, meals (home-made and pre-prepared), meat & fish, and dairy & eggs. The waste is classified as 'avoidable', 'possibly avoidable' or 'unavoidable' although, for the purposes of this study, it is important to consider all wastes arising in the household for disposal. Unavoidable household food waste items such as egg shells, tea leaves, chicken bones and banana skins remain available for valorization since it is possible to collect this material via kerbside food waste collections.

Looking at the most wasted foods (Table 17), this is dominated by fresh potato waste, tea waste and standard bread with major contributions from banana, milk, poultry, composite meals, mixed semi-solid food[25] and carbonated soft drinks. Together these account for almost half of all food waste in the UK.

[25] Mixed semi-solid food is classified as food which was not identifiable during the compositional analysis and includes food that has decomposed and is no longer identifiable.

Table 17. List of the most commonly wasted food products in the UK (WRAP 2012)

Food product	Total quantity of waste (tonnes, 2012)	Percentage
Potato	730,000	10
Tea waste	550,000	8
Standard bread	460,000	7
Banana	310,000	4
Milk	290,000	4
Poultry	280,000	4
Composite meals	270,000	4
Mixed semi-solid food	250,000	4
Carbonated soft drinks	230,000	3
Sub-total	3,370,000	48
Total of all food waste	7,000,000	

A study by Parfitt et al., (Food waste within food supply chains: quantification and potential for change to 2050) gives details of household food waste composition across 5 countries. Whilst considerable variation can be seen between the various studies, fresh fruit & vegetables, salads, bakery and dairy products usually account for the bulk of household food waste.

Table 18 Studies reporting the composition of domestic food waste

	Netherlands (Thönissen, 2009)	Austria (Lechner & Schneider, 2009)	USA (Jones, 2002)	Turkey (Pekcan, 2006)
Fresh vegetables & salads	15 %	12 %	27 %	34 %
Drink	-	6 %	9 %	-
Fresh fruit	9 %	8 %	16 %	35 %
Bakery products	20 %	20 %	19 %	6 %
Meals	-	9 %	-	-
Meat & fish	7 %	11 %	11 %	12 %
Dairy & eggs	30 %	16 %	-	9 %
Other	20 %	16 %	17 %	4 %

A study by Silvennoinen et al, [3] (Food waste volume and composition in the Finnish supply chain: special focus on food service sector)

Table 19 List of the most commonly wasted food products in the Finnish household (Silvennoinen et al)

Food product	Percentage
Vegetables	19
Home-cooked food	18
Milk produce	17
Bread	13
Fruits & berries	13
Meat, fish & eggs	7
Convenience food	6

Tinned & non-perishable foods	2.5
Sub-total	95.5

WRAP, AD Workshop – Optimising Processes for the Stable Digestion of Food Waste [4]

The above workshop gives the composition of collected kitchen waste which is discussed as a feedstock for anaerobic digestion. The quantity collected amounted to 2.91kg household^{-1} week^{-1} with a total solids content of 23%, volatile solids content of 21% and a carbon to nitrogen ratio of 14:1.

Food product	Percentage
Uncooked fruit & vegetables	60
Cooked meat	12
Teabags	10
Bread	7
Cooked fruit & vegetables	7
Meat, fish & eggs	7
Uncooked meat	1
Eggs	1
Cheese	1

Zhang et al, Compositional analysis of food waste entering the source segregation stream in four European regions and implications for valorization via anaerobic digestion [5]

	UK	Finland	Portugal	Italy	Average
Fruit and vegetable waste	60.9 %	44.5 %	59.2 %	69.0 %	58.4 %
Pasta/rice/flour/cereals	1.5 %	0.4 %	0.2 %	12.4 %	3.6 %
Bread and bakery	9.0 %	3.8 %	3.1 %	2.8 %	4.7 %
Meat and fish	6.7 %	4.3 %	7.3 %	6.2 %	6.1 %
Dairy	1.7 %	2.0 %	0.7 %	1.4 %	1.4 %
Drinks	7.1 %	27.5 %	0.2 %	-	8.7 %
Confectionery, snacks, etc	0.7 %	3.2 %	0.3 %	-	1.0 %
Mixed meals	12.3 %	6.3 %	29.0 %	1.4 %	12.2 %
Other food	0.2 %	8.0 %	-	6.9 %	3.8 %

5.1.3 Catering sector

Information on the composition of the estimated 10.5 million tonnes of food waste (FUSIONS) from the food service sector is not available on an EU-wide basis. However, again, several key publications from individual member states give an insight into compositional characteristics of this waste.

WRAP Overview of Waste in the UK Hospitality and Food Service Sector, November 2013 [6] gives a detailed breakdown of the various constituent parts of the UK hospitality and food service sector and the wastes arising therein. Whilst relatively high rates of packaging waste are recycled, only 12% of food waste is currently recycled. The composition of food waste will vary depending on the type of establishment (e.g. staff canteen, hotel, quick service restaurant, etc.) and the amount of food preparation taking place on-site.

The following table shows the average composition of food being wasted across the whole UK hospitality & food service sector.

Table 2. Composition of the food being wasted in the UK Hospitality & Food Service sector

Constituent	Total quantity of waste (tonnes per year)	Percentage
Avoidable potato/potato products	193,000	21
Avoidable fruit & veg	139,000	15
Avoidable bread & bakery	105,000	12
Avoidable inseparable plate scrapings	67,000	7
Avoidable pasta & rice	64,000	7
Avoidable meat & fish	57,000	6
Avoidable whole servings	20,000	2
Avoidable where categories <2%	35,000	4
Unavoidable fruit & veg	139,000	15
Unavoidable other	52,000	6
Unavoidable potato/potato products	20,000	2
Unavoidable where categories <2%	28,000	3

Silvennoinen et al, Food waste volume and composition in the Finnish supply chain: special focus on food service sector [3] found that food waste in restaurants was heavily influenced by the restaurant type and this affected the relative proportions of kitchen waste, service waste and leftovers. In self-service buffets, the main cause of food waste was service waste i.e. cooking too much food. Fast food restaurants had the lowest proportion of food waste although additional preparation wastes may occur elsewhere for pre-prepared items.

Table 20. List of the plate waste contribution of plate leftovers in the Finnish food service sector

Food product	Schools and day-care centres (%)	Dinner restaurants (%)
Vegetables and fruits	16	26
Potato, rice and pasta	-	28
Breads and grains	3	14
Cheese and other dairy	4	3
Main course, fish	63	5
Main course, meat		9
Main course, vegetarian	3	<1
Dessert	-	<1
Other	11	15

Stare, M., Food waste volumes in Sweden, Report for Swedish Environmental Protection Agency [7]

The above report once again shows the marked dependency of type of catering establishment on the composition of food waste. Much higher wastage of meat, pasta, rice and potato occurred in Swedish restaurants than in catering facilities such as school canteens.

Food product	Catering facilities (%)	Restaurants (%)
Fruit and vegetables	72	12
Pasta, rice and potato	11	37
Meat	9	40
Bread	5	5
Dairy products	2	3

Gomez et al, In-Vessel Composting of Food Waste – A Catering Waste Management Solution [8] gives useful analytical information on typical food wastes although the individual foods contained within the waste are not discussed.

Constituent	Mean
Moisture content (%)	76.7
Ash (%)	1.7
Volatile matter (%)	98.3
Total Carbon	50.1
Total Nitrogen	4.4
C:N Ratio	12:1

5.1.4 Compositional model for mixed food waste experiments

In summary, the different studies indicate that households and catering food waste composition is variable (Table 21). It is not possible to define a typical average composition for food waste from either source. Neither are there enough studies within countries to understand how variable waste composition is nationally, geographically and also seasonally.

The mix will however be rich in fruit and vegetable waste, have a moisture content in the region of 75% and a carbon-nitrogen ratio in the region of 12 to 14:1.

Based on this a conceptual model of mixed waste of more specific waste stream reflecting sandwich and lunch service related wastes has been derived from retail purchased food products, (Table 22). The approximated nutritional composition is presented in Table 23.

Figure 10 Summary for putrescible food waste composition taken from studies across Europe (% average in category and range of values in for the 10 reported studies).

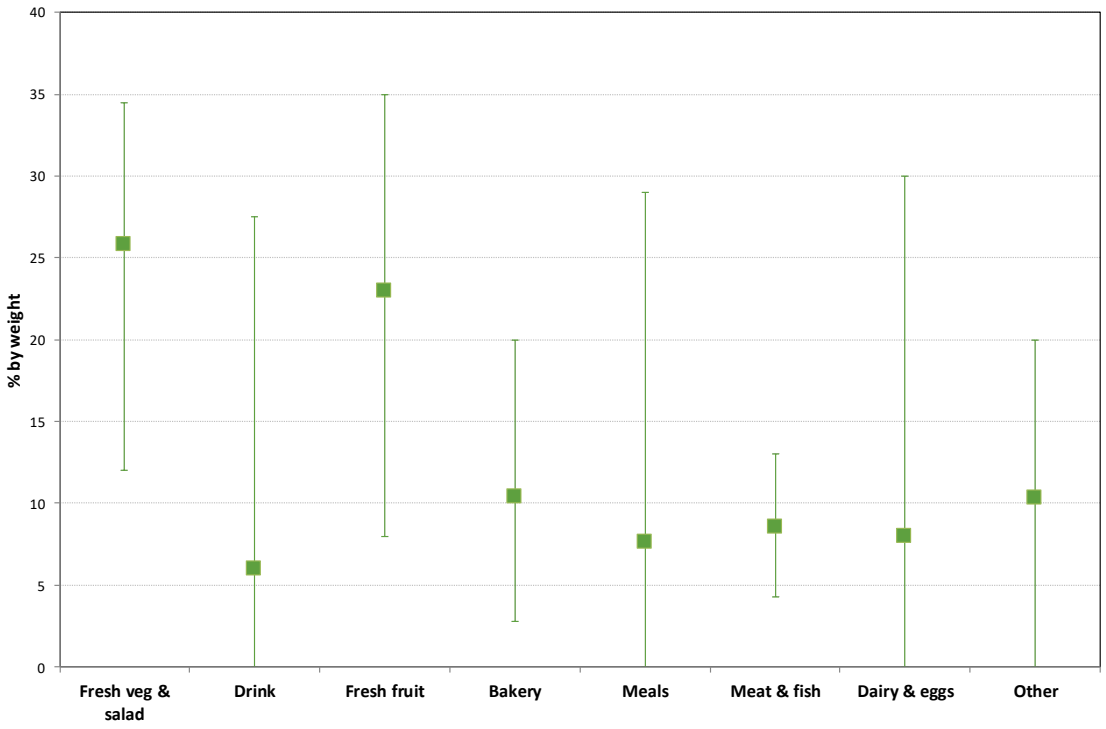

Table 21 Literature summary for putrescible food waste composition taken from studies across Europe.

Studies	Fresh veg & salad	Drink	Fresh fruit	Bakery	Meals	Meat & fish	Dairy & eggs	Other
Netherlands (Thönissen, 2009)	15	0	9	20	0	7	30	20
Austria (Lechner & Schneider, 2009	12	6	8	20	9	11	16	16
USA (Jones, 2002)	27	9	16	19	0	11	0	17
Turkey (Pekcan, 2006)	34	0	35	6	0	12	9	4
Silvennionen (finland)	19	0	13	13	18	7	17	13

UK (WRAP AD)	33	10	33	7	0	13	2	0
Zhang (UK figures)	31	7.1	30	9	12.3	6.7	1.7	2.2
Zhang (Finland figures)	22.5	27.5	22	3.8	6.3	4.3	2	11.6
Zhang (Portugal figures)	30	0.2	29.2	3.1	29	7.3	0.7	0.2
Zhang (Italy figures)	34.5	0	34.5	2.8	1.4	6.2	1.4	19.2
Average % w/w	26	6	23	10	8	9	8	10
Range of % w/w	12 - 34.5	0 - 27.5	8 - 35	2.8 - 20	0-29	4.3-13	0-30	0-20

Table 22 Components of the test putrescible food waste substrate

Component	Weight (g)	%	Brand /product name
Peach, stoned	107.2	11%	Co-op /Ripen at Home Peaches
Potato, diced, unpeeled	147.8	15%	Co-op /Maris Piper Potato
Carrot wedges	74.1	7%	Co-op/ Loved by Us Cook in 3 min Carrot Wedges
Cherry tomatoes	35.4	4%	Co-op /Sweet & Crunchy Salad Bowl 170g
Cucumber, sliced	30.4	3%	Co-op/ Sweet & Crunchy Salad Bowl 170g
Lettuce	81.7	8%	Co-op /Sweet & Crunchy Salad Bowl 170g
Carrot strips	23.4	2%	Co-op/ Sweet & Crunchy Salad Bowl 170g
Total & salad vegetables, fruit	500.0	50%	
Bakery	100.4	10%	Morrisons/ 800g Farmhouse Sliced Bread
Composite meals	100.0	10%	Co-op/ Ready to eat Quiche Lorraine
Meat & fish	100.2	10%	Co-op/ Thin Sliced Honey Roast Ham
Dairy & eggs	100.6	10%	Co-op/ Truly Irresistible Madagascan Vanilla Yogurt

Drink	100.0	10%	Robinsons/ Summer Fruits Squash (diluted to 5%)
Total	1001.2		

Table 23 Nutritional composition of the test food waste substrate

Food item	Weight (g)	Energy (kcal)	Fat (g)	Carbohydrate (g)	(of which sugars)	Fibre (g)	Protein (g)	Salt (g)
Peach, stoned	107.2	40.7	0.5	8.1	8.1	1.6	1.1	0.0
Potato, diced, unpeeled	147.8	119.7	0.7	25.9	1.2	2.4	2.7	0.0
Carrot wedges	74.1	31.1	0.4	5.7	5.3	2.2	0.4	0.0
Cherry tomatoes	35.4	7.1	0.2	1.1	1.1	0.4	0.2	0.0
Cucumber, sliced	30.4	3.3	0.2	0.4	0.4	0.2	0.2	0.2
Lettuce	81.7	11.4	0.4	1.1	1.1	1.1	1.0	0.0
Carrot strips	23.4	6.8	0.1	1.0	1.0	0.7	0.1	0.0
Bread	100.4	243.0	0.9	48.3	3.2	2.7	9.1	0.9
Quiche	100.0	274.0	18.0	19.0	2.1	1.6	8.9	0.6
Ham	100.2	113.2	3.1	2.7	2.7	0.5	19.0	1.8
Yogurt	100.6	150.9	8.3	16.1	16.1	0.5	2.9	0.1
Drink, squash	100.0	4.7	0.0	0.3	0.3	0.0	0.0	0.1
Total	1001.2	1006.0	32.9	129.7	42.6	13.8	45.8	3.7

5.1.5 References

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Thönissen R. 2009 Food waste: The Netherlands. Presentation to the EU Presidency Climate Smart Food Conf., November 2009, Lund, Sweden.

The following citations are related to data published in Parfitt et al 2010:

Netherlands (Thönissen, 2009)

Austria (Lechner & Schneider, 2009

USA (Jones, 2002)

Turkey (Pekcan, 2006)

6 Appendix 2

Essential genes identified in Pseudomonas Fluorescens cultured in the model putrescible (sandwich) food waste substrate but not identified in control substrates.

PFL2_0037	trpB	tryptophan synthase beta chain
PFL2_0302	hyp	hypothetical protein PVLB_24070
PFL2_0944	unk	proline dehydrogenase superfamily protein
PFL2_0947	unk	major facilitator superfamily protein
PFL2_1020	ptsH	phosphocarrier protein HPr
PFL2_1026	ostA	organic solvent tolerance protein
PFL2_1398	ompH	outer membrane protein OmpH
PFL2_1412	LysR	putative LysR-family regulatory protein
PFL2_3614	hyp	secretin
PFL2_3615	ipsJ	isocitrate lyase
PFL2_3842	unk	response regulator/EAL domain protein
PFL2_3843	unk	sensor histidine kinase/response regulator
PFL2_3849	hyp	hypothetical protein PFLU4059
PFL2_5547	ptsP	phosphoenolpyruvate-protein phosphotransferase

7 Appendix 3(i):

PhbC (*Pseudomonas* sp. 61-3)

MDNNAHTFKTYWSGQVPFIASFAVQQLRLWVSTNPWFSGHEHGAWFELPRETLDSLQADYQVQWGQLGQK
LLTGQPFSFDDRRFASGNWSEPLFGSLAAFYLLNSSFLLKLLDMLLIDEKKPRQRLRYLVEQAIAASAPS
NFLVSNPDALQRVVETQGASLVTGMQHLASDMNEGKMRQCDSGAFKVGIDLANTPGEIVFENHLFQLIHY
YPQSETQYRHPVFVVPPSINKYYILDLRPDNSMVRHLLEQGHPVFLMSWRNFDEEHAGTTWDDLIELGVI
DGLQVAREISGEQRLNCVGFCIGGTLLSTALAVLAARGDREIASVSLFTTFLDYHDTGPIDIFVDEELVA
HRERTIGGVNGPIGLFRGEDMGNTFSLLRPNDLWWNYNVDKYLKGQKPIPLDLLFWNNDSTNLPGPMYCW
YLRHTYLQNDLKSGELECCGNKLDLRAIDAPAYILATHDDHIVPWKSAYASTNLLSGSKRFVLGASGHIA
GVINPPAKQKRHYWTNNRVTKNPETWFKNAEQHPGSWWNDWFTWLAGHSGERQPAVAHTGNNKYPPLEPA
PGRYVKL

PhbA (*Pseudomonas* sp. 61-3)

MNEVVIVAATRTAIGSFQGALSAIPATELGAAVIRRLLEQTGIDAAQIDEVILGQVLTAGAGQNPARQTA
IKAGLPHTTPALTLNKVCGSGLKAVHLAVQAIRCGDAELVIAGGQENMSLAPYVLPKARTGLRMGHAQLQ
DSMIQDGLWDAFNDYHMGITAENLAQKYEISREAQDAFSAASQQKAAAAIEGGRFQSEITPILIPQRKGE
PLVFDTDEQPRIDSTAQALAKLKPAFQKDGSVTAGNSSTLNDGAAVLLLASAAKAQELGLPVLARIKAYA
SAGVDPSIMGIGPVPATRLTLQKAGWSIEDLDLIEANEAFAAQSLAVGKELGWDTSKVNVNGGAIALGHP
IGASGARILVSLVHELIRRDGKKGLATLCIGGGQGVGLAIER

PhbB (*Pseudomonas* sp. 61-3)

MESRSRIAVVTGGMGGIGTSISQRLYKEGFKVIVGCSANSSRKDDWMATQLAAGYQFECVETDITDWEST
RKAFEMVREHFGPIDVLVNNAGITRDASFRKLTPEDWNTVIGTNLNSLFNTSKQVIEGMLSKGWGRIINI
SSINGQRGQFGQTNYSAAKAGIHGFTMALAREVSGKGVTVNTVSPGYIQTDMTAAIRKDILDGMIAGIPV
GRLGQPEEIASIVAWLASDESAYSTGADFSVNGGMNMQ

PhbR (*Pseudomonas* sp. 61-3)

MYRMSSGYASVLANTLSAQGLDVARLCDEAGLDVKLVNESGAFCERSVIYRLWDLAAQASDDPNIGLKAY
GNFHPGSFQIVGYTMMSSSNLKRALERLVRFSPLISTGFSLFFVQEQQNYRLASLDHQQRGSVKPRQYTD
AGLASLLGFCRWLAGGKSPQPLSVEFTYPEPEDTSEHQRLFGCPLRFGAAYDSILFDGQELLSPLSMANE
ALATLHDSFAEAQLDLLSGYSFVCRIRALITERLSQGQGQGQCDMESIAAALSISKRTLQRGLEKDGTQF
KDVLNDVRRQLADFYLRHSNFNMKHVTYLLGFHDHSSFHKACTRWFGMTPGQYRADESLFAVEEAMPVPV
PVRLMNPVGLRRDQKIGGFASAYAERIHR

Pseudomonas 61-3_nucleotide sequence

AAGCTTGCGCAGCCTGCCCTTGCTACTCCCGGACACCAAGGCACGCCAACAGGTCCTTGCGGCCGTT
GGGCAATTGATGCCTGAACTGCTGACAATTGCTGGAGCTGAAGGTGAATTCTGGCGTGAACTGCACG
CGCTACTCGAAGTCCCCCTGCCAGGTTTTAGCCTGACCCAACCGCCCAGTGAAACAGATGTCATTCAG
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GGCATGGTCTCTGTAGTTCGTTTCTCGCAAACCGGCGCCCCTGAGGTGATGAACATCGAGCACGTGA
CAGCGTGTGAACCGGGGCCC

PhbC (Pseudomonas extremaustralis 14-3/14-3b)

MNNSHSFAHYWSGQAPFITSFALQQLRLYVAQNSWFNGHDQSQWFNVSPEALEQLQVDYQQQWTALG
QQLLARQSFDFDDRRFASGNWSEPLFGSMAAFYLLNSGFLLKLLELLTIKEEKPRQRLRYLIEQAIAASAPS
NFLLSNPDALKCLVETQGASLLSGLLHLAGDLQEGKLRQCDRGDFEVGVNLAVTPGEVVLETPLFQLIQYL
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IGGHGGKCGLFRGEDMGNTFSLLRPNELWWNYNVDKYLKGQKPRALDLLFWNNDSTNLPGPMYCWYLR
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PPASNKRHYWVNEHITPIADDWLQSAQQHAGSWWVDWFAWLAGHAGELRPAITRMGNAEYPPLEQAP
GRYVKQ

PhbA (Pseudomonas extremaustralis 14-3/14-3b)

MIDVVIVAATRTAIGSFQGSLAEIPAPELGAIVIRRLLEQTGLDAAQVDEVILGQVLTA
ASGQNPARQAVIRAGLPHVVPAMTLNKVCGSGLKALHLAAQAIRCGDAEVIIAGGME
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EAQDAFAAASQQKAVAAIEAGRFDAEITPVLIPQRKGDPIAFARDEQPRAGTTAESLA
KLKPAFKKDGSVTAGNASSLNDGAAAVLLMSAAKAKALGLSVLAKISAYANAGVDPA
IMGIAPVSASRHCLDKAGWSLNELDLIEANEAFAAQALAVGQELGWDAAKVNVNGG
AIALGHPIGASGCRVLVSLLHEMIRRDAKKGLATLCIGGGQGVALAIER

PhbB (Pseudomonas extremaustralis 14-3/14-3b)

MATSSNSTRIALVTGGMGGIGTAISQRLHQDGFTVVVSCSPYSSRKASWMAKQLEA
GFHFHCIDCDITDWDSTRQAFEMVRENVGPIDVLVNNAGITRDGTLRKMPPENWKA
VIDTNLTGLFNTTRQVIESMLTKGWGRVINISSINGQRGQFGQTNYSAAKAGIHGFS
MALAREVSGKGVTVNTVSPGYIKTDMTAAIR
PDILEGMIAGIPVGRLGQPEEIASIVAWLASDQSAYATGADFSVNGGMNMQ

PhbR (Pseudomonas extremaustralis 14-3/14-3b)

MYRISSSYVGMLVAMLQAEGLDAVRLCREAGIDMCLLAQEDAFFTRASAYRLMALAE
LDSDNPNLGLKAAVHFKPGSFQLVGYVMMSSANLKQALEHFVRFHLLLGNGVTLALS

PEQGGRLRLTCLEHPEEGCPRNRAFEDAGAAALLGFCRWLMGGGLPQPLQFDFVHD
EPGNLPEYQQLFGCSLRFGAQHTSVLFDQQELLRPLSTANEALALLHKRFAEFRLGQL
GGVSLGGRVRGLIIERLGQEGCDMESIGLALCMSKRTLQRGLEKEGLKFKDILGDVR
RQSADYYLCRSSYSLTQVAEYLGFHDQSSFHKACLRWFGRSPGRYRLDEGEQSTPH
EDVSDAAHQADDQCIDDIDEEAADQRNDNERLVRGSVALSNCRHVDDGRCS

Pseudomonas extremaustralis 14-3/14-3b_nucleotide sequence

ATTGCTGGGTTTCCCCTGCATGGGCCGCAACTACGAAGCCAGCGTGATAGCCGCCGGCTTCGGCGG
CATCGCCCTGGGTTCAACTGCAACGGCCATCGTCAACATGACGGCAGTTACTCAGCGCTACGGAGCC
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GCGCCGACTTCTCGGTGAACGGCGGCATGAATATGCAGTGATGCGCAATCGACTAGTCATAACATGA
GGCATTCCAGATGATCGACGTCGTTATTGTCGCTGCAACCCGCACCGCCATCGGTAGCTTCCAAGGC
AGCCTGGCCGAGATTCCGGCACCGGAACTCGGCGCCATCGTCATCCGGCGCCTGCTCGAGCAGACC
GGCCTCGATGCTGCCCAGGTCGATGAAGTGATCCTCGGCCAGGTGCTCACTGCCGCGTCCGGACAG
AACCCCGCACGCCAGGCTGTGATTCGCGCCGGCCTGCCCCACGTCGTTCCTGCGATGACCCTGAACA
AGGTCTGCGGCTCGGGCCTCAAGGCCCTGCACCTGGCTGCCCAGGCTATCCGTTGCGGCGATGCCG
AGGTGATCATTGCCGGCGGCATGGAGAACATGAGCCTGTCGCCCTACGTGCTGCCCAAAGCCCGCAC
CGGCCTGCGCATGGGCCATGCGCAGATGCTCGACAGCATGATCGTCGATGGCCTGTGGGATGCCTT
CAACGATTACCACATGGGCATCACCGCCGAGAACCTGGTGGACAAGTACGGCATCAGCCGTGAAGCC
CAGGACGCCTTTGCCGCCGCCTCGCAGCAGAAGGCCGTGGCCGCCATCGAAGCTGGTCGTTTCGAC
GCCGAGATAACCCCGGTGCTGATCCCGCAGCGCAAGGGCGATCCAATCGCCTTCGCCCGTGACGAG
CAGCCGCGTGCCGGCACCACCGCCGAGTCGCTGGCCAAACTCAAGCCGGCGTTCAAGAAGGACGGC
AGCGTTACCGCCGGCAATGCCTCCAGCCTCAACGATGGCGCCGCCGCCGTGCTGCTGATGAGCGCA
GCCAAGGCCAAAGCGCTGGGCCTGTCGGTGCTGGCGAAAATCAGCGCCTATGCCAACGCAGGCGTC
GATCCGGCGATCATGGGTATCGCCCCGGTCTCGGCCAGCCGCCACTGCCTGGACAAAGCCGGCTGG
AGCCTGAACGAGCTAGACCTGATCGAAGCGATCGCAGCATGGCCGTTGGTAGGAGCTGGGCTGGGA
TGCCGCCAAGGTCAACGTCAACGGCGGCGCCATCGCCCTGGGTCACCCGATCGGCGCCTCCGGCTG
CCGCGTGCTGGTCAGCCTGCTGCACGAGATGATCCGCCGCGATGCCAAGAAAGGCCTGGCGACGCT
GTGCATCGGCGGCGGCCAGGGCGTGGCGCTGGCCATTGAACGCTGACTAACGATTTCGCGGATCTA

TCGGGCGAGCTCCCCTGCACCCTCACCGCCTGGATGGCGGTGAGGGTATTCCCCCAAATGATCGCAC
CGTGCCCTTTGCCGGGCGCGGCCTTTTTTCATTTTCTTGGACAAACCATGAACATGAACAATTCACATT
CTTTCGCTCACTACTGGTCAGGACAGGCGCCTTTCATCACCAGCTTCGCCCTACAGCAACTGCGCCTG
TACGTAGCGCAAAACTCTTGGTTCAACGGGCATGACCAAAGCCAGTGGTTCAATGTCTCCCCCGAAG
CGTTGGAACAATTGCAGGTTGACTACCAGCAACAATGGACAGCGCTTGGCCAGCAACTGCTGGCCCG
TCAATCGTTCGACTTCGACGACCGGCGTTTCGCTAGTGGCAACTGGAGTGAACCGTTGTTCGGTTCCA
TGGCCGCCTTCTACCTGCTGAATTCCGGATTTCTGCTGAAATTGCTGGAGCTGCTCACCATCAAAGAA
GAGAAGCCACGTCAACGTCTGCGCTACCTGATCGAGCAGGCAATCGCCGCAAGCGCCCCGAGCAAC
TTCCTGCTGAGCAATCCCGATGCCCTGAAATGCCTGGTGGAAACCCAGGGGGCCAGCCTGCTCAGTG
GTCTATTGCATCTGGCCGGAGACCTACAAGAAGGCAAGTTGCGTCAATGCGACCGAGGCGACTTCGA
AGTTGGCGTGAATCTCGCCGTCACTCCCGGTGAGGTGGTGCTGGAAACTCCGCTGTTCCAGTTGATC
CAATACCTGCCGCTCACCGATACACAGTACCAGAGGCCAATATTTATCGTCCCGCCGGCCATCAACAA
GTACTACATCCTCGACCTCAGCCCGGAAAATTCTCTGGTTCGCCATCTGCTGGAACAGGGTCACCCG
GTATTCCTGATGTCCTGGCGCAACTTCACCCAGAAACAGGCTGACATCACCTGGGAGCAGATCATCCA
GGACGGAGTGATCAGCGCCCTACGCACTACCCGGGTCATCAGTGGCGAGCGTCATCTGAACTGCCTG
GGCTTCTGCATCGGCGGCACCCTGCTGAGCTGCGCGTTGGCAGTGCTGGCTGCGAGGGGCGACCAC
GACATTGCCAGTCTGAGCCTGTTCGCCACCTTCCTCGACTACCTTGATACCGGGCCGATCAGTGTCTT
TGTCGATGAGCAACTGGTGGCCTACCGCGAGCGCACCATCGGTGGCCATGGTGGTAAATGTGGCCT
GTTCCGCGGAGAGGATATGGGCAACACCTTCTCCCTGCTGCGGCCCAACGAGCTGTGGTGGAACTAC
AACGTCGACAAGTACCTCAAGGGACAGAAACCACGCGCCCTAGATCTGCTGTTCTGGAACAACGACA
GTACCAACCTACCTGGCCCCATGTACTGCTGGTATCTGCGCCACACCTACCTGCAGAACGACCTCAAG
TCGGGCGAGCTGGAGCTGTGCGGGGTCAAGCTGGATCTGCGCTCCATAGAGGCGCCAGCCTACCTC
CTCGGAACCCACGATGACCACATAGTCCCATGGCGCAGTGCCTATGCCAGCACGGCTCTTCTTGGGGG
GATCGAAACGCTTCGTCCTCGGATCTTCTGGCCACATCGCCGGAGTGATTAACCCACCAGCAAGCAA
CAAACGCCATTACTGGGTAAACGAGCACATCACGCCGATTGCTGACGACTGGCTACAAAGCGCTCAA
CAACACGCAGGTAGTTGGTGGGTCGATTGGTTCGCCTGGCTGGCCGGGCATGCCGGCGAGCTCCGG
CCCGCCATCACGCGGATGGGCAATGCCGAATACCCTCCCCTAGAACAAGCGCCTGGGCGCTACGTGA
AGCAATGACCGCCCGAAGTTTAGGAAAACCCTCGATGAAGCCACAACAGAAGAAAACCCTGATCCAC
ACGGCCTCCGCCGTCGCCCTGGCCGCCGTGGCCGCACTGGTCTGGCT

PhbB (*Pseudomonas* USM 4-55)

MATSSNSTRIALVTGGMGGIGTAISQRLHQDGFTVVVSCGPYSSRKASWMAKQLEAGFHFHCIDCDITD
WDSTRQAFEMVRENVGPIDVLVNNAGITRDGTLRKMPPENWKAVIDTNLTGLFNTTRQVIESMLTKGW
GRVINISSINGQRGQFGQTNYSAAKAGIHGFSMALAREVSGKGVTVNTVSPGYIKTDMTAAIRPDILEGM
IAGIPVGRLGQPEEIASIVAWLASDQSAYATGADFSVNGGMNMQ

PhbA (*Pseudomonas* USM 4-55)

MIDVVIVAATRTAIGSFQGSLAEIPAPELGAIVIRRLLEQTGLDAAQVDEVILGQVLTAASGQNPARQAVIR
AGLPHVVPAMTLNKVCGSGLKALHLAAQAIRCGDAEVIIAGGMENMSLSPYVLPKARTGLRMGHAQMLD
SMIVDGLWDAFNDYHMGITAENLVDKYGISREAQDAFAAASQQKAVAAIEAGRFDAEITPVLIPQRKGDP
IAFARDEQPRAGTTAESLAKLKPAFKKDGSVTAGNASSLNDGAAAVLLMSAAKAKALGLSVLAKISAYAN
AGVDPAIMGIAPVSASRHCLDKAGWSLNELDLIEANEAFAVQALAVGQELGWDAAKVNVNGGAIALGHP
IGASGCRVLVSLLHEMIRRDAKKGLATLCIGGGQGVALAIER

PhbR (*Pseudomonas* USM 4-55)

MYRISSSYVGMLVAMLQAEGLDAVRLCREAGIDMCLLAQEDAFFTRASAYRLMALAELDSDNPNLGLKAA
VHFKPGSFQLVGYVMMSSANLKQALEHFVRFHLLLGNGVTLALSPEQGGRLRLTCLEHPEEGCPRNRAFE
DAGAAALLGFCRWLMGGGLPQPLQFDFVHDEPGNLPEYQQLFGCSLRFGAQHTSVLFDQQELLRPLSTA
NEALALLHKRFAEFRLGQLGGVSLGGRVRGLIIERLGQEGCDMESIGLALCMSKRTLQRGLEKEGLKFKDI
LGDVRRQSADYYLCRSSYSLTQVAEYLGFHDQSSFHKACLRWFGRSPGRYRLDEGEQSTPHEDVSDAA
HQADDQCIDDIDEEAADQRNDNERLVRGSVALSNCRHVDDGRCS

PhbC (*Pseudomonas* USM 4-55)

MNNSHSFAHYWSGQAPFITSFALQQLRLYVAQNSWFNGHDQSQWFNVTPEALEQLQVDYQQQWTALG
QQLLARQPFDFDDRRFASGNWSEPLFGSMAAFYLLNSGFLLKLLELLTIKEEKPRQRLRYLIEQAIAASAPS
NFLLSNPDALKCLVETQGASLLSGLLHLAGDLQEGKLRQCDRGDFEVGVNLAVTPGEVVLETPLFQLIQYL
PLTDTQYQRPIFIVPPAINKYYILDLSPENSLVRHLLEQGHPVFLMSWRNFTQKQADITWEQIIQDGVISAL

RTTRVISGERHLNCLGFCIGGTLLSCALAVLAARGDHDIASLSLFATFLDYLDTGPISVFVDEQLVAYRERT
IGGHGGKCGLFRGEDMGNTFSLLRPNELWWNYNVDKYLKGQKPRALDLLFWNNDSTNLPGPMYCWYLR
HTYLQNDLKSGELELCGVKLDLRSIEAPAYLLGTHDDHIVPWRSAYASTALLGGSKRFVLGSSGHIAGVIN
PPASNKRHYWVNEHITPIADDWLQSAQQHAGSWWVDWFAWLAGHAGELRPAITRMGNAEYPPLEQAP
GRYVKQ

Pseudomonas USM 4-55_nucleotide sequence

phbB

GTGGCTACTTCGAGTAATTCGACACGTATAGCACTGGTCACCGGCGGCATGGGCGGCATCGGCACAG
CGATCAGCCAGCGCCTGCACCAGGATGGTTTCACCGTAGTGGTGAGTTGCGGCCCCTACTCAAGCCG
CAAAGCCTCTTGGATGGCGAAGCAATTAGAGGCTGGCTTCCACTTCCACTGCATCGACTGCGACATCA
CCGACTGGGACAGCACCCGCCAGGCTTTTGAGATGGTGCGCGAAAATGTCGGCCCGATTGATGTACT
GGTCAACAATGCGGGGATCACCCGAGACGGTACGCTACGCAAGATGCCTCCCGAAAACTGGAAAGC
AGTGATCGATACCAACCTCACCGGCCTGTTCAACACAACCCGTCAGGTCATCGAGAGCATGCTGACC
AAGGGCTGGGGGCGTGTCATCAACATATCCTCGATCAATGGACAGCGGGGTCAGTTCGGCCAGACC
AACTACTCTGCAGCCAAAGCCGGTATCCATGGTTTCAGCATGGCTTTGGCCCGAGAAGTGAGTGGCA
AGGGCGTGACCGTCAATACGGTTTCCCCTGGCTATATCAAGACAGATATGACTGCGGCAATTCGCCC
AGACATCCTTGAAGGCATGATTGCCGGAATCCCCGTCGGTCGCCTCGGCCAGCCTGAGGAAATTGCC
TCGATCGTAGCCTGGCTAGCCTCTGATCAATCCGCCTATGCCACCGGCGCCGACTTCTCGGTGAACG
GCGGCATGAATATGCAGTGA

phbC

ATGAACAATTCACATTCTTTCGCTCACTACTGGTCAGGACAGGCGCCTTTCATCACCAGCTTCGCCCTA
CAGCAACTGCGCCTGTACGTAGCGCAAAACTCTTGGTTCAACGGGCATGACCAAAGCCAGTGGTTCA
ATGTCACCCCCGAAGCGTTGGAACAATTGCAGGTTGACTACCAGCAACAATGGACAGCGCTTGGCCA
GCAACTGCTGGCCCGTCAACCGTTCGACTTCGACGACCGGCGTTTCGCTAGTGGCAACTGGAGTGAA
CCGTTGTTCGGTTCCATGGCCGCCTTCTACCTGCTGAATTCCGGATTTCTGCTGAAATTGCTGGAGCT
GCTCACCATCAAAGAAGAGAAGCCACGTCAGCGTCTGCGCTACCTGATCGAGCAGGCAATCGCCGCA
AGCGCCCCGAGCAACTTCCTGCTGAGCAATCCCGATGCCCTGAAATGCCTGGTGGAAACCCAGGGG
GCCAGCCTGCTCAGTGGTCTATTGCATCTGGCCGGAGACCTACAAGAAGGCAAGTTGCGTCAATGCG
ACCGAGGCGACTTCGAAGTTGGCGTGAATCTCGCCGTCACTCCCGGTGAGGTGGTGCTGGAAACTCC
GCTGTTCCAGTTGATCCAATACCTGCCGCTCACCGATACACAGTACCAGAGGCCAATATTTATCGTCC
CGCCGGCCATCAACAAGTACTACATCCTCGACCTCAGCCCGGAAAATTCTCTGGTTCGCCATCTGCTG
GAACAGGGTCACCCGGTATTCCTGATGTCCTGGCGCAACTTCACCCAGAAACAGGCTGACATCACCT
GGGAGCAGATCATCCAGGACGGAGTGATCAGCGCCCTACGCACTACCCGGGTCATCAGTGGCGAGC
GTCATCTGAACTGCCTGGGCTTCTGCATCGGCGGCACCCTGCTGAGCTGCGCGTTGGCAGTGCTGGC
TGCGAGGGGCGACCACGACATTGCCAGTCTGAGCCTGTTCGCCACCTTCCTCGACTACCTTGATACC
GGGCCGATCAGTGTCTTTGTCGATGAGCAACTGGTGGCCTACCGCGAGCGCACCATCGGTGGCCAT
GGTGGTAAATGTGGCCTGTTCCGCGGAGAGGATATGGGCAACACCTTCTCCCTGCTGCGGCCCAACG
AGCTGTGGTGGAACTACAACGTCGACAAGTACCTCAAGGGACAGAAACCACGCGCCCTAGATCTGCT
GTTCTGGAACAACGACAGTACCAACCTACCTGGCCCCATGTACTGCTGGTATCTGCGCCACACCTACC
TGCAGAACGACCTCAAGTCGGGCGAGCTGGAGCTGTGCGGGGTCAAGCTGGATCTGCGCTCCATAG
AGGCGCCAGCCTACCTCCTCGGAACCCACGATGACCACATAGTCCCATGGCGCAGTGCCTATGCCAG
CACGGCTCTTCTTGGGGGATCGAAACGCTTCGTCCTCGGATCTTCTGGCCACATCGCCGGAGTGATT
AACCCACCAGCAAGCAACAAACGCCATTACTGGGTAAACGAGCACATCACGCCGATTGCTGACGACT
GGCTACAAAGCGCTCAACAACACGCAGGTAGTTGGTGGGTCGATTGGTTCGCCTGGCTGGCCGGGC
ATGCCGGCGAGCTCCGGCCCGCCATCACGCGGATGGGCAATGCCGAATACCCTCCCCTAGAACAAG
CGCCTGGGCGCTACGTGAAGCAATGA

phbR

ATGTACAGGATCAGTTCCAGTTACGTCGGGATGCTGGTGGCTATGCTGCAGGCGGAGGGACTGGAT
GCGGTGCGTTTGTGTCGTGAGGCAGGAATCGATATGTGTTTGCTGGCACAGGAGGATGCGTTCTTCA
CGCGCGCTAGCGCCTATCGACTGATGGCGCTGGCCGAGTTGGATTCGGACAATCCGAATCTCGGCCT
CAAGGCTGCCGTGCATTTCAAACCTGGCAGTTTCCAGTTGGTGGGCTACGTGATGATGTCCAGTGCC
AACCTGAAACAGGCTCTGGAGCATTTTGTGCGTTTTCACTTGCTACTTGGCAATGGTGTCACTCTCGC
ACTGTCTCCTGAGCAAGGCGGGCGTCTGCGTTTGACATGTTTGGAGCATCCTGAAGAGGGCTGTCCC
AGAAATCGAGCGTTTGAGGATGCCGGTGCCGCAGCCCTGTTGGGTTTTTGCCGTTGGCTGATGGGG

GGAGGGCTGCCGCAACCGCTGCAATTCGATTTTGTCCATGACGAGCCGGGTAATCTCCCTGAATATC
AGCAGTTATTCGGGTGTTCGTTACGTTTCGGTGCCCAGCACACTAGTGTCCTGTTCGACCAGCAAGAA
TTGTTGCGCCCGTTGAGCACGGCCAATGAGGCGCTTGCGTTGTTACATAAGCGCTTCGCCGAGTTTC
GTCTGGGGCAGTTGGGTGGTGTCTCCTTGGGGGGGCGCGTGCGTGGCCTGATAATCGAGCGCCTGG
GTCAGGAAGGATGCGACATGGAGTCGATCGGACTGGCTCTTTGCATGAGCAAGCGTACCCTGCAGC
GAGGCCTGGAAAAGGAAGGGCTGAAGTTCAAGGACATTCTCGGTGATGTTCGCCGGCAGTCGGCCG
ATTATTACCTGTGCCGCAGCTCATACAGTCTGACCCAGGTGGCCGAGTACCTGGGGTTCCACGATCA
GAGCAGTTTCCACAAGGCCTGCCTGCGCTGGTTCGGGAGGTCTCCGGGTCGATACCGGCTGGACGA
AGGCGAGCAGAGTACGCCCCATGAGGATGTCTCAGATGCCGCTCATCAGGCTGATGATCAGTGCATT
GACGATATCGATGAAGAAGCCGCAGACCAGCGGAACGATAATGAACGCCTGGTGCGCGGCTCCGTA
GCGCTGAGTAACTGCCGTCATGTTGACGATGGCCGTTGCAGTTGA

phbA

ATGATCGACGTCGTTATCGTCGCTGCAACCCGCACCGCCATCGGTAGCTTCCAAGGCAGCCTGGCCG
AGATTCCGGCACCGGAACTCGGCGCCATCGTCATCCGGCGCCTGCTCGAGCAGACCGGCCTCGATG
CTGCCCAGGTCGATGAAGTGATCCTCGGCCAGGTGCTCACTGCCGCGTCCGGACAGAACCCCGCAC
GCCAGGCTGTGATTCGCGCCGGCCTGCCCCACGTCGTTCCTGCGATGACCCTGAACAAGGTCTGCGG
CTCGGGCCTCAAGGCCCTGCACCTGGCTGCCCAGGCTATCCGTTGCGGCGATGCCGAGGTGATCATT
GCCGGCGGCATGGAGAACATGAGCCTGTCGCCCTACGTGCTGCCCAAAGCCCGCACCGGCCTGCGC
ATGGGCCATGCGCAGATGCTCGACAGCATGATCGTCGATGGCCTGTGGGATGCCTTCAACGATTACC
ACATGGGCATCACCGCCGAGAACCTGGTGGACAAGTACGGCATCAGCCGTGAAGCCCAGGACGCCT
TTGCCGCCGCCTCGCAGCAGAAGGCCGTGGCCGCCATCGAAGCTGGTCGTTTCGACGCCGAGATAA
CCCCGGTGCTGATCCCGCAGCGCAAGGGCGATCCAATCGCCTTCGCCCGTGACGAGCAGCCGCGTG
CCGGCACCACCGCCGAGTCGCTGGCCAAACTCAAGCCGGCGTTCAAGAAGGACGGCAGCGTTACCG
CCGGCAATGCCTCCAGCCTCAACGATGGCGCCGCCGCCGTGCTGCTGATGAGCGCAGCCAAGGCCA
AAGCGCTGGGCCTGTCGGTGCTGGCGAAAATCAGCGCCTATGCCAACGCAGGCGTCGATCCGGCGA
TCATGGGTATCGCCCCGGTCTCGGCCAGCCGCCACTGCCTGGACAAAGCCGGCTGGAGCCTGAACG
AGCTAGACCTGATCGAAGCCAACGAAGCCTTCGCCGTCCAGGCACTGGCCGTTGGTCAGGAGCTGG
GCTGGGATGCCGCCAAGGTCAACGTCAACGGCGGCGCCATCGCCCTTGGCCACCCGATCGGCGCCT
CCGGCTGCCGCGTGCTGGTCAGCCTGCTGCACGAGATGATCCGCCGCGATGCCAAGAAAGGCCTGG
CGACGCTGTGCATCGGCGGCGGCCAGGGCGTGGCGCTGGCCATTGAACGCTGA

8 Appendix 3(ii)

Similarity of the PHB synthase (PhbC) from *Pseudomonas* strain 61-3 to PHA synthases (PhaC1 and C2) found in *Pseudomonas* strain 61-3:

PHB synthase (PhbC) from *Pseudomonas* strain 61-3:

MDNNAHTFKTYWSGQVPFIASFAVQQLRLWVSTNPWFSGHEHGAWFELPRETLDSLQADYQVQWGQLGQK
LLTGQPFSFDDRRFASGNWSEPLFGSLAAFYLLNSSFLLKLLDMLLIDEKKPRQRLRYLVEQAIAASAPS
NFLVSNPDALQRVVETQGASLVTGMQHLASDMNEGKMRQCDSGAFKVGIDLANTPGEIVFENHLFQLIHY
YPQSETQYRHPVFVVPPSINKYYILDLRPDNSMVRHLLEQGHPVFLMSWRNFDEEHAGTTWDDLIELGVI
DGLQVAREISGEQRLNCVGFCIGGTLLSTALAVLAARGDREIASVSLFTTFLDYHDTGPIDIFVDEELVA
HRERTIGGVNGPIGLFRGEDMGNTFSLLRPNDLWWNYNVDKYLKGQKPIPLDLLFWNNDSTNLPGPMYCW
YLRHTYLQNDLKSGELECCGNKLDLRAIDAPAYILATHDDHIVPWKSAYASTNLLSGSKRFVLGASGHIA
GVINPPAKQKRHYWTNNRVTKNPETWFKNAEQHPGSWWNDWFTWLAGHSGERQPAVAHTGNNKYPPLEPA
PGRYVKL

PHA synthases found in *Pseudomonas* strain 61-3:

PhaC2

MREKPTPGLLPTPATFINAQSAITGLRGRDLFSTLRSVAAHGLRHPVRSARHVLALGGQLGRVLLGETLHT
PNPKDNRFADPTWRLNPFYRRSLQAYLSWQKQVKSWIDESGMSDDDRARAHFVFALLNDAVSPSNTLL
NPLAIKELFNSGGNSLVRGLSHLFDDLMHNNGLPSQVTKHAFEIGKTVATTAGSVVFRNELLELMQYKPM
SEKQYAKPLLIVPPQINKYYIFDLSPGNSFVQYALKNGLQVFVVSWRNPDVRHREWGLSSYVEALEEALN
VCRAITGARDVNLMGACAGGLTIAALQGHLQAKRQLRRVSSASYLVSLLDSQIDSPATLFADEQTLEAAK
RHSYQRGVLEGRDMAKIFAWMRPNDLIWNYWVNNYLLGKEPPAFDILYWNSDNTRLPAAFHGDLLDFFK
HNPLTHPGGLEVCGTPIDLQKVNVDSFSVAGINDHITPWDAVYRSTLLLGGDRRFVLSNSGHIQSILNPP
SNPKSNYIENPKLSGDPRAWYYDGTHVEGSWWPRWLSWIQERSGTQRETLMALGNQNYPPMEAAPGT
YVRVR

