Communication

DOI: 10.5582/ddt.2022.01013

Probiotic microbes: Are their anti-melanogenicity and longevity promoting activities closely linked through the major "pathogenic" kinase PAK1?

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SUMMARY PAK1-deficient mutant of *C. elegans* lives 60% longer than the wild-type. Interestingly, PAK1-deficient mutant of melanocytes produces less melanin (only a half compared with the wild-type) in the presence of either serum (PDGF) or α-MSH (alpha-melanocyte stimulating hormone). These observations indicate that the major "pathogenic" kinase PAK1 is responsible for both shortening the healthy lifespan, and PDGF/α-MSH-dependent melanogenesis. For screening of PAK1-blocking probiotic bacteria or their products, their anti-melanogenic as well as longevity promoting properties were examined. Recently it was found that *C. elegans* fed with *Lactobacillus rhamnosus* in Xinjiang cheese lives 40% longer than the worm fed with the standard *E. coli*. Interestingly, a Chinese traditional medicine called "ChiBai" fermented with the *Lactobacillus rhamnosus* also inhibited the α-MSH-induced melanogenesis, and this bacteria itself produces butyric acid that blocks the oncogenic HDAC (histone deacetylase)-PAK1 signaling pathway. These findings strongly suggest, if not proven, that anti-melanogenic activity of *Lactobacillus* and many other probiotic bacteria might serve as a reliable indicator for their longevity promoting activity. In this context, a popular Japanese *Lactobacillus*-fermented milk drink called "Calpis", developed a century ago, and recently proven to inhibit the melanogenesis by suppressing the PAK1-dependent tyrosinase gene expression, may potentially prolong our healthy lifespan.

Keywords PAK1, *Lactobacillus*, melanogenesis, longevity, *C. elegans*, *Bacillus*, COVID

1. Introduction

In 1908, a Russian/Ukranian physiologist, Ilya Mechnikov (1845-1916), shared a Nobel Prize in Medicine with Paul Ehrlich (1854-1915), a German/Jewish pathologist who developed the first chemotherapeutic called "Salvarsan" (or 606) against Syphilis in 1909. The former was an expert in phagocytes called macrophages, and published a rather sensational book entitled "Prolongation of life: Optimistic Studies" in 1907. In this book he proposed a theory that *Lactobacillus* from stomach from long-living Bulgarians who ingest routinely the Bulgarian local yogurts fermented with *Lactobacillus* could potentially be useful for our longevity. More than a century later, there are increasing biochemical evidences, provided mainly from the Far-East research groups, supporting this theory in principle.

In this century, the majority of scientists studying on the longevity opt for testing the potential longevity-promoting (so-called elixir) effect of given chemicals or organisms on a tiny worm called *C. elegans*, mainly because its lifespan is the shortest among aminal kingdom, around 15 days at 20°C. Not suprisingly among the genes responsible for shortening the life-span of this organism shared with mammals are "oncogenic" genes encoding PI-3 kinase (AGE), PAK1, ILK, AKT and TOR (*1-4*). PI-3 kinase deficient mutant of this worm lives 100% longer than the wild-type (*1*), and PAK1-deficient mutant lives 60% longer than the wild-type (*2*). Interestingly in both cases, these mutants show a very low fertility (less than 14% of the wild-type) (*1,2*), indicating that these oncogenes are essential for their fertility. In other words, longevity trades fertility (*1,2*).

Unfortunately, however, any chemical compounds such as LY3023414 which block the oncogenic PI-3 kinase-AKT signalling cannot be used clinically for promoting the longevity, simply because this pathway is essential for heart development/function as well (*2,5*). Tiny "experimental" invertebrates such as *C. elegans* and

Drosophila have no cardiovascular system. Fortunately, in 2021, PAK1-deficient mutant of mice was proven to live significantly longer than the wild-type without any complication on either heart or brain (6). In addition, mice treated with rapamycin, a TOR-inhibitor, have been shown to live longer than the control mice (7). However, this drug has been used mainly to suppress the immune response against grafted organs (2). Therefore it may be rather risky for ordinary people, in particular during pandemics of COVID and other deadly viruses.

2. Natural chemicals, that prolong the lifespan of *C. elegans*, inhibit melanogenesis by blocking PAK1

Using *C. elegans* as a target, a number of natural longevity promoters have been identified. Among are curcumin (CC), caffeic acid (CA), caffeic acid phenethyl ester (CAPE), and melatonin (*8-11*). Interestingly, all these longevity promoters are known to inhibit melanogensis, without affecting directly the enzymatic activity of tyrosinase which is responsible for biosynthesis of melanin from tyrosine (*12-15*). Since the first three chemicals (CC, CA and CAPE) at least have been known to block PAK1, during 2015-2017 we examined whether melanogenesis of melanoma (B16F10) requires PAK1 or not. We found that treatment of melanoma with si-RNA specific for PAK1 (silencing *PAK1* gene) clearly reduces the melanin synthesis to a half of the control cells only when cells are activated with either serum (PDGF) or alpha-MSH (*16*), indicating that the "induced" melanogenesis depends on PAK1, although the "basic" melanogenesis without PDGF or alpha-MSH does not (Figure 1A).

3. *Lactobacillus rhamnosus* extends the lifespan of *C. elegans* and inhibits melanogenesis

In 2016, to our great surprise, researchers found that *C. elegans* fed with *Lactobacillus rhamnosus* which is used for fermentation of Xinjiang cheese lives 40% longer than the worm fed with the standard *E. coli*. In 2020, other researchers found that an extract from a Chinese traditional herb mixture called "ChiBai" fermented with *Lactobacillus rhamnosus* inhibits alpha-MSH-induced melanogenesis of B16F10 melanoma by suppressing the tyrosinase gene expression (*18*) which depends on PAK1 (Figure 1B, *16*). These two independent findings altogether indicate that both longevity-promoting and anti-melanogenic activities of this bacterium closely link to each other, and perhaps suggesting its PAK1-blocking activity. Incidentally, in 2021, another group found that *Lactobacillus rhamnosus* inhibits COVID fibrosis in part by producing butyric acid (*19*), which is known to inhibit HDAC (histone de-acetylase), thereby blocking PAK1 (*20,21*) that is responsible for inflammation, melanogenesis, oncogenesis and so many other diseases (for a review. *22*).

4. *Bacillus subtilis* also extends the lifespan of *C. elegans* and inhibits melanogenesis.

It is well known that vitamin D3, a PAK1-blocker, is also anti-melanogenic and extends the healthy lifespan of *C. elegans* by 40% at 1 mg/mL (*23,24*). Interestingly, another vitamin called K2 or menaquinone 7 (Figure 2 left), derived from a traditional Japanese soybean product called "Natto" (fermented by *Bacillus subtilis* natto), also blocks PAK1 and is anti-melanogenic (*25*), although its longevity-promoting activity has not been tested as yet. In 2019, however, it was found that *C. elegans* fed with *Bacillus subtilis*, instead of the standard *E. coli*, at 20°C has 30% lesser size (number of eggs laid) than the *E. coli*-fed, and far more resistant to heat-shock at 34°C than

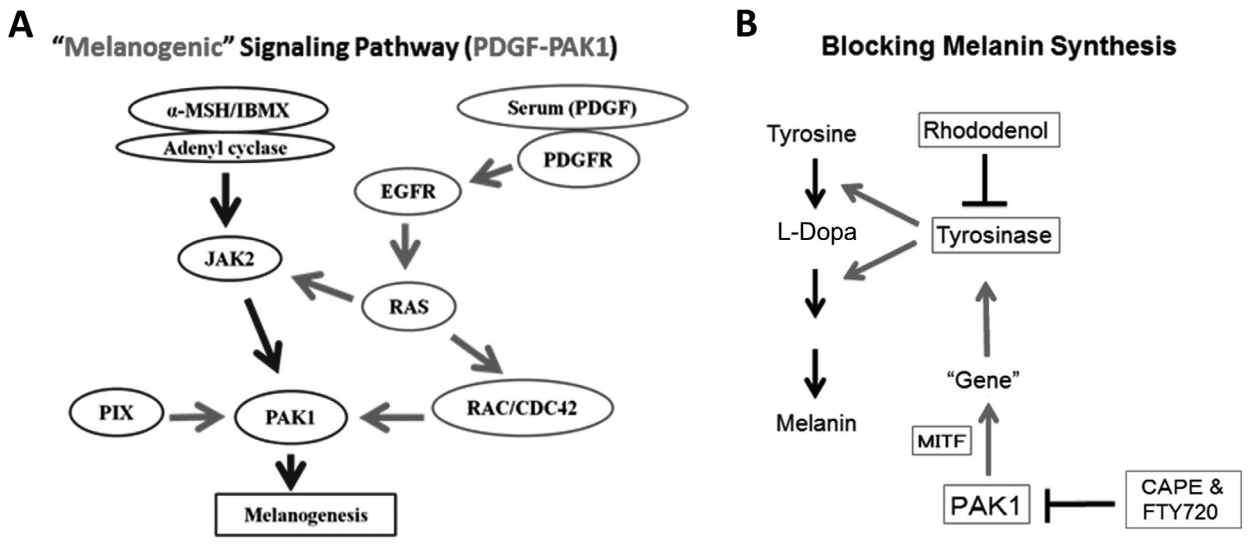
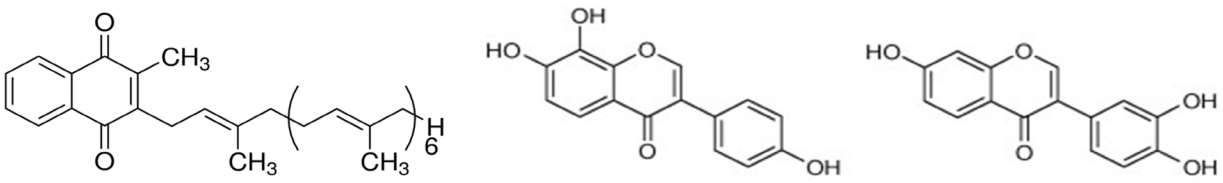