PhaC1

MSNKNSDDLNRQASENTLGLNPVIGLRGKDLLTSARMVLTQAIKQPIHSVKHVAHFGIELKNVMFGKSKL
QPESDDRRFNDPAWSQNPLYKRYLQTYLAWRKELHDWIGNSKLSEQDINRAHFVITLMTEAMAPTNSAA
NPAAVKRFFETGGKSLLDGLTHLAKDLVNNGGMPSQVDMGAFEVGKSLGTTEGAVVFRNDVLELIQYRP
TTEQVHERPLLVVPPQINKFYVFDLSPDKSLARFCLSNNQQTFIVSWRNPTKAQREWGLSTYIDALKEAV
DVVSAITGSKDINMLGACSGGITCTALLGHYAALGEKKVNALTLLVSVLDTTLDSQVALFVDEKTLEAAKR
HSYQAGVLEGRDMAKVFAWMRPNDLIWNYWVNNYLLGNEPPVFDILFWNNDTTRLPAAFHGDLIEMFK
NNPLVRANALEVSGTPIDLKQVTADIYSLAGTNDHITPWKSCYKSAQLFGGKVEFVLSSSGHIQSILNPPG
NPKSRYMTSTDMPATANEWQENSTKHTDSWWLHWQAWQAERSGKLKKSPTSLGNKAYPSGEAAPGT
YVHER

The PHB and PHA synthases in *P. fluorescens* 61-3 had only low similarity (52% - 53%) confirming that the PHB and PHA pathways are easily distinguished.

10 Appendix 3(iii)

P. fluorescens NCIMB-11764 PHB pathway:

PhbC

MDNNAHTFNTFWSGQVPFIASFAVQQLRLWVSTNPWFTGQEYNEWFDLPRDTLDSLQSDYQLQWADL
GHRLMSGQPFTFEDRRFASGNWREPLFGSLAAFYLLNAGFLLKLLDKLPIKDKKPRQRLLYLVEQAIAAGA
PSNFLVSNPDALQRAADTQGASLLTGLLHLASDLQEGKMRQCDSGAFKVGVDLASTPGEVVFENDLFQLI
QYYPQSDTQYRRPVFIVPPSINKYYILDLRPDNSMVRHLLQQGHPVFLMSWRNFDQAHAGTTWDDLIET
GIIKGLQVTREISGEQRPNCVGFCIGGTLLSTALAVLAARGDREIGSVSLLTTFLDYLDTGPIDIFVDEQLV
AYRERTIGGVDGPIGLFKGEDMGNTFSLLRPNDLWWNYNVDKYLKGQKPIPLDLLFWNNDSTNLPGPMY
CWYLRHTYLQNDLKSGELDCCGARLDLRAIDAPAYILATHDDHIVPWRSAYASTELLSGTKRFVLGASGH
IAGVINPPAKEKRHYWTNNRVTKNPDTWFKNAEQHPGSWWNDWFAWLAEHGGERQPSAPHSGNAQY
PALESAPGRYVMQ

PhbR

MYKMSSGYASVLVNTLSAQGLDVASLCREAGLDIDLANKPGAFCERKAIYRLWELAAEASGDPDIGLRAY
GSFHPGSFQIVGYTMMSSLNLKKALERLVRFSPLIGTGFSLFFTSEQQHYRLSGLDHQQQGSVKPRQYTD
AGLASLLGFCRKLSGGNAPQPLSVEFTYPEPEDISEHQRLFGCNLQFDAAYDSILFDREELMRPLSMANEA
LAVLHDSFAEAQLDLLFGFCIVGRIRALITERLSQGQGQCDMESIAAALNISKRTLQRALEKEGTQFRDVL
NAVRRQLADFYLRHSHFNMKHVAYLLGFHDHSSFNKACSRWFGMTPGQYRSDESFEIEEAAPV

PhbA

MNEVVIVAATRTAIGSFQGALSAIPATELGATVIRRLLEETGLDGAQIDEVILGQVLTAGSGQNPARQTAI
KAGLPFTTPALTLNKVCGSGLKAVQLAVQAIRCGDAELVIAGGQENMSLAPYVLPKARTGLRLGHAQLQD
SVIQDGLWDVFNDYHMGITAENLATRYSLTREDQDAFAAASQQKAAAAIEAGYFKREITPILIPQRKGDPL
VFDTDEQPRPGSTLQALSNLKPAFQKDGSVTAGNSSTLNDGAAVLLLTSATTAQALGLPVLARIKAYASA
GVDPSIMGIGPVPATRLV0LEKAGWRLDDLDLIEANEAFAAQSLAVGKELGWDTRKVNVNGGAIALGHP
IGASGARILVSLVHELIRRDGKKGLATLCIGGGQGVSLVIER

PhbB

MKSLGRIALVTGGMGGIGTAISQRLYKEGFKVIVGCSADSARKNDWIATQLAAGYQFECIYGDITDWES
TRKAFETAHEQFGAVDVLVNNAGITRDASFRKLTPEDWNAVIGTNLSGLFNTTKQVIEGMLAKGWGRVI
NISSINGQRGQFGQTNYSAAKAGIHGFTMALAREVSGKGVTVNTVSPGYIQTSMTAAIRPDILDTMIAAT
PVGRLGQPEEIASIVAWLASDESGYSTGADFSVNGGMNMQ

Pseudomonas arsenicoxydans strain CECT 7543 PHB pathway:

PhbC

MDNNAHTFNTFWSGQVPFIASFAVQQLRLWVSTNPWFTGQDYNEWFELPRATLDSLQADYQMEWGDL
GQRLLTGQPFSFEDRRFASGNWNTPLFGSLAAFYLLNAGFLLKLLDKLPIKDKKPRQRLLYLVEQAIAAGA
PSNFLASNPDALQRVVDTQGASLLTGLLHLASDLQEGKMRQCDSGAFKVGVDLANTPGEVVFENELFQLI
QYYPQSETQYRRPVFIVPPSINKFYILDLRPDNSMVRHLLQQGHPVFLMSWRNFDAAHAGTTWDDLIETG
IIKGLQVTREISGEQRPNCVGFCIGGTLLSTALAVLAARGDKDIASVSLLTTFLDYLDTGPIDIFVDEQLVAY
RERTIGGLDGPVGLFKGEDMGNTFSLLRPNDLWWNYNVDKYLKGQKPNALDLLFWNNDSTNLPGPMYC
WYLRHTYLQNDLKSGDLDCCGVKLDLRAIEAPAYILATHDDHIVPWRSAYASTRLLSGPTRFVLGASGHI
AGVINPPAREKRHYWTNKRVTKNPETWFMNAQQHPGSWWNDWFAWLAEQAGERQTSALHTGNAQYP
ALESAPGRYVMQ

PhbR

MYRMSSGYASVLVNTLSAQGLDVASLCQEAGLDIELANKPGAFCERRAIYRLWDLAAQASGDPDIGLKA
YGSFHPGSFQIVGYTMMSSLNLKNALERLVRFSPLIGTGFSLFFTSEQQNYRLSGLDHQQVGSVKPRQYT

DAGLASLLGFCRKLAGGTLPHPLSVEFTYPEPADTREHRRLFGDDLHFGAAYDSILFDGQELMRPLSMAN
EALAVLHDSFAEAQLDLLFGFCIVGRIRALITERLSQGNGQCDMESIAAALNISKRTLQRALEKEGTQFKD
VQNAVRRQLADFYLRHSHFNMKHVAYLLGFHDHSSFNKACSRWFGMTPGQYRSDESFELEETAPV

PhbA

MNEVVIVAATRTAIGSFQGALSAIPATDLGAAVIRSLLEQAGVDAAQIDEVILGQVLSAGSGQNPARQSAI
KAGLPFTTPALTLNKVCGSGLKAVQLAVQAIRCGDAELVIAGGQENMSLAPYVLPKARTGLRLGHAQLQD
SVIQDGLWDAFNDYHMGITAENLATQYSLSREDQDAFAAASQQKAAAAIEAGYFKREITPILIPQRKGDP
LAFDTDEQPRPGSTLQALSNLKPAFQKDGSVTAGNSSTLNDGAAVLLLASAAKALVLGLPVLARIKAYAG
AGVDPSIMGIGPVPATRLALTKAGWSLDDLDLIEANEAFAAQSLAVGKELGWDTQKVNVNGGAIALGHP
IGASGARILVSLVHELIRRDGKKGLATLCIGGGQGVSLLIER

PhbB

MKSLGRIALVTGGMGGIGTAISQRLYKEGFKVIVGCSSDSARKNDWIAGQLAAGYQFECIYGDITDWES
TRKAFEMAREQFGPVDVLVNNAGITRDASFRKLQPEDWNAVIGTNLTGLFNTTKQVIESMLAKGWGRVI
NISSINGQRGQFGQTNYSAAKAGIHGFTMALAREVSGKGVTVNTVSPGYIQTSMTAAIRQDILDTMIAAT
PVGRLGQPEEIASIVAWLASDESGYSTGADFSVNGGMNMQ

11 Appendix 3(iv)

RpsB 30S ribosomal protein S2 [*Pseudomonas fluorescens* NCIMB 11764, accession AKV06965.1]

MSQVNMRDMLKAGVHFGHQTRYWNPKMGKYIFGARNKIHIINLEKTLPMFNEALTFVERLAQGKNKILFVGTKRSAGKIVAEEAAR
CGSPYVDHRWLGGMLTNFKTIRASIKRLRDLEVQAEDGTFAKLTKKEALMRTRDLEKLDRSLGGIKDMGGLPDALFVIDVDHERIAITE
ANKLGIPVIGVVDTNSSPEGVDYIIPGNDDAIRAIQLYMGSMADAVIRGRNHVAGGTEQFVEEAPAAAAE

Uridylate kinase [*Pseudomonas fluorescens* NCIMB 11764, accession AKV06967.1]

MAQQGSGYQARYKRILLKLSGEALMGSEEFGIDPKVLDRMALEVGQLVGIGVQVGLVIGGGNLFRGAALSAAGMDRVTGDHMGM
LATVMNALAMRDALERANISAIVMSAISMVGVTDHYDRRKAMRHLNAKEVVIFAAGTGNPFFTTDSAACLRAIEIDADVVLKATKVD
GVYTADPFKDPHAEKFDHLTYDEVLDRKLGVMDLTAICLCRDHKMPLRVFNMNKPGALLNIVHGGAEGTLIEEGQQ

Antibiotic transporter [*Pseudomonas fluorescens* NCIMB 11764, accession AKV07119.1]

MQFKPAVTALVTAVALASLLSGCKKEEAAPAAPPPQVGVVTLQPQAFTLTSELPGRTSAYRIAEVRPQVNGIILKRLFKEGGDVKAGQ
QLYQIDPAVYEATLKSAEANLQQTKSISDRYKQLVDEQAVSRQEYDTALANRLQSEAALQSAQINVRYTKVYAPLSGRIGRSNVTEGAL
VTSGQADAMAVIQQLDPIYVDVTQSSVELLELRRELESGRLQKAGDNSAAVKLTLEDGSQYKLDGKLEFSEVSVDQTTGSVTLRAVFP
NPDHTLLPGMFVHAQLQAGVNAAAILAPQQGVTRDLKGTPTALVVGPDNKVELRQLKASRTVGSQWLIEDGLKAGDRLITEGLQFV
KPGIEVKATEATNVGAKNPAPAQAASKASSGKGE

Multidrug transporter [*Pseudomonas fluorescens* NCIMB 11764, accession AKV07118.1]

MSRFFIDRPIFAWVIALVIMLVGALSILKLPINQYPSIAPPAIAIQVTYPGASAQTVQDTVVQVIEQQLNGIDNLRYVSSESNSDGSMTIT
ATFEQGTNSDTAQVQVQNKLNLATPLLPQEVQQQGIRVTKSVKNFLLVIGVVSRDGSMTKDDLSNYIVSNMQDPISRTAGVGDFQV
FGSQYAMRVWLDPAKLNNFSLTPVDVKAAIAAQNIQVSSGQLGGLPAAPGQQLNATIIGKTRLQTAEQFNKILLKVNKDGSQVRLSD
VADVGLGGENYSINAQFNGAPASGLAVKLANGANALDTAKALRNTINTLKPFFPQGMEVVFPYDTTPVVTESIKGVVETLVEAIVLVF
LVMFLFLQNFRATVITTMTVPVVLLGTFGILAAFGFSINTLTMFGMVLAIGLLVDDAIVVVENVERVMSEEGLSPKEATKKSMGQIQG
ALVGIALVLSAVLLPMAFFSGSTGVIYKQFSITIVSAMALSVLVALIFTPALCATMLKAIPKGEHGTPKRGFFGWFNRSFDRGVKSYERG
VGNMLAHKAPYLLAYLIIVVGMIWLFTRIPTAFLPEEDQGVLFAQVQTPAGSSAQRTQVVIDEMRSYLLDKESSAVASVFTVNGFNFA
GRGQSSGLAFIMLKPWDQRDAENSVFKLAARAQQHFFTFRDAMVFAFAPPAVLELGNATGFDVFLQDRAGIGHEKLMEARNQFLG
MAAQSKVLYQVRPNGLNDEPQYQLEIDDEKASALGITLSDINSTLSIALGSSYVNDFIDRGRVKKVYVQGQPGSRMSPEDIKKWYVRN
SAGTMVPFSAFAKGQWIYGAPKLSRYNGVEAMEILGAPAPGYSTGEAMAEVEALAKKLPAGVGISWTGLSYEERLSGSQAPALYALS
LLMVFLCLAALYESWSIPIAVMLVVPLGIIGALLATSLRGLSNDVYFQVGLLTTIGLAAKNAILIVEFAKELHEQGRSLRDAAIEACRMRLR
PIIMTSLAFVLGVVPLAISTGAGSGSQHAIGTGVIGGMITATVLAIFWVPLFFVTVSSIGQRKTADQDDAIEPSKEAG

12 Appendix 3(v)

Pgm

MTLSPFAGKPAPAESLVDIPRLVTAYYTGQPDAAISTQRVAFGTSGHRGSSFDLSFNEWHVLAISQAICL
YREAQGIDGPLFVGIDTHALSTPAGASALEVLAANGVTVMIAEGDEYTPTPAISHAILCYNRGRTSGLAD
GIVITPSHNPPQSGGYKYNPTNGGPADTHITKWIEAKANELLGNKLAGVKRISYEQALKASTTHRHDYLN
TYVADLINVIDFDAIRGAKLHLGVDPLGGAGVRYWSAIAEHYKLDLDVVNKQVDPTFRFMTVDWDGQIRM
DPSSSHAMQGLIGLKERFDVAFACDPDHDRHGIVTPSGGLLAPNSYLAVSIDYLFQNRPQWRADAAVGKT
VVSSGLIDRVAKRLGRRLYEVPVGFKWFADGLFDGSLGFGGEESAGASFLRKDGGVWSTDKDGLIPALLA
AEMTARTGRDPSQAYRALTDELGEPFSVRVDAKANPEQKALLSKLSPEQVTSTQLAGEAIQSILSHAPGN
DQAIGGLKVMTENGWFAARPSGTEDIYKIYAESFVSDEHLKQLVAEAQTLVDGAISAK

Glk

MKLALVGDIGGTNARFALWKNQQLESVQVLATADHASPEEAIAIYLGGLGLAPGSIGSVCLSVAGPVSGD
EFKFTNNHWRLSRKAFCQTLQVDQLLLVNDFSAMALGMTRLQPGEFRVVCEGTPEPLRPAVVIGPGTGLG
VGTLLDLGEGRFAALPGEGGHVDLPLSSLRETQLWQHIFNEIGHVSAETALSGGGLPRVYRAICAVDGHT
PVLETPEAITAAGLAGDPIALEVLEQFCCWLGRVAGNNVLTTGARGGVYIVGGVIPRFADFFIESGFARC
FADKGCMSHYFKGIPVWLVTAPYSGLVGAGVALEQSSLA