Figure 1. (A), "Melanogenic" signaling pathway (PDGF-PAK1). (B), Blocking melanin synthesis. PAK1-blockers do not inhibit directly tyrosinase, but suppress its gene expression.



Vitamin K2 **Isoflavone derivative 1** **Isoflavone derivative 2**

Figure 2. Vitamin K2 and isoflavone derivatives from soybeans fermented with *Bacillus subtilis*. These PAK1-blockers, derived from Japanese "Natto" and Korean "Doenjang", are anti-melanogenic and anti-carcinogenic/anti-angiogenic, and most likely to promote the longevity.

the *E. coli*-fed, while a half of the latter die within 6 h (*26*). Since the litter size is reciprocal to the lifespan, and heat-resistance is proportional to the lifespan (*1,2*), it is most likely that *Bacillus subtilis* is a longevity promoter, just like *Lactobacillus*. Interestingly a traditional Korean soybean paste (or cake) called "Doenjang" fermented with *Bacillus subtilis* is mainly produced in the Southern west region (Sunchang) of Korea, which is well known as the "longevity" town.

According to two Korean groups, "Doenjang" contains a PAK-blocker called "ortho-dihydroxyisoflavone" (Figure 2 right) that suppresses cancer growth, angiogenesis and melanogenesis (*27,28*). More interestingly, in 2015, another Korean group found that Genistein (4',5,7-trihydroxyisoflavone), which is often produced by yeast fermentation, indeed extends the lifespan of *C. elegans* significantly, and increases its heat resistance by boosting *HSP16* gene expression at 50 μM (*29*). More recently genistein was found to boost the tumor suppressor p21 (CDK inhibitor) by blocking the JAK-PAK1 signaling pathway (*30,31*).

5. Anti-melanogenic activity might be used as a reliable indicator for both PAK1-blocking and longevity-promoting activities

Indeed, it has been shown in 2005 that HDAC inhibitors such as butyrate and TSA (trichostatin A), which eventually block PAK1 (*21*), extend the healthy lifespan of *Drosophila* (*32*). Thus, if a given bacterium or chemical (natural or synthetic) inhibits alpha-MSH/PDGF-induced melanogenesis of B16F10 melanoma by suppressing tyrosinase gene expression, instead of inhibiting tyrosinase activity itself, it is hypothesized that this bacterium or chemical would be a PAK1-blocker, and therefore might extend the healthy lifespan. In other words, the inhibition of the inducible melanogenesis (without any inhibition of cell growth *per se*) might serve as an indicator for screening any PAK1-blocking probiotic bacteria, foods or chemicals/drugs that contribute to the longevity.

In this context, it would be worth noting that two independent Chinese and Japanese groups in 2016 and 2020, respectively found that an old Japanese *Lactobacillus* fermented milk drink called "Calpis", which was developed a century ago by a Japanese monk (Kaiun Mishima) using *L. helveticus*, inhibits the inducible melanogenesis of B16F10 melanoma by suppressing PAK1-dependent tyrosinase gene expression (*33,34*). Thus, it is quite possible that this popular fermented milk could contribute to both COVID prevention/therapy and the longevity eventually (for review, *35*).

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Received February 9, 2022; Revised February 24, 2022; Accepted February 27, 2022.

